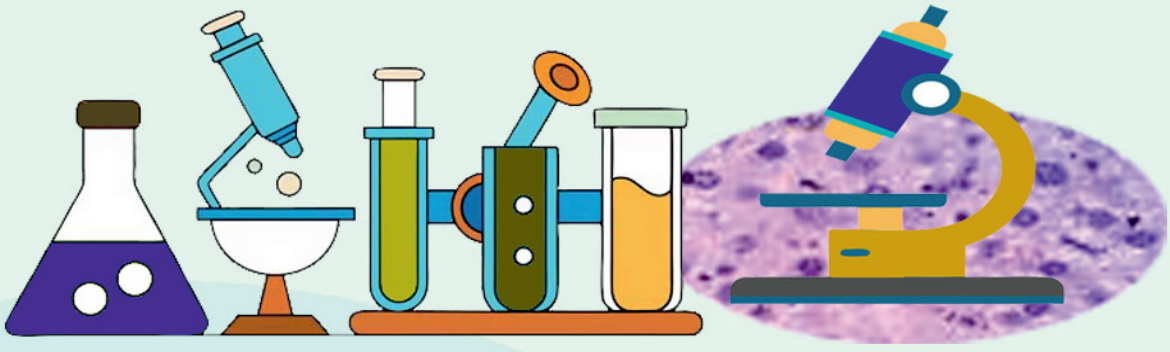
Preclinical Studies

A Compendium of Abstracts

(2014-2025)



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CCRUM

Central Council for Research in Unani Medicine

PRECLINICAL STUDIES
A COMPENDIUM OF ABSTRACTS
(2014-2025)

Central Council for Research in Unani Medicine

Ministry of Ayush, Government of India

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PREFACE

Unani Medicine enjoys a long legacy of education, research, and clinical practice, evolving over centuries into a well-structured and institutionalized discipline in India. Recognizing its significance, the Government of India has been extending sustained support for the comprehensive development of Unani Medicine, along with other traditional systems, through focused policies, funding, and institutional strengthening. As a result, India today possesses a robust infrastructure of academic, research, and healthcare institutions dedicated to Unani Medicine.

Globally, there is a growing acceptance and utilization of traditional medicine in healthcare delivery. This renewed interest has simultaneously underscored the need for rigorous scientific validation of the safety, efficacy, and quality of traditional formulations and therapeutic practices. Addressing this need through systematic research is essential for the wider acceptance and integration of Unani Medicine into contemporary healthcare frameworks.

The Central Council for Research in Unani Medicine (CCRUM), an autonomous organization under the Ministry of Ayush, Government of India, has been at the forefront of advancing research in Unani Medicine. The Council is actively engaged in both fundamental and applied research, and has made noteworthy contributions through innovative research outcomes, patent filings, and high-quality scientific publications in national and international journals. The Council is implementing the Preclinical Research program at the National Research Institute of Unani Medicine for Skin Disorders, Hyderabad, and the Regional Research Institute of Unani Medicine, Srinagar. The Research Laboratories at these Institutes, equipped with state-of-the-art facilities, are actively involved in the safety and efficacy evaluation of single and compound Unani formulations. Efficacy studies of Unani formulations are undertaken using the reverse pharmacology approach, employing validated animal models. A wide range of pharmacological activities, have been systematically investigated for various single and polyherbal Unani formulations.

Preclinical Studies- A Compendium of Abstracts brings together the preclinical research carried out by the Council and reflects our commitment to generating credible evidence through rigorous research. It is hoped that this publication will serve as a valuable reference for researchers, scholars, academicians, clinicians, and policymakers and will further strengthen the scientific foundation and global acceptability of Unani Medicine.

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Prior to 2013

Pharmacology and Toxicology Laboratory

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BACKGROUND

Pharmacology and Toxicology Research Laboratory at National Research Institute of Unani Medicine for Skin Disorders, Hyderabad is involved in conducting safety and efficacy evaluation of single and compound Unani formulations. The Institute has CCSEA (Committee for Control and Supervision of Experiments on Animals) approved laboratory animal facility (Reg. No. 1034/GO/ReRc/S/2007/CCSEA).

Pharmacology & Toxicology Research laboratory is equipped with state-of-the-art facilities carried out acute and repeated dose oral toxicity studies of different duration (i.e., 28-day, 90-day and 180-day) to establish the safety of Unani formulations on prolonged use. Toxicity studies on Unani formulations are being conducted in rats and mice as per AYUSH, OECD, ICH, and Schedule-Y of Drugs and Cosmetics Act guidelines. Efficacy of Unani formulations are being conducted in Reverse Pharmacology mode using various validated animal models. Pharmacological activities like hepatoprotective, nephroprotective, haematopoietic activity, diuretic activity, anti-inflammatory activity, analgesic activity, antiepileptic activity, anti-depressant activity, anxiolytic activity and effect on cognitive function have been conducted so far on various single/compound Unani formulations.

All studies were conducted after prior approval from Institutional Animals Ethics Committee of NRIUMSD Hyderabad, duly authorised and constituted by CCSEA, New Delhi. The animal studies are being conducted at this facility with full adherence to ethics guidelines issued by CCSEA from time to time.

2014-2015

1 Preclinical Safety Evaluation of *Jawārish-i-Jālīnūs* (classical and modified version)

Sub-chronic (repeated dose 90-day) oral toxicity study on Jawārish-i-Jālīnūs (classical and modified version) in rats.

Jawārish-i-Jālīnūs (JJ) is a classical Unani formulation indicated for the treatment of weakness of vital organs, liver and stomach. Though JJ has been widely used for several decades, no scientific report is available for its safety. In the present study, JJ and its sugar-free tablet version (SFJJ) were assessed for safety in Sprague Dawley rats.

Repeated dose 90-day oral toxicity study was performed as per the OECD test guideline no. 408. JJ was orally administered at the dose of 2,000 mg/kg bw/day whereas SFJJ was orally administered at the doses of 506, 1,012 and 2,024 mg/kg bw/day for 90-days. Animals were periodically observed for clinical sign of toxicity, mortality, morbidity, body weight changes and feed consumption. At the end of study, haematology, clinical biochemistry, electrolytes, gross pathology, relative organ weight and histological examination were performed.

Clinical examinations of rats made at different time intervals did not reveal any incidence of abnormal clinical signs / behaviour suggestive of any systemic toxicity among the rats treated with SFJJ at the dose levels of 506, 1,012 and 2,024 mg/kg or 2,000 mg/kg of classical JJ version or control group animals. All the rats in the study survived throughout the 90-days study period. No significant difference in the body weight gain was observed between control and drug treated groups during the study. There was no statistically significant

difference noted in the feed intake of any drug treated groups in any sex compared to the vehicle treated control rats. Haematological parameters such as Hb, RBC, WBC count and HCT of drug-treated rats were comparable to that of control rats. No dose-dependent treatment related significant difference was observed in the blood biochemical parameters such as AST, ALT, ALP, total protein, albumin, bilirubin, creatinine and BUN between control and drug treated groups. There was no significant difference observed between the motor performance in rota-rod and grip strength (as assessed by GSM), among drug treated and control animals.

Further, no significant differences were observed in the relative organ weight of brain, heart, thymus, liver, lungs, spleen, kidneys, adrenals, testes/ovaries and uterus between the control and drug treated groups. No gross pathological changes were observed during necropsy in any group. Based on the data of 90-day repeated dose oral toxicity study, the No Observed Adverse Effect Level (NOAEL) of SFJJ and JJ may be considered greater than 2,024 and 2,000 mg/kg bw/day, p.o. in rats, respectively. In conclusion, both formulations were found to be safe up to the tested dose levels and experimental conditions, and have similar safety profile.

Publications

Husain GM, Ahmed SS, Azhar M, Siddiqui JI, Waheed MA, Kazmi MH. Comparative toxicity study on Classical and modified version of *Jawārish-i-Jālīnūs* (A traditional Unani Formulation) in rats. *Integrative Medicine Research.* 2017; 6(1):66–78. https://doi.org/10.1016/j.imr.2017.01.001

2 Preclinical Safety Evaluation of *Iṭrīfal Usṭūkhūdūs* and Sugar-Free Tablet Version of *Iṭrīfal Usṭūkhūdūs*

2.1 *Acute Oral Toxicity Study on Iṭrīfal Usṭūkhūdūs in Rats*

This study was conducted to evaluate acute oral toxicity potential of *Iṭrīfal Usṭūkhūdūs* (IU) in Sprague Dawley rats. Considering the low acute toxicity potential, the limit test as per OECD-425 was conducted at the dose of 5000 mg/kg body weight. Animals were weighed, observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with IU in three consecutive animals, dosing to further animals was stopped. All the three animals were observed daily and sacrificed on Day-15. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the tested dose of 5000 mg/kg bw of IU. Therefore, oral LD_{50} of the IU in the female Sprague-Dawley rats was estimated to be greater than 5,000 mg/kg body weight.

2.2 *Acute Oral Toxicity Study on Sugar-Free Version of Iṭrīfal Usṭūkhūdūs in Rats*

This study was designed to evaluate acute oral toxicity potential of sugar free version of *Iṭrīfal Usṭūkhūdūs* (SFIU) in Sprague Dawley rats. Considering the low acute toxicity potential, the limit test as per OECD-425 was conducted at the dose of 5000 mg/kg body weight. Animals were weighed, observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with SFIU in three consecutive animals, dosing to further animals was stopped. All the three animals were sacrificed on Day-15 and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 5000 mg/kg bw of SFIU. Therefore, oral LD_{50} of the SFIU in the female Sprague-Dawley rats was estimated to be greater than 5,000 mg/kg body weight.

2.3 *Sub-chronic (repeated dose 90-day) oral toxicity study of Iṭrīfal Usṭūkhūdūs and sugar-free tablet version of Iṭrīfal Usṭūkhūdūs in rats*

Sub-chronic toxicity study of IU and SFIU was carried out in SD rats in accordance with OECD guideline number 408. Animals were divided into various groups (n=10 per sex per group). Classical formulation of IU was administered at the doses of 1028 and 2000 mg/kg bw/day p.o. and SFIU was administered at doses of 357(1X), 1070 (3X) and 1783 (5X) mg/kg bw/day p.o., respectively for 90 days.

All the rats in the study survived throughout the 90-days study period. No significant difference in the body weight gain was observed between control and drug treated groups during the study. There was no statistically significant difference noted in the feed intake of any drug treated groups in any sex compared to the respective vehicle treated control rats. Haematological parameters and clinical biochemical parameters were found normal as compared to control group. Nevertheless, there were few alterations observed in terms of haematology and biochemistry. However, these changes were not dose dependent and remained within the normal physiological range. No gross pathological changes were observed during necropsy in any group. Changes of histological significance (such as focal lobular inflammation) were observed only in the liver of one rat of SFIU and two rats of IU group.

As there were no signs of toxicity observed with respect to haematology, biochemistry, relative organ weight, gross, and histological examinations in IU, SFIU and control groups, up to the highest tested dose levels, the No Observed Adverse Effect Level (NOAEL) of SFIU and IU in SD rats is considered as >1783 mg/kg bw and >2000 mg/kg bw, respectively. Furthermore, based on the present findings, it can be concluded that both classical and sugar-free versions of IU have similar safety profiles.

2015-2016

3 Preclinical Safety Evaluation Studies on *Ma'jūn-i-Kundur*

3.1 Acute oral toxicity study of *Ma'jūn-i-Kundur* in rats

Ma'jūn-i-Kundur (MK) is a compound Unani Pharmacopoeial formulation used in *Taqṭīr al-Bawl* (Dribbling of urine), *Salas al-Bawl* (Urinary incontinence), *Bawl fi'l Farāsh* (Nocturnal enuresis), *Sur'a al-Inzāl* (Premature ejaculation) and *Ḍu'f al-Mathāna* (Weakness of urinary bladder). This study was conducted to evaluate acute oral toxicity potential of *Ma'jūn-i-Kundur* (MK) in Sprague Dawley rats. Considering the low acute toxicity potential, the limit test as per OECD-425 was conducted at the dose of 5000 mg/kg body weight. Rats were weighed, observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with MK in three consecutive animals, dosing to further animals was stopped. All the three animals were sacrificed on Day-15 and necropsy was performed. No treatment related gross pathological abnormality was observed during necropsy. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 5000 mg/kg bw of MK. Therefore, oral LD_{50} of the MK in the female Sprague-Dawley strain rat was estimated to be greater than 5,000 mg/kg body weight.

3.2 Sub-chronic (repeated dose 90-day) oral toxicity study of *Ma'jūn-i-Kundur*

Repeated dose 90-day oral toxicity study of MK was carried out on SD rats of both sexes. Animals were divided into three groups (n=10 per sex per group) including control. MK was administered at the dose of 1028 and 2000 mg/kg bw/day p.o. for 90 days. Control animals were administered with vehicle. There was no statistically significant difference noted in the feed intake and body weight of MK treated group in any sex compared to the vehicle treated control rats. There was no toxicologically significant observation with respect to clinical signs of toxicity, body weight, feed consumption, haematology, biochemistry, organ weight, and gross necropsy in MK treated rats at 1028, 2000 mg/kg bw or in control animals. The histopathological finding did not reveal any treatment related toxicologically significant changes. Thus, the No Observed Adverse Effect Level (NOAEL) of MK may be considered >2000 mg/kg bw in rats.

3.3 Chronic (repeated dose 180-day) oral toxicity study of Ma'jūn-i-Kundur

The study was carried out to evaluate the 180-day repeated dose toxicity of MK in rats. Sprague Dawley (SD) rats of both sexes were used in the study. Animals were divided into two groups (n=15). MK was administered at a limit dose of 2000 mg/kg bw/day p.o. for 180 days. After completion of 180 days blood samples were collected for haematological and biochemical analysis and animals were sacrificed and organs were harvested for relative organ weight determination followed by histopathological evaluation.

Rats of control group and group treated with MK at 2000 mg/kg were subjected to clinical examination at regular time points. Both groups did not show any abnormal behaviour or clinical signs indicative of systemic toxicity. All the rats in the study survived throughout the 180-days study period. Body weight gain in control and MK treated groups did not show any significant difference throughout the study. There was no statistically significant difference noted in the feed intake of any drug treated groups in any sex compared to the vehicle treated control rats.

There was no significant difference observed in haematological parameters such as Hb, RBC, WBC count and HCT of control and MK treated rats of either sex. There was significant rise in monocyte and platelet count however the values remained within normal limits. No dose-dependent treatment related significant difference was observed in the blood biochemical parameters such as AST, Bilirubin, ALP, Total Protein, Albumin, Globulin, A/G Ratio, BUN, Creatinine, Triglycerides, HDL and VLDL between control and drug treated groups. No significant changes were observed in the gross necropsy of any animal. Changes of histological significance were observed only in the lungs and livers of animals of both control and MK treated rats.

No toxicologically significant observation was noticed with respect to body weight, feed consumption, behavioural pattern, haematology, biochemistry, organ weight and histopathology following chronic oral administration of MK at 2000 mg/kg for 180-day or in control animals. Therefore, MK would be considered safe and the reported data support the long-term clinical use of this valuable Unani formulation.

Publications

Khan MA, Urooj M, Razvi SH, Ahmed SS, Kazmi MH, Husain GM. 90-Days Repeated Dose Oral Toxicity Study of a Traditional Polyherbal Formulation Used in Urinary Disorders. *International Journal of Pharmaceutical Research.* 2018;10(3):311-320. https://doi.org/10.31838/ijpr/2018.10.03.015

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4 Preclinical Safety Evaluation Studies on *Jawārish Shāhī*

4.1 Acute oral toxicity study on Jawārish Shāhī in rats

Jawārish Shāhī (JS) is a compound Unani Pharmacopoeial formulation indicated for management of *Khafaqān* (Palpitation), *Nafkh-i-Mi'da* (Flatulence) and *Waswās* (Insanity; false perception and hallucinations). This study was designed to evaluate acute oral toxicity potential of *Jawārish Shāhī* (JS) in Sprague Dawley rats. Considering the low acute toxicity potential, the limit test as per OECD-425 was conducted at the dose of 5000 mg/kg body weight. Rats were weighed, observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with JS in three consecutive rats, dosing to further animals was stopped. All the three rats were sacrificed on Day 15 and necropsy was performed. No treatment related gross pathological abnormality was observed during necropsy. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 5000 mg/kg bw of JS. Therefore, oral LD_{50} of the JS in the female Sprague-Dawley rats was estimated to be greater than 5,000 mg/kg body weight.

4.2 Sub-chronic (repeated dose 90-day) oral toxicity study of Jawārish Shāhī in rats

The present study was carried out to evaluate repeated dose 90-days oral toxicity of JS in accordance with OECD guideline number 408. The study was carried out in SD rats. Sixty rats were divided into three groups (n=10 per sex per group). JS was administered at dose levels of 1028 and 2000 mg/kg bw/day, p.o., for 90 days. After completion of 90 days of drug administration, blood samples were collected for haematological and biochemical analysis; animals were sacrificed and organs were harvested for weight determination and histopathological evaluation.

The animals of control group and JS treated at dose 1028 mg/kg bw and 2000 mg/kg bw were subjected to clinical examination. The observations did not reveal any abnormal clinical sign and symptoms indicative of systemic toxicity.

There was no significant difference observed in body weight gain and feed intake pattern in both control and JS treated animals. The consistent observation of normal and expected pattern of body weight gain and feed intake in both sexes throughout the study suggested normal growth pattern.

Haematological parameters and clinical biochemical parameters were found normal as compared to control group. Few alterations were observed in terms of haematology and biochemistry. However, these changes were not dose-dependent and remained within the normal physiological range. No gross pathological changes were observed during necropsy in any group. No significant changes were observed related to histopathology in control as well as drug treated animals.

Since, there was no toxicologically significant observation with respect to clinical signs of toxicity, haematology, biochemistry, relative organ weight, gross necropsy and histopathological findings in JS treated rats at 1028 mg/kg bw and 2000 mg/kg bw (90-day repeated administration) as compared to control group, JS may be considered as safe up to the tested dose levels in rats.

4.3 Chronic (repeated dose 180-day) oral toxicity study of Jawārish Shāhī in rat

JS has not been evaluated for its long-term safety. Present study was conducted to evaluated long term toxicity of this Unani formulation following repeated administration for 180 days in SD rats. Animals were divided into two groups (n=15). JS was administered at dose of 2000 mg/kg bw/day, p.o., for 180 days. After completion of 180 days blood samples were collected for haematological and biochemical analysis and animals were sacrificed and organs were harvested for weight determination and histopathological evaluation was carried out.

No abnormal behaviour or clinical signs indicative of systemic toxicity was observed in any of the group during functional observation. All animals in both groups survived throughout the study period of 180-days. Rats in control and treatment group showed expected pattern of body weight gain and feed consumption throughout the study. The haematological profile of control and JS group did not show any significant difference in haematological parameters

such as Hb, RBC, WBC count and HCT in rats of either sex. Platelet count in Control and JS treated groups were also comparable. Blood biochemical parameters such as Albumin, Globulin, A/G Ratio, BUN, ALT, AST, Creatinine, HDL, LDL, Chol/HDL Ratio, HDL/LDL Ratio, in control and drug treated groups did not show treatment related difference. No significant alteration was observed in relative organ weights of Brain, Thymus, Heart, Lungs, Liver, Spleen, Adrenals, Kidney, Testis, Epididymis, Uterus and Ovaries in JS treated group and it was found comparable to Control.

Rats treated with JS did not show any toxicologically significant changes with respect to behavioural signs of toxicity, body weight, feed intake, haematology parameters, biochemistry profile, gross necropsy, relative organ weight and histopathology compared to control group. Based on the present findings, No Observed Adverse Effect Level (NOAEL) of JS in SD rats is 2000 mg/kg bw.

Publications

Khan MA, Urooj M, Thejaswini G, Ahmed SS, Kazmi MH, Husain GM. 180-Days repeated dose oral toxicity study of polyherbal Unani formulation: Jawarish Shahi. Journal of Clinical and Experimental Toxicology. 2017;1(1):21-29.

Urooj M, Khan MA, Thejaswini G, Kazmi MH, Husain GM. Toxicity Evaluation of a Traditional Polyherbal Unani Formulation Jawarish Shahi in Rats. The Journal of Phytopharmacology. 2018;7(5):412-418. DOI: https://10.31254/phyto.2018.7502

5 Preclinical Safety Evaluation of *Kushta-i-Fawlād*

Sub- chronic (repeated dose 90-day) oral toxicity study in rats

Kushta is the finest powder form of the Unani medicinal preparation obtained by the calcinations of metal, mineral and animal drugs. *Kushta-i-Fawlād* (KF) is a compound Unani Pharmacopoeial formulation recommended for the treatment of *Su-ul-Qiniya* (anaemia with hypoproteinemia), *Ḍu'f al-Bāh* (sexual debility), *Ḍu'f-i-Dimāgh* (weakness of brain/ cerebrasthenia), and *Ḍu'f al-Kabid* (hepatic insufficiency). There is no availability of scientific evidence in support of safety of this formulation; hence the present study was designed to evaluate repeated dose 90-day oral toxicity study of KF in Sprague Dawley (SD) rats.

Safety of KF was assessed in SD rats by conducting repeated dose 90-day oral toxicity study as per the OECD test guideline number 408. The drug was administered orally in the form of aqueous suspension in 0.3% carboxymethyl cellulose (CMC) at three dose levels i.e., 06, 30 and 60 mg/kg bw. Animals were periodically observed for clinical sign of toxicity, mortality, morbidity, body weight changes and feed consumption. At the end of study, haematology, biochemistry, electrolytes, gross pathology, relative organ weight and histological examination were performed. The animals subjected to clinical examination at different time interval suggest no abnormality in clinical signs and behaviour at all the tested dose levels. All animals survived throughout 90 days of the study duration in all drug treated and control group. The outline changes in body weight in drug treatment group were comparable to control in both sexes. No statistically significant differences were observed in feed consumption in KF-treated groups compared to vehicle control group.

Treatment with KF showed no significant changes in body weight gain, feed consumption and clinical signs of systemic toxicity. Haematological parameters did not reveal any statistically significant differences as compared to control group. There are alterations in few biochemical parameters as compared to control group which are toxicologically not significant because all the observed values remained within normal physiological range. A significant increase in total bilirubin in high dose male group ($p<0.05$ vs. control) and ALP in low dose male group ($p<0.01$ vs. control) was observed, though values remained within normal physiological limits and may not be considered toxicologically significant. The level of ALT in male group was significantly increased in low, mid dose ($p<0.001$ vs. control) and high dose group ($p<0.05$ vs. control). However, effect on ALT were not dose dependant and consistent and only observed in male rats and hence may be considered as toxicological insignificant in the absence of derangement of other associated liver biomarkers such as ALP and total bilirubin. Gross necropsy performed at the termination of the study revealed no alteration in any KF-treated group or control animals. Relative organ weight of control and KF treated groups were found to be comparable. Changes of histological significance were observed only in the lungs and livers of animals of both control and KF treated rats.

Based on the results of this study, it may be concluded that KF is safe at the therapeutically recommended dose levels and liver might be the target organ of toxicity at higher doses after prolonged administration.

2016-2017

6 Preclinical Safety Evaluation of *Khamīra-i- Banafsha* in Rats

Sub-chronic (repeated dose 90-day) oral toxicity study of Khamīra-i- Banafsha in rats

Khamīra-i-Banafsha (KB) is a classical semisolid traditional Unani formulation clinically used as *Munaffith-i-Balgham* (expectorant) and *Mulayyin* (laxative) and it is recommended for the treatment of *Qabḍ* (constipation), *Nazla* (catarrh) and *Su'āl* (cough). KB is widely used clinically in Unani system of medicine; however, there is no availability of scientific data to support the safety of this valuable formulation. Hence, the present study is designed to investigate repeated dose 90-day oral toxicity study in rats.

Safety of KB was assessed by conducting repeated dose 90-day oral toxicity study as per the OECD guideline number 408 in SD rats. KB was orally administered at the dose of 2,000 mg/kg bw/day (limit dose). Animals were periodically observed for clinical sign of toxicity, mortality, morbidity, body weight changes and feed consumption. At the end of study, haematology, clinical biochemistry, electrolytes, gross pathology, relative organ weight and histological examination were performed. The observations of clinical examination done at different time interval suggested no incidence of abnormal clinical signs / behaviour suggestive of any systemic toxicity among the rats treated with KB at the dose level of 2,000 mg/kg. All the rats in the study survived throughout the 90-days study period. No significant difference in the body weight gain was observed between control and KB treated group during the study. No significant changes were reported in feed intake of drug treated group of both sexes as compared to control group. Haematological parameters of drug-treated rats were comparable to that of control rats. Biochemical parameters such as AST, ALT, total Protein, Albumin, BUN, glucose and triglyceride of drug-treated rats were comparable to that of control rats. No lesion or abnormalities were

observed during gross necropsy in control or KB treated rats. No significant differences were observed in the relative organ weight of various organs. No treatment related histological changes were observed in brain, heart, liver, lungs, trachea, spleen, kidneys, adrenals, pancreas, stomach, testes/ovaries of KB treated or control rats.

There were no changes in body weight, feed intake, haematological, biochemical, gross pathological and histological alterations observed following oral treatment with KB at the dose of 2,000 mg/kg bw/day. The present investigation confirms the safety of classical KB, in line with the long history of its clinical use in Unani system of medicine.

7 Preclinical Safety Evaluation of *Sharbat-i-Dīnār* in Rats

Sub-chronic (repeated dose 90-day) oral toxicity study of Sharbat-i-Dīnār in rats

Sharbat-i-Dīnār (SDR) is a compound Unani Pharmacopoeial formulation recommended for the treatment of *Waram al-Kabid* (inflammation of liver), *Waram al-Raḥim* (metritis/ Pelvic Inflammatory Diseases), *Yarqān Suddī* (obstructive jaundice), *Istisqā' Ziqqī* (ascites), and *Qabḍ* (constipation). The present study was designed to investigate repeated dose 90-day oral toxicity study of SDR in rats.

Safety of SDR was assessed by conducting repeated dose 90-day oral toxicity study as per the OECD guideline number 408 in Sprague Dawley rats. SDR was orally administered (gavage) at the doses of 04, 10 and 20 mL/kg bw/ day (i.e., 1X, 2.5X and 5X of therapeutically equivalent dose). Animals were periodically observed for clinical sign of toxicity, mortality, morbidity, body weight changes and feed consumption. After the treatment duration of three months, animals were anaesthetized using isoflurane, blood samples were collected from retro-orbital sinus puncture. Blood samples were subjected to haematological investigation and serum was subjected to different biochemical estimation. Animals were sacrificed using CO_2 euthanasia, gross necropsy was performed and internal organs/ tissues were harvested and preserved in neutral buffered formalin for histopathological investigation.

Oral administration of SDR for 90 consecutive days did not cause any mortality in male or female rats at any tested dose level. Daily general examination and detailed clinical examination conducted at various time points did not reveal any abnormal clinical signs in SDR treated or control animals at any tested dose. Oral administration of SDR did not induce any significant effect on body weight gain and feed intake in all drug treated animal as compared to control animals.

Oral administration of SDR for 90 consecutive days at three dose levels did not alter the haematological profile. There is no statistically significant difference in haemoglobin, RBC, WBC, HCT or differential leukocytes levels in SDR treated rats compared to control group in any sex. The serum level of ALT, AST, ALP, Bilirubin, and Albumin of SDR treated groups were found comparable to control group in both sexes. The oral administration of SDR at three dose levels i.e., 04, 10 and 20 mL/kg bw did not result any alterations in relative organ weight of Brain, Thymus, Heart, Lungs, Liver, Spleen, Adrenals, Kidney, Testis, Epididymis, Uterus and Ovaries. No toxicologically relevant histological changes were observed in Heart, brain, kidneys, spleen, pancreas, adrenals, trachea, stomach, small intestine, sternum and bone marrow, testes/ uterus and ovaries of high dose SDR (20 mL/kg bw) treated rats and control group. Based on the results of this study, the no-observed adverse effect level (NOAEL) of SDR may be considered as 20 mL/kg bw/day, i.e., the highest tested dose.

Publications

Husain GM, Ahmad T, Fatima SH, Javed G, Kazmi MH, Urooj M. 90-Days Repeated Dose Oral Toxicity Study of *Sharbat-i-Dīnār* (A Hepato-protective Unani Herbal Formulation). Traditional and Integrative Medicine. 2021;6(1):5-18. https://doi.org/10.18502/tim.v6i1.5924

8 Preclinical Safety Evaluation of *Ma'jūn-i-Najāḥ* in Rats

Chronic (repeated dose 180-day) oral toxicity of Ma'jūn-i-Najāḥ in rats

Ma'jūn-i-Najāḥ (MN) is a compound Unani Pharmacopoeial formulation mentioned in the National Formulary of Unani Medicine, Part-I and other classical text of Unani system of medicine. It is recommended for the treatment of *Mālankhūliyā* (melancholia), *Qūlanj* (colic), and *Ikhtināq al-Raḥim* (hysteria). However, long-term toxicity study of MN has not been reported in literature. The study was carried out to evaluate the 180 days repeated dose toxicity of MN in SD rats. Study was carried out on SD rats of both sexes. Animals were divided into various groups (n=15). MN was administered at doses of 1000 and 2000 mg/kg bw/day, p.o., for 180 days. Body weight and feed consumption was recorded at regular interval. Clinical examination of rats of control group and group treated with MN at 1000 and 2000 mg/kg was carried out at different time points. After completion of 180 days, blood samples were collected for haematological and biochemical analysis and animals were sacrificed and organs were harvested for weight determination and histopathological evaluation was carried out.

No abnormal behaviour or clinical signs indicative of systemic toxicity was observed in any of the group. Mortality was observed in male treatment groups where two animals in MN-1000 mg/kg treatment group, one animal in MN-2000 mg/kg treatment group and two animals in control group died during the treatment period. Body weight gain in control and MN treated groups did not show any significant difference throughout the study. Similarly, feed intake of control and MN groups was also comparable.

Mortality rate in MN treated groups was almost comparable to vehicle treated control group. The haematological profile of control and MN group did not show any significant in haematological parameters such as Hb, RBC, WBC count and HCT in rats of either sex. However, platelet count in MN treated male groups showed significant decrease in platelet count in both 1000 ($P<0.05$ vs control) and 2000 mg/kg dose ($P<0.01$ vs control). Although, there was significant decrease in blood glucose level in MN treated male rats, the values were within the physiological range and did not exhibit any toxicological relevance. Levels of hepatic enzyme AST was significantly increased in MN treated male animals at the doses of 1000 ($p<0.001$ vs control) and 2000 mg/kg ($p<0.01$ vs control). AST was significantly increased in MN treated male animals at the doses of 1000 ($p<0.001$ vs control) and 2000 mg/kg ($p<0.01$ vs control). ALT was also significantly increased in the same groups at the doses of 1000 ($p<0.01$ vs control) and 2000 mg/kg ($p<0.01$ vs control). However, in the present study alteration observed in ALT and AST levels in male animals were marginal and had an inverse relationship with dose. Further, the increased AST and ALT levels were not associated with corresponding increase in bilirubin or ALP and hence cannot be considered toxicologically relevant. In this group BUN level was also significantly increased ($p<0.01$ vs control). Serum creatinine level was significantly increased in female group treated with 2000 mg/kg of MN ($p<0.01$ vs control) and male groups treated with 1000 mg/kg of MN ($p<0.01$ vs control). The increase in renal markers BUN and creatinine in various treatment groups was also found to be inconsistent and did not show any dose dependent pattern. Despite significant alteration, the values were within the normal physiological range and did not carry any relevance from toxicological perspective as no associated histopathological changes were found in liver, kidney and other organs treated with MN.

Based on study data, no toxicologically significant changes were observed in behavioural observations, body weight, feed-intake, relative organ weight, gross necropsy and histopathology in MN treated group and control animals. Few changes observed in haematology and clinical biochemistry parameters may be considered incidental as these changes are not consistent with respect to dose and sex and observed values were within normal physiological limits. No Observed Adverse Effect Level (NOAEL) of MN in SD rats may be considered as 2000 mg/kg bw.

9 Preclinical Safety Evaluation of *Jawārish-i-Bisbāsa* in Rats

Sub-chronic (repeated dose 90-day) oral toxicity study of Jawārish-i-Bisbāsa in rats

Jawārish-i-Bisbāsa (JBS) is a polyherbal Unani formulation clinically indicated for the treatment of *Ḍu'f-i-Mi'da* (Weakness of the stomach), *Ḍu'f al-Haḍm* (Delayed digestion), *Bawāsīr Amya* (Blind piles), *Nafkh-i-Mi'da* (Flatulence) and *Ghathayān* (Nausea). The present study was designed to investigate repeated dose 90-day oral toxicity study in rats.

Safety of JBS assessed by conducting 90-day repeated dose oral toxicity study as per the OECD guideline number 408 in SD rats. JBS was orally administered at the dose of 1000 and 2000 mg/kg bw/day (i.e., 1X and 2X, respectively). Animals were periodically observed for clinical sign of toxicity, mortality, morbidity, body weight changes and feed consumption. At the end of study duration, assessment of haematology, clinical biochemistry, gross necropsy, relative organ weight and histopathology of organs/tissues was performed.

No treatment related effect was reported on survival of both male and female rats after oral administration of JBS for three months. There were no abnormal clinical signs based on clinical examination conducted at different time points in JBS treated animals at 1000 mg/kg bw and 2000 mg/kg bw as compared to animals of control group. The expected pattern of weight gain was observed in both male and female rats of drug treated group as compared to control group. Male rats treated with JBS at all tested dose levels did not show significant difference in food consumption as compared to control group. No consistent difference observed in food consumption in female group receiving test drug as compared to control group.

JBS did not produce any significant alterations in haematological parameters like Hb, RBC, WBC, HCT, neutrophils, lymphocyte and eosinophils except significant increase ($p<0.05$) of platelets count in female group at low dose level (1000 mg/kg bw) compared to control group. JBS did not produce any significant alteration in biochemical parameters except significant decrease ($p<0.01$) in serum glucose level in drug treated female group at JBS-2000 mg/kg bw as compared to control group. There was a significant increase ($p<0.05$) in total protein in drug treated female group at JBS-2000 mg/kg bw as compared to control animals. No alterations in relative organ weight was observed in JBS-treated rats compared to vehicle control. Heart, brain, kidneys, spleen, thymus, pancreas, adrenals, testes/ ovaries of JBS-2000 mg/kg bw and control group did not reveal any toxicologically significant findings following histological investigations. Histopathological examination revealed abnormality in liver and lungs of few animals. Vascular congestion observed in hepatocytes could possibly be due to inflammation, blockage and vasoconstrictor effect of JBS or vehicle on the walls of blood vessel of hepatocytes. The pneumonitis in lungs may be attributed due to aspiration of drug / vehicle in lungs for repeated oral administration for 90 days. As abnormalities were observed both in control and drug treated animals, findings may not be attributed to drug treatment.

Based on the findings, it may be concluded that oral administration of JBS at 1000 and 2000 mg/kg bw/day for 90 days did not show any significant adverse effects on clinical observations, body weight and feed intake, haematology, clinical biochemistry, relative organ weight, gross necropsy and histopathology. Therefore, no-observed-adverse-effect-level (NOAEL) may be considered as 2000 mg/kg body weight.

Publications

Urooj M, Reddy MA, Alam M, Kazmi MH, Husain GM. 90-days Repeated Oral Dose Toxicity Study on *Jawārish-i-Bisbāsa* (A Polyherbal Unani Formulation). Toxicology International. 2018;25(4):224–231. https://doi.org/10.18311/ti/2018/v25i4/23868

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10 Preclinical Safety Evaluation of Coded Unani formulation *UNIM-N-2000* in Rats

10.1 *Acute Oral Toxicity Study of UNIM-N-2000 in rats*

UNIM-N-2000 is a polyherbal coded Unani formulation clinically indicated for the treatment of renal dysfunctions and as nephroprotective. This study was designed to evaluate acute oral toxicity potential of *UNIM-N-2000* in Sprague Dawley rats. Considering the low acute toxicity potential, a limit test as per OECD-425 was conducted at the dose of 2000 mg/kg body weight. Animals were observed for lethality and toxic signs and symptoms for 14 days post-treatment. As no lethality was observed following treatment with *UNIM-N-2000* in three consecutive animals, dosing to further animals was stopped. All the three animals were sacrificed on Day 15 and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 2000 mg/kg bw of *UNIM-N-2000*. Therefore, oral LD_{50} of the *UNIM-N-2000* in the female Sprague-Dawley rats was estimated to be greater than 2,000 mg/kg body weight.

10.2 *Sub- chronic (repeated dose 90-day) oral toxicity on UNIM-N-2000 in rats*

The present study was designed to investigate repeated dose 90-day oral toxicity study as per the OECD guideline number 408 in SD rats. *UNIM-N-2000* was orally administered at the dose of 300, 900 and 1500 mg/kg bw/day (i.e., 1X, 3X and 5X of therapeutically equivalent dose, respectively). Animals were periodically observed for clinical sign of toxicity, mortality, morbidity, body weight changes and feed consumption. At the end of study, assessment of haematology, clinical biochemistry, electrolytes, gross necropsy and relative organ weight was performed.

No treatment related adverse effect was observed on survival of both male and female rats after oral administration of *UNIM-N-2000* for 90 consecutive days. No incidences of mortality were reported in *UNIM-N-2000* treated male and female rats at any tested dose levels. There were no report for any abnormal clinical signs based on clinical examination conducted at regular interval following *UNIM-N-2000* treatment at 300, 900 and 1500 mg/kg bw as compared to animals of control group. There were no significant differences in body weight and feed intake of *UNIM-N-2000* treated animals as compared to control group. The expected pattern of weight gain and feed intake was observed in both male and female rats of drug treated group as compared to control group.

The effect of *UNIM-N-2000* on haematological and biochemical parameters in both male and female did not produce any significant effect after 90 days administration except few alterations which were found within normal physiological range. *UNIM-N-2000* did not induce any alterations in relative organ weight. Changes of histological significance were observed only in the lungs and livers of experimental animals. However, it was reported that similar changes were also observed in the control group. Hence, these changes could not be attributed to the administration of test compound.

It may be inferred that repeated oral administration of *UNIM-N-2000* at doses 300, 900 and 1500 mg/kg bw for 90-days did not show any significant adverse effects on survival, body weight, haematology, clinical biochemistry, gross necropsy and histopathology of organs. Therefore, no-observed-adverse-effect-level (NOAEL) in rats may be considered as 1500 mg/kg body weight, i.e., the highest tested dose.

11 Nephroprotective Activity of *UNIM-N-2000* Coded Unani Formulation Against Cisplatin Induced Acute Renal Damage in Experimental Animals

UNIM-N-2000 is a coded polyherbal Unani formulation intended to treat kidney disorders. Long term use of certain drugs like antimicrobials and anticancer drugs cause structural or functional damage to kidney tissue leading to nephrotoxicity, as these drugs selectively accumulate in kidney as by-product of metabolism. The present study was designed to evaluate the nephroprotective potential of *UNIM-N-2000* against cisplatin-induced renal toxicity in rats by measuring different renal and inflammatory biomarkers as well as to assess the histopathological alterations in kidney.

A total of 48 male Wistar rats were randomly assigned into six groups as control, cisplatin control and experimental groups with 08 rats in each group. The classical formulation (300 and 600 mg/kg bw) and its extract (35 and 70 mg/kg bw, equivalent doses based on percentage yield of extract) at two dose levels were tested. Body weight and feed intake of all the animals was recorded at weekly intervals throughout study duration. At the end of experiment, blood samples were collected for estimation of serum biomarkers (BUN, creatinine and Uric acid). After necropsy, left kidney tissue homogenate was prepared for the estimation of inflammatory markers like TNF-α, IL-1β and Kim-1. Urine sample was collected for the estimation of micro-albumin level. Additionally, relative organ weight was calculated, and right kidney tissues were subjected to histopathological examination.

No significant effect was observed on primary serum biomarkers BUN and creatinine. Additionally, there was no significant reduction of Kim-1, a specific kidney injury maker, although a reduction was observed in TNF-α, IL-1β and urine micro-albumin level following treatment with *UNIM-N-2000*. Histopathological examination indicated that kidney tissue showed degenerative changes in the cisplatin control (6/8) and *UNIM-N-2000* (4/8) group as compared to the normal control. Only one rat in *UNIM-N-2000* extract high dose (70 mg/kg bw) group (1/8), showed regenerative changes. The findings of the study were not in favour of concluding that *UNIM-N-2000* possesses significant nephroprotective activity against cisplatin-induced renal damage in rats in the experimental condition.

12 Preclinical Safety Evaluation of Coded Unani formulation *UNIM-N-2002* in Rats

12.1 Acute oral toxicity study of UNIM-N-2002 in rats

UNIM-N-2002 is a coded Unani formulation. This study was designed to evaluate acute oral toxicity potential of a coded Unani formulation *UNIM-N-2002* in Sprague Dawley rats. Considering the low acute toxicity potential, the limit test as per OECD-425 was conducted at the dose of 2000 mg/kg body weight. Animals were observed for body weight, lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with *UNIM-N-2002* in 3 consecutive animals, treatment to further animals was stopped. All the three animals were sacrificed on 15th day and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 2000 mg/kg bw of *UNIM-N-2002*. Therefore, oral LD_{50} of the *UNIM-N-2002* in the female Sprague-Dawley rats was estimated to greater than 2,000 mg/kg body weight.

12.2 Sub- chronic (repeated dose 90-day) oral toxicity of UNIM-N-2002 in rats

Present study was designed to investigate repeated dose oral toxicity study of coded Unani formulation *UNIM-N-2002* in rats as per the OECD guideline number 408 in Sprague Dawley rats. *UNIM-N-2002* was orally administered (gavage) at the doses of 300, 900 and 1500 mg/kg bw/day (1X, 3X and 5X of therapeutically equivalent dose, respectively). Animals were periodically observed for clinical sign of toxicity, mortality, morbidity, body weight changes and feed consumption. After the treatment duration of three months, animals were anaesthetized using isoflurane, blood samples were collected from retro-orbital sinus puncture. Blood samples were subjected to haematological investigation and serum was subjected to different biochemical estimation. Animals were sacrificed using CO_2 euthanasia, gross necropsy was performed and internal organs/ tissues were harvested and preserved in neutral buffered formalin for histopathological investigation.

Oral administration of *UNIM-N-2002* for 90 days did not cause any adverse effect on the normal behaviour and survival of both male and female rats. Body weight gain and feed intake in all drug treated rats were comparable to control throughout the study duration. The effect of *UNIM-N-2002* on haematological parameters in both male and female rats did not reveal any significant effect. *UNIM-N-2002* did not produce any significant alteration in biochemical profile. *UNIM-N-2002* did not induce any alterations in relative organ weight of brain, thymus, heart, lungs, liver, spleen, adrenals, kidney, testis and epididymis/ uterus and ovaries. No toxicologically significant changes were observed in histopathology of organs/tissues of *UNIM-N-2002* treated rats compared to vehicle control. The data from this study suggested that no-observed adverse effect level (NOAEL) of *UNIM-N-2002* may be considered as 1500 mg/kg bw/ day in rats.

13 Nephroprotective Activity of *UNIM-N-2002* Coded Unani Formulation Against Cisplatin Induced Acute Renal Damage in Experimental Animals

UNIM-N-2002 is a coded polyherbal Unani formulation intended to treat kidney disorders. Long-term use of certain drugs like antimicrobial and anticancer drugs lead to nephrotoxicity as these drugs accumulate in kidney as by-product of metabolism and causes structural or functional damage to kidney tissue. Nephroprotective potential of *UNIM-N-2002* was evaluated against cisplatin induced renal toxicity in rats by estimating different renal and inflammatory biomarkers as well as to assess the histopathological alterations in kidney.

A total of 48 male Wistar rats were randomly assigned into 06 groups including control, cisplatin control and experimental groups (n=8). The classical formulation (300 and 600 mg/kg bw) and its 50% hydro-alcoholic extract (50 and 100 mg/kg bw, equivalent doses based on percentage yield of extract of *UNIM-N-2002*) at two dose levels were evaluated. At the end of experiment, blood samples were collected for estimation of serum biomarkers (BUN, creatinine and Uric acid). Additionally, left kidney tissue homogenate was prepared for the estimation of inflammatory markers TNF-α, IL-1β and Kim-1. Urine sample was collected for the estimation of micro-albumin level. Body weight and feed intake was recorded weekly throughout study duration. After necropsy relative organ weight was calculated and kidney tissues were subjected to histopathological examination.

The findings of the study indicate that no significant effect was observed on conventional serum biomarkers of renal functions like creatinine and BUN. The inhibitory effect on proinflammatory cytokines like TNF-α, IL-1 β, and urine micro-albumin level, as well as KIM-1 (only extract), showed a positive effect in this model. Kidney tissue subjected to histopathological examination showed degenerative changes, including cystic dilatation, accumulation of cystic fluid and tubular membrane damage and degeneration in tubular epithelial cells in tubules of the kidney in all treatment groups, including the cisplatin-control as compared to the normal control group. Regeneration of tubules was observed only in *UNIM-N-2002* group animals (not in extract group). This observation strongly support the positive response of *UNIM-N-2002*. It may be concluded that *UNIM-N-2002* may have an ameliorative effect on proinflammatory markers in rats and *UNIM-N-2002* induced regenerative changes in kidneys after cisplatin-induced damage.

14 Preclinical Safety Evaluation of Coded Formulation *UNIM-N-2003* in Rats

14.1 Acute oral toxicity study of UNIM-N-2003 in rats

UNIM-N-2003 is a coded Unani formulation. This study was designed to evaluate acute oral toxicity potential of *UNIM-N-2003* in Sprague Dawley rats. Considering the low acute toxicity potential, the limit test as per OECD test guideline number 425 was conducted at the dose of 2000 mg/kg body weight. Animals were observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with *UNIM-N-2003* in three consecutive animals, dosing to further animals was stopped. All the three animals were sacrificed on 15th day and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 2000 mg/kg body weight of *UNIM-N-2003*. Therefore, oral LD_{50} of the *UNIM-N-2003* in the female Sprague-Dawley strain rat was estimated to be greater than 2,000 mg/kg body weight.

14.2 Sub-chronic (repeated dose 90-day) oral toxicity study of UNIM-N-2003 in rats

Safety of *UNIM-N-2003* was assessed by conducting 90-day repeated dose oral toxicity study as per the OECD test guideline number 408 in SD rats. *UNIM-N-2003* was orally administered at the dose of 300, 900 and 1500 mg/kg bw/day (1X, 3X and 5X of therapeutically equivalent dose, respectively). Animals were periodically observed for clinical sign of toxicity, mortality, morbidity, body weight changes and feed consumption. At the end of study, haematology, clinical biochemistry, electrolytes, gross pathology, relative organ weight and histological examination were performed.

No treatment-related adverse effect was reported on survival of both male and female rats after oral administration of *UNIM-N-2003* for 90 consecutive days. No abnormal clinical sign was observed on clinical examination conducted at regular interval following *UNIM-N-2003* treatment. There were no significant differences in body weight and feed intake of *UNIM-N-2003* treated animals as compared to control group. The expected pattern of weight gain and feed intake was observed in both male and female rats of drug treated group as compared to respective control group.

Based on study findings, it may be concluded that *UNIM-N-2003* did not show any significant adverse effects on behaviour, body weight and feed intake, haematology, clinical biochemistry, relative organ weight, gross necropsy and histopathology. Therefore, no-observed-adverse-effect-level (NOAEL) of *UNIM-N-2003* may be considered as 1500 mg/kg body weight.

15 Nephroprotective Activity of *UNIM-N-2003* Coded Unani Formulation Against Cisplatin Induced Acute Renal Damage in Experimental Animals

UNIM-N-2003 is a coded polyherbal Unani formulation developed to treat kidney disorders. Long term use of certain drugs like antimicrobial and anticancer drugs lead to nephrotoxicity as these drugs accumulate in kidney as by-product of metabolism and causes structural or functional damage to kidney tissue. The present study was designed to evaluate the nephroprotective potential of *UNIM-N-2003* against cisplatin-induced renal toxicity in rats by estimating different renal and inflammatory biomarkers as well as to assess the histopathological alterations in kidney.

A total of 48 male Wistar rats were randomly assigned into 06 groups, including control, cisplatin control and experimental groups of 08 rats in each group. The classical formulation (300 and 600 mg/kg bw) and its extract at two dose levels (50 and 100 mg/kg bw; doses of extract were based on percentage yield of extract) were evaluated. At the end of experiment blood samples were collected for estimation of serum biomarkers (BUN, creatinine and Uric acid). Additionally, left kidney tissue homogenate was prepared for the estimation of inflammatory markers TNF-α, IL-1β and Kim-1. Urine sample was collected for the estimation of micro-albumin level. The body weight and feed intake of all animals was recorded weekly throughout study duration. After necropsy relative organ weight was calculated and tissues were subjected for histopathological examination.

No significant effect was observed on primary serum biomarkers creatinine and BUN. There was no significant reduction of Kim-1, a specific kidney injury maker, although a decrease was observed in TNF-α, IL-1β and urine micro-albumin level. Kidney tissue subjected to histopathological examination showed degenerative changes, including cystic dilatation, accumulation of cystic fluid and tubular membrane damage and degeneration in tubular epithelial cells in tubules of the kidney in all treatment groups, including cisplatin control, as compared to the normal control group. Regeneration of tubules was noticed in *UNIM-N-2003* (both in classical formulation and extract). Regeneration of tubules coupled with inhibition of inflammatory markers suggests that *UNIM-N-2003* may have mild nephroprotective activity against cisplatin-induced renal damage in rats.

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16 Preclinical Safety and Efficacy Evaluation of *Ma'jūn Māsik al-Bawl* and its Hydroalcoholic Extract

16.1 Acute Oral Toxicity Study of classical form and hydro-alcoholic extract of Ma'jūn Māsik al-Bawl in rats

Ma'jūn Māsik al-Bawl (MMB) is a compound Unani formulation clinically indicated for the treatment of *Kathra al-Bawl* (Polyuria), *Jarayān* (semenorrhoea) and *Bawl fi'l Farāsh* (Nocturnal enuresis). This study was designed to evaluate acute oral toxicity potential of both classical form and 50% hydro-alcoholic extract of *Ma'jūn Māsik al-Bawl* (MMB and MMBE, respectively) in Sprague Dawley rats. Considering the low acute toxicity potential, the limit test as per OECD test guideline number 425 was conducted at the dose of 2000 mg/kg body weight. Animals were administered with single dose of test drug and observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with *Ma'jūn Māsik al-Bawl* classical as well as extract in three consecutive animals, respectively, dosing to further animals was stopped. Blood samples were collected on 15th day for haematological and biochemical investigation from all the three animals of each test drug. Animals were sacrificed on Day-15 and necropsy was performed. No treatment related gross pathological abnormality was observed. Biochemical and haematological parameters were found within normal limits. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 2000 mg/kg bw for both classical and hydro-alcoholic extract of *Ma'jūn Māsik al-Bawl*. Therefore, oral LD_{50} of the MMB and MMBE in the female Sprague-Dawley rats was estimated to be greater than 2,000 mg/kg body weight.

16.2 Repeated dose 90-day oral toxicity study of Ma'jūn Māsik al-Bawl in rats

Background: The present study was designed to investigate repeated dose 90-day oral toxicity study of MMB formulation in rats.

Methods: Safety of MMB was assessed by conducting 90-day repeated dose oral toxicity study as per the OECD guideline 408 in Sprague Dawley rats. MMB was orally administered (gavage) at the doses of 1000 and 2000 mg/kg bw/day. Animals were periodically observed for clinical sign of toxicity, mortality, morbidity, body weight changes and feed consumption. After the treatment duration of three months, animals were anaesthetized using isoflurane, blood samples were collected from retro-orbital sinus puncture. Blood samples were subjected to haematological investigation and serum was subjected to different biochemical estimation. Animals were sacrificed using CO_2 euthanasia, gross necropsy was performed and internal organs/ tissues were harvested and preserved in neutral buffered formalin for histopathological investigation.

Results: Treatment with MMB showed no significant differences in survival, body weight gain, and haematology and biochemistry profile except certain isolated changes in serum platelets, alkaline phosphatase and uric acid, which were considered toxicologically insignificant as the values remained in the normal physiological range. There were no changes observed in the gross necropsy and relative organ weight data of control and MMB-treated rats. Histology of brain, heart, lungs, liver, kidney, spleen, testis, ovaries and uterus, adrenals, pancreas, stomach, and sternum was normal in MMB and vehicle treated rats.

Conclusion: No mortality or adverse changes in clinical signs, body weight and feed-intake were noted. No toxicologically significant changes in haematology, clinical chemistry, and relative organ weights were noted. Gross pathological examinations and histology on organs/ tissues did not reveal treatment-related abnormalities. Based on the results of this study, MMB may be considered safe up to 2000 mg/kg bw/day in rats.

16.3 Efficacy evaluation for the effect of classical formulation (MMB) and its 50% hydroethanolic extract (MMBE) on urine output, saluresis, and natriuresis in rats

Ma'jūn Māsik al-Bawl (MMB) is a compound Unani formulation used in *Salas al-Bawl* (urinary incontinence), *Bawl fi'l Farāsh* (nocturnal enuresis), and *Ḍu'f-i- Mathāna* (weakness of bladder). This study aims to evaluate the effect of classical formulation (MMB) and its 50% hydroethanolic extract (MMBE) on urine output, saluresis, and natriuresis in rat.

Sprague-Dawley rats were randomly distributed in six groups with six animals in each. One group served as the control and another group received furosemide (10 mg/kg, p.o.) which served as diuretic control. Remaining four groups received two doses of classical formulation (1000 or 2000 mg/kg MMB) or equivalent doses (based on percentage yield) of hydroethanolic extract (50 or 100 mg/kg MMBE). Cumulative urine output after 5 h after respective drug administration, urinary electrolytes, saluretic and natriuretic activity of all groups were estimated and then compared with control groups.

Furosemide induced significantly high ($p<0.001$) urine excretion (4.737±0.611) compared to vehicle control (0.376±0.031) with diuretic index of 12.61. Diuretic indices for low and high doses of MMB were 0.22, 0.14 and for MMBE were 0.26 and 0.25, respectively. Classical MMB showed significant dose dependent reduction in urine output and both doses and resulted around 22% and 13.5% reduction in urine volume (0.082±0.036, 0.051±0.025, respectively) compared to vehicle control. Both doses of MMBE resulted about 25% urine excretion (0.097±.035, 0.092±0.045, respectively) compared to control and no dose dependent activity was observed in case of extract. Furosemide treatment induced a significant increase ($p<0.001$) in urinary excretion of Na^+, K^+ and Cl^- compared to vehicle control. A pattern of reduced urine Na^+ excretion was observed in all test groups; however, this reduction was statistically significant ($p<0.05$) only in rats that received the MMB 2000 mg/kg and MMBE 50 mg/kg. A significant decrease ($p<0.05$) in urine K^+ excretion was observed in rats that received all test doses except MMBE 50 mg/kg compared to vehicle control group. No significant difference was observed in any test group in urine Cl^- excretion compared to vehicle control. Effect of various treatments on saluretic activity (Na^+ and Cl^-), natriuretic activity (Na^+/K^+) and saluretic index. No

significant changes were observed in saluretic or natriuretic activity in any test drug treated group. On the contrary, furosemide showed a significant (p<0.001) saluretic and natriuretic effect.

It may be concluded that MMB and MMBE have reduced urine output in rats without significantly altering saluresis and natriuresis. This is an important and therapeutically useful finding which provides scientific evidence in favour of its traditional claims and widespread usage in urinary incontinence and nocturnal enuresis.

16.4 Nephroprotective Evaluation of Classical Formulation (MMB) and its 50% Hydroethanolic Extract (MMBE) in rats

Ma'jūn Māsik al-Bawl (MMB) is a compound traditional polyherbal Unani formulation indicated for the treatment of urinary and kidney disorders. The present study was designed to evaluate the nephroprotective effect of MMB classical formulation and MMBE extract against cisplatin induced nephrotoxicity in rats. The study was performed using male Sprague Dawley rats. Animals were divided into six groups of eight animals each. Group I served as normal control, Group II as cisplatin control treated with cisplatin 7 mg/kg bw, i.p., Group III and IV were orally treated with MMB 1000 and 2000 mg/kg bw, respectively, as per study protocol. Group V and VI were orally treated with hydroalcoholic extract of MMB i.e., MMBE 50 and 100 mg/kg bw, respectively. The study duration was 21 days where cisplatin (7 mg/kg bw, i.p.) was administered on 19th, 20th and 21st day. At the end of study biochemical parameters (liver and kidney biomarkers) were estimated in serum. The kidney homogenate was used for the estimation of inflammatory markers like TNF-α, IL-1β, and KIM-1. Liver and kidney were subjected to histopathological examination.

The results showed that test drugs MMB 2000 mg/kg bw, MMBE 50 mg/kg bw and MMBE 100 mg/kg bw resulted significant reduction of BUN and creatinine as compared to cisplatin control. The inflammatory markers (TNF-α, IL-1β, and KIM-1) found to be significantly reduced in treatment group as compared to cisplatin control group. The histopathological examination revealed that treatment group MMB 2000 mg/kg bw, MMBE 50 mg/kg bw and MMBE 100 mg/kg bw showed tubular regeneration. The above findings suggested that tested drug MMB and MMBE found to be effective as nephroprotective in cisplatin induced nephrotoxicity model in rats.

17 Preclinical Safety and Efficacy Studies on *Qurṣ-i-Damawī* and its Hydroalcoholic Extract in Rats

17.1 Acute Oral Toxicity Study of Classical form and Hydro-alcoholic Extract of Qurṣ-i-Damawī in Rats

Qurṣ-i-Damawī (QD) is a polyherbal Unani formulation. It contains *Rewand Chīnī* (Rheum emodi), *Hira Kasees* (sulphates of Iron), *Zanjabīl* (*Zingiber officinale* Roscoe), *Samagh-e-Arabi* (*Acacia arabica*). DM is indicated for *Faqr-ud-Dam* (Anaemia). This study was designed to evaluate acute oral toxicity potential of both classical form and 50% hydro-alcoholic extract of *Qurṣ-i-Damawī* (QD) in Sprague Dawley rats. Considering the low acute toxicity potential, the limit test as per OECD-425 was conducted at the dose of 2000 mg/kg body weight. Animals were observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with QD classical as well as extract in 3 consecutive animals respectively, dosing to further animals was stopped. Blood samples were collected on Day-15 for haematological and biochemical investigation from all the three animals of each formulation. Animals were sacrificed on Day 15 and necropsy was performed. No treatment related gross pathological abnormality was observed. Biochemical and haematological parameters were found within normal limits. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 2000 mg/kg bw for both classical and 50% hydro-alcoholic extract of *Qurṣ-i-Damawī*. Therefore, oral LD_{50} of the *Qurṣ-i-Damawī* the female Sprague-Dawley strain rat was estimated to be greater than 2,000 mg/kg body weight.

17.2 Repeated dose 90-day Oral Toxicity Study of Classical form and Hydro-alcoholic Extract of Qurṣ-i-Damawī in Rats.

The present study was conducted to investigate repeated dose 90-day oral toxicity study in rats. Safety of *Qurṣ-i-Damawī* (QD) was assessed by conducting a 90-day repeated dose oral toxicity study performed as per the OECD guideline 408 in SD rats. QD was orally administered at the dose of 125 and 250 mg/kg bw/day. Animals were periodically observed for clinical sign of toxicity, mortality, morbidity, body weight changes and feed consumption. At the end of study, assessment of haematology, clinical biochemistry, gross necropsy and relative organ weight was performed. Treatment with QD showed no adverse effect on survival and body weight gain in animals throughout study duration. No overt findings were observed in the behaviour of animals and no clinical signs indicative of any systemic toxicity were observed during clinical examination. There were no adverse effects observed in haematology and biochemistry profile except certain changes which are clinically not significant as the effects observed were not dose dependant and the values remained within normal physiological range. There were no remarkable changes observed in the relative organ weight data of control and QD-treated rats. Gross necropsy of QD-treated or control animals did not reveal any adverse findings. The liver of the majority of animals in both the control and QD treated group were normal. However, 25% animals (5/20) of QD-treated group showed mild degenerative changes in the form of micro vacuolation involving <33% of the section, which might be treatment related.

Based on findings, it can be concluded that oral administration of QD at doses 125 and 250 mg/kg bw did not show any significant adverse effects on survival, body weight gain, haematology, clinical biochemistry, and gross necropsy. Based on micro vacuolation observed in the liver of the QD-250 mg/kg group, it may be concluded that the liver might be the target organ of toxicity at 250 mg/kg body weight on prolonged administration in rats.

17.3 Efficacy Evaluation of Qurṣ-i-Damawī and its Hydroethanolic Extract in Cyclophosphamide Induced Haemotoxicity in Rats

Qurṣ-i-Damawī (QD) is a polyherbal Unani formulation used in conditions like anaemia. The aim of the current study was to validate the use of QD and its hydroethanolic extract (QDE) in cyclophosphamide induced haemotoxicity in rats for the assessment of erythropoietic activity.

QD was prepared as per classical methodology. Extract of *Qurṣ-i-Damawī* (QDE) was obtained from crude formulation (QD) with ethanol and water (1:1, v/v). Haemotoxicity was induced by intraperitoneal administration of cyclophosphamide 3 mg/kg (i.p.) bw in rats for seven consecutive days. Oral drug treatment was started from day-8 and continued till day-22. Blood samples were collected and analysed on day-7 and day-22 using haematology analyser.

Rats treated with cyclophosphamide 3 mg/kg (i.p.) for seven days (group II-VI) showed marked decrease (statistically significant in most of cases) in haematological parameters such as RBC, Hb, WBC, HCT and PLT compared to vehicle control. Haematological perturbations persisted on day-22 in cyclophosphamide control rats (group-II) and there was a significant reduction in RBC ($p<0.01$), Hb ($p<0.05$), WBC ($p<0.05$), and HCT ($p<0.05$) compared to vehicle control. Treatment with QD at 25 and 50 mg/kg bw significantly normalised these haematological parameters and all values were comparable to vehicle control except a significant decrease ($p<0.01$) in WBC count at QD 25 mg/kg bw (4000±184.4 *vs.* 5840±201.5 of control). QDE 10 mg/kg treatment normalised Hb and PLT count, however, RBC, WBC and HCT values were still significantly lower ($p<0.05$) compared to vehicle control. QDE 20 mg/kg treatment normalised all haematological parameters except a significant decrease ($p<0.001$) in WBC count was persisted on day-22 (3700±312.0 *vs.* 5840±201.5 of control).

Treatment with QD at 25 and 50 mg/kg bw restored the haematological parameters in rats induced by cyclophosphamide. QDE effectively restored haematological parameters only at 20 mg/kg bw. Observed effect may be exerted by the presence of iron and other constituents of QD such as flavonoids, terpenoids, and steroids. Present findings validate the indication of this traditional Unani formulation in the management of iron deficiency anaemia and QD could be a potential formulation for erythropoietic activity.

Publications

Husain GM, Urooj M, Mustehsan, Javed G, Kumar P, Kazmi MH. Beneficial Effect of *Qurs-e-Damavi*, A Traditional Unani Formulation in Cyclophosphamide Induced Haematological Perturbations in Rats. Advances in Complementary & Alternative Medicine. 2020;6(2):576–579. DoI: https://10.31031/ACAM.2020.06.000635

18 Preclinical Safety and Efficacy Evaluation of *Khamīra-i- Gā'ozabān 'Ambarī Jadwār Ūd Salīb Wālā*

18.1 Acute Oral Toxicity Study of KGAJOS in Rats

This study was designed to evaluate acute oral toxicity potential of *Khamīra-i-Gā'ozabān'Ambarī Jadwār Ūd Salīb Wālā* (KGAJOS) in Wistar rats. Considering the low acute toxicity potential, the limit test as per OECD guideline number 425 was conducted at the dose of 2000 mg/kg body weight. Rats were weighed and orally administered with single dose of test drug and observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with KGAJOS in three consecutive animals, dosing to further animals was stopped. Blood samples were collected on 15th day for haematological and biochemical investigation from all the three animals. Animals were sacrificed on Day-15 and necropsy was performed. No treatment related gross pathological abnormality was observed. Biochemical and haematological parameters were found within normal limits. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 2000 mg/kg bw of KGAJOS. Therefore, oral LD_{50} of the KGAJOS in the female Wistar rats was estimated to be greater than 2,000 mg/kg body weight.

18.2 Chronic (Repeated Dose 180-day) Oral Toxicity Study of Khamīra-i-Gā'ozabān'Ambarī Jadwār Ūd Salīb Wālā

Chronic toxicity study on *Khamīra-i-Gā'ozabān'Ambarī Jadwār Ūd Salīb Wālā* (KGAJOS) has been conducted in rats as per limit test method. 15 Male and 15 Female Wistar rats per group were orally treated with vehicle or KGAJOS (2000 mg/kg bw) as an aqueous suspension in water at the maximum volume of 2 mL/100 gm bw, once daily. The control animals were administered with equal amount of vehicle (water) only. Rats were observed for clinical signs, morbidity and mortality twice daily. Detailed clinical observations were made, once before

the first exposure and at regular intervals during course of treatment. Body weight and feed consumption were recorded throughout the course of study. After competition of study period of 180-days, animals were sacrificed. Haematological and biochemistry parameters were performed from the blood samples collected from retro-orbital sinus under anaesthesia. All rats were subjected to gross necropsy and internal vital organs were stored in formalin. Tissue samples submitted for histopathological investigation.

No abnormal behaviour or clinical signs indicative of systemic toxicity was observed in any of the group. There were four mortalities (04 females and 01 male) in vehicle control group and three mortalities (02 females and 01 male) in KGAJOS -2000 mg/kg treated group during the treatment period (mostly during 5^{th} & 6^{th} month of treatment). Mortality rate in KGAJOS treated groups was less compared to vehicle treated control group and may not be considered treatment related. Body weight and feed consumption pattern in control and KGAJOS-2000 mg/kg treated groups did not show any significant difference throughout the study. No toxicologically significant changes were observed in haematological and biochemical parameters between KGAJOS-2000 mg/kg and vehicle control group except few isolated changes where values of parameters remained within the physiological range and did not exhibit any toxicological relevance. Relative organ weights of organs and tissues were comparable. Gross necropsy of animals survived through 180-day treatment duration revealed no anomalies. However, necropsy of morbid animals during the mid of the study revealed changes related to aspiration pneumonia. Therefore, mortality may not be attributable to the test substance. Changes of histological significance were observed only in the lungs, livers and kidneys of control and experimental group of animals. In lungs, low grade inflammatory changes were observed in both the groups. However, grade-3 chronic interstitial pneumonitis (CIPn) changes were observed only in the KGAJOS-2000 mg/kg group, thus indicating probable inflammation in this group when compared to the control group. In kidneys, majority of animals in both groups were normal, the focal interstitial nephritis (FIN) changes observed (equal in both groups) are not considered significant. In livers, majority of the histopathological changes (except the low grade micro vacuolation (MiV) in combination with portal tract inflammation (PTI) and parenchymal inflammation (PI) were observed equally in both the groups. However, these changes (low grade MiV in combination

with PTI and PI) if considered individually, were present in equal frequency in both the groups, due to which these changes may not be attributed to the test compound administered.

Based on study data, no toxicologically significant changes were observed in behavioural observations, body weight, feed-intake, relative organ weight and gross necropsy in KGAJOS treated group and control animals. Few changes observed in haematology and clinical biochemistry parameters may be considered incidental as these changes were not consistent and observed values were within normal physiological limits. Considering higher frequency of CIPn grade-3 in KGAJOS-2000 mg/kg group, lungs may be the target organ of toxicity at higher doses on prolonged administration.

18.3 Evaluation of Cognitive Functions of KGAJOS Using Morris Water Maze Test in C57BL/6 Mice.

Khamīra-i-Gā'ozabān'Ambarī Jadwār Ūd Salīb Wālā (KGAJOS) is a polyherbal compound Unani Pharmacopoeial formulation described in various traditional Unani texts as *Muqawwī-i-A'ḍā' Ra'īsa* (tonic for brain, heart, liver and stomach) and is used in epilepsy, *Umm al-Ṣibyān* (infantile epilepsy) and *Ikhtināq al-Raḥim* (hysteria). KGAJOS is also reported to possess anxiolytic and antidepressant activity in mice. Though it is used clinically for various neurological conditions, preclinical efficacy of this formulation in learning and memory is not established.

Khamīra-i-Gā'ozabān'Ambarī Jadwār Ūd Salīb Wālā was evaluated for cognitive function improvement activity using Morris water maze test in C57BL/6 mice. Three dose levels of KGAJOS i.e., 500, 1,000 and 1,500 mg/kg bw/day (i.e., equivalent to 0.5X, 1X and 1.5X of therapeutically equivalent dose) were orally administered for seven consecutive days before recording the water maze performance and treatment was continued throughout the procedure till probe trial. Piracetam (400 mg/kg bw, i.p.) was used as positive control for comparison. Maze video tracking software (Stoelting) was used for tracking the path of mice in pool as per standard protocol.

There was a significant increase ($p<0.01$) in time spent in platform quadrant in piracetam treated group compared to vehicle control group. No significant

difference was observed at KGAJOS 500 mg/kg bw compared to vehicle. KGAJOS significantly increased the time spent in the target quadrant at 1000 and 1500 mg/kg bw as compared to vehicle control ($p<0.01$ and 0.001, respectively). Latency to reach the platform quadrant (escape latency) was significantly reduced ($p<0.001$) in piracetam and KGAJOS group at 1000 and 1500 mg/kg bw compared to vehicle control. No change in escape latency was observed at 500 mg/kg bw of KGAJOS. Further, average distance travelled by mice in platform quadrant is significantly higher ($p<0.001$) in piracetam group compared to vehicle control. KGAJOS significantly increased distance travelled by mice in platform quadrant at 1000 mg/kg ($p<0.01$) and 1500 mg/kg ($p<0.001$) while no difference was observed at 500 mg/kg bw compared to vehicle.

Morris water maze experiment conducted in mice revealed improved learning and memory function of KGAJOS at the dose levels of 1000 and 1500 mg/kg bw whereas 500 mg/kg bw was not found to be effective. Observed efficacy of KGAJOS confirmed the traditional claims and usage of this formulation in conditions associated with cognition and memory.

Publications

Husain GM, Nadeem M, Javed G, Urooj M, Alam M, Reddy MA, Kazmi MH. A traditional poly-herbal formulation improves cognitive function in C57BL/6 mice. Ars Pharmaceutica. 2021;62(1):6-14. http://doi.org/10.30827/ars.v62i1.15432

19 Preclinical Evaluation of Cognitive Effect of *Ma'jūn IQ*

Evaluation of Ma'jūn IQ for Cognitive Functions Improvement Using Morris Water Maze Test in C57BL/6 Mice

Ma'jūn IQ (MIQ) is a compound Unani formulation used as brain tonic. Acute and sub-acute toxicity studies have been conducted on MIQ in Wistar rats and it is found to be safe up to 4800 mg/kg bw in repeated dose 28-days oral toxicity study in rats. MIQ is clinically used as brain tonic however, no data is available regarding efficacy of this valuable compound Unani formulation on cognitive function.

Ma'jūn IQ was evaluated for cognitive function improvement activity using Morris water maze test in C57BL/6 mice. Therapeutic dose of MIQ is 3-5g in human. Therapeutic Equivalent Dose (TED) for 5g human dose in mouse is ~1000 mg/kg bw per day as per body surface area conversion method. Accordingly, present study was performed at three dose levels of MIQ i.e., 500, 1,000 and 1,500 mg/kg bw/day, orally administered for seven consecutive days before recording the water maze performance and treatment was continued throughout the procedure till probe trial. Piracetam (400 mg/kg bw, i.p.) was used as positive control for comparison. Anymaze video tracking software was used for tracking the path of mice in pool as per standard protocol.

During probe trial (i.e., on day-6), there was a significant increase ($p<0.01$) in time spent in platform quadrant in piracetam treated mice compared to vehicle control group. No significant difference was observed at MIQ 500 or 1000 mg/kg bw compared to vehicle. MIQ significantly ($p<0.05$) increased the time spent in the target quadrant only at 1500 mg/kg bw as compared to vehicle control. Latency to reach the platform quadrant (escape latency) was significantly reduced ($p<0.05$) in piracetam group compared to vehicle control while no change in escape latency was observed in any MIQ treated group. Further, average distance travelled by mice in platform quadrant is significantly higher ($p<0.001$) in piracetam group compared to vehicle control whereas MIQ did not alter the distance travelled by mice in platform quadrant at any tested dose level compared to vehicle.

20 Antiepileptic and Antidepressant Activity of Classical form and Hydro-alcoholic Extract of *Ma'jūn-i-Najāḥ* in Experimental Animals

Morris water maze experiment conducted in mice revealed no significant improvement on learning and memory function by MIQ except a mild effect only at 1500 mg/kg bw. Further studies are warranted using alternate model(s) of cognitive dysfunction to explore the potential benefits of this formulation on learning and memory.

*Ma'jūn-i-Naj*āḥ (MN) is a compound Unani Pharmacopoeial formulation mentioned in the National Formulary of Unani Medicine, Part-I and other classical text of Unani system of medicine. It is recommended for the treatment of *Mālankhūliyā* (melancholia), *Qūlanj* (colic), and *Ikhtināq al-Raḥim* (hysteria). The efficacy study of MN for the management of epilepsy and depression need to be conducted to generate evidence to support the clinical use. This study was carried out to evaluate the antiepileptic and antidepressant activity of MN classical and 50% hydro-ethanolic extract in experimental animals.

Antiepileptic Activity: The antiepileptic activity was evaluated using Maximal Electroshock (MES) - Induced Convulsion in SD rats. Animals were divided into six groups of eight animals each. Group I was normal control, Group II was positive control treated with Diazepam (3 mg/kg, p.o.). Group III & IV were given MN classical at dose administered 500 and 1000 mg/kg bw per day, respectively, for seven days. While Group V & VI were treated with 50% hydro-alcoholic extract at the dose level of 170 and 340 mg/kg bw per day (equivalent doses based on percentage yield), respectively, for seven days. The second model used for screening of antiepileptic activity was Pentylenetetrazol (PTZ) induced convulsion in mice. Swiss albino mice were divided into six groups of eight animals each. Treatment was same as mentioned above except administration of PTZ 65 mg/kg bw intra-peritoneally 45 minutes after oral administration of vehicle and test formulation (MN) and 30 minutes after administration of standard (Phenytoin; 25 mg/kg i.p.).

Antidepressant Activity: The antidepressant activity was evaluated using Porsolt's Forced Swim Test model in rats. Rats were divided into six groups of six animals each. Group I served as control. Group II was positive control which received Imipramine (20 mg/kg, p.o.). Group III and IV were test dose groups for classical form of MN which were orally administered at the doses of 500 and 1000 mg/kg bw of MN per day consecutively for two weeks. Group V and VI were orally treated with 50% hydro alcoholic extract at dose levels of 170 and 340 mg/kg bw per day respectively, for two weeks.

The results showed that there was a decrease in clonic convulsion and stupor phase in MN treated group as compared to control group. However, there were no statistically significant reduction in both classical and extract treated animals at both dose levels as compared to animals of control group. Statistically significant reduction ($p<0.05$ or $p<0.01$) were observed in positive control animals treated with diazepam as compared to control animals for all measured parameters. The seizure score of epileptic behaviour measured 30 min after PTZ administration showed reduction in all MN treated groups compared to control, however, statistically significant reduction was observed only in phenytoin treated group as compared to control ($p < 0.01$). The results found in FST model indicated statistically significant reduction ($p<0.001$) in immobility duration in all treatment group except extract low dose group (170 mg/kg bw) as compared to control.

The finding of the study showed that MN both classical and extract does not possess significant antiepileptic property when subjected to MES and PTZ induced models. MN showed promising antidepressant activity at all tested dose levels for both classical and extract in experimental animals except low dose extract. Findings of the present study support that MN has potent antidepressant activity and may play a promising role in elevation of mood in depressed patients.

Publications

Urooj M, Husain GM, Nadeem M, Naikodi MAR, Alam M, Kazmi MH. Antiepileptic and Antidepressant Activity of Majoon Najah (A Traditional Unani Formulation) in Experimental Animals. International Journal of Pharmaceutical Investigation. 2020;10(3):396-401. DOI: https://10.5330/ijpi.2020.3.70

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21 Preclinical Safety Evaluation of *Qurṣ-i-Dīdān*

21.1 Acute Oral Toxicity Study of Qurṣ-i-Dīdān in Rats

Qurṣ-i-Dīdān (QD) is a compound Unani formulation considered as *Qātil-i-Dīdān-i-Am'ā'* and used for treatment of worm infestations. Presently, no data is available regarding toxicity of this valuable Unani formulation. Accordingly, this study was designed to evaluate acute oral toxicity potential of *Qurṣ-i-Dīdān* in Wistar rats. Considering the low acute toxicity potential, the limit test as per OECD test guideline no 425 was conducted at the dose of 2000 mg/kg body weight. Animals were weighed and single oral dose of test drug administered. Rats were observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with *Qurṣ-i-Dīdān* in three consecutive animals, respectively dosing to further animals was stopped. All the three animals were sacrificed on Day 15 and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 2000 mg/kg bw. Therefore, oral LD_{50} of the *Qurṣ-i-Dīdān* in the female Wistar rat was estimated to be greater than 2,000 mg/kg body weight.

21.2 Sub-acute (Repeated Dose 28- day) Oral Toxicity Study of Qurṣ-i-Dīdān in Rats

Present study was designed to evaluate repeated dose 28-day oral toxicity. The study was carried out in Wistar rats. Animals were divided into three groups (n=5 per sex per group). *Qurṣ-i-Dīdān* (QD) was orally administered at the dose of 50, 250 and 500 mg/kg bw/day (i.e., 1X, 5X and 10X, respectively) for 28 days. Control animals were administered with vehicle (water) only. Body weight and feed intake for all animals was measured weekly throughout study duration. Detailed clinical observations were made periodically to detect signs of toxicity. After completion of 28-days, blood samples were collected for haematological and biochemical analysis and animals were sacrificed, organs were harvested for weight determination. Vital organs & tissues were preserved in the neutral buffered formalin and tissues of control and high dose were subjected for histopathological evaluation.

There was no mortality after administration of QD at different dose in both male and female rats consecutively for 28 days. Detailed clinical examination in both male and female rats did not reveal any incidence of adverse effect or abnormal clinical sign

which was carried out at different time points at different dose levels throughout study duration. No significant changes in terms of body weight and feed intake observed in both male and female animals treated with QD at all dose levels as compared to control group measured weekly throughout study duration.

Haematological and biochemical indices have not shown any significant alteration in both male and female rats at all three tested dose level as compared to control animals except few alterations which were within normal physiological range and found toxicologically insignificant. There was significant reduction of total protein in male animals treated with QD at dose level of 250 mg/kg bw ($p<0.01$) and 500 mg/kg bw ($p<0.001$) as compared to control animals. Significant reduction of urea was observed in male animals QD at dose level of 250 mg/kg bw ($p<0.01$) and 500 mg/kg bw ($p<0.05$) as compare to control animals. The lipid profile showed significant reduction in HDL level in male animals treated with QD at dose level of 250 mg/kg bw ($p<0.05$) and 500 mg/kg bw ($p<0.01$) as compared to control animals.

The biochemical analysis in female animals revealed significant reduction of AST at QD dose 50 mg/kg bw as compare to control animals. It was observed that there was significant reduction of total protein at QD 50 mg/kg bw ($p<0.01$), 250 mg/kg bw ($p<0.001$) and 500 mg/kg bw ($p<0.001$) as compare to control animals. There was significant reduction ($p<0.001$) of urea at all tested dose levels as compared to vehicle treated control animals. Significant ($p<0.01$) reduction of cholesterol level was observed at QD 500 mg/kg bw as compared to control animals. The relative organ weight data have not showed alteration in relative organ weight at all tested dose levels in the organs such as brain, thymus, heart, lungs, liver, adrenals, kidney, testis, epididymis, uterus and ovaries. However, there was significant increase ($p<0.05$) in relative organ weight of adrenal in female animals at tested dose of 50 mg/kg bw and 250 mg/kg bw as compared to control animals. There was significant ($p<0.01$) increase in relative organ weight of liver in male animals at QD dose 500 mg/kg bw compared to control animals. No dose-related toxic changes were observed in the histology of lungs, liver, spleen, kidney, heart, pancreas, testis/ovaries, stomach and brain of the treatment group when compared with the vehicle control group.

No treatment-related toxicological significant observations were noted in body weight, feed intake, behavioural pattern, haematology, biochemistry and relative organ weight in QD-treated group at 50, 250 and 500 mg/kg bw dose levels compared to control animals. Histopathological findings revealed that QD may not produce any major reactive and toxic changes in any systemic organ up to the highest tested dose i.e., 500 mg/kg bw. Therefore, QD may be considered safe based on the above observations up to the highest tested dose of 500 mg/kg bw in rats.

22 Preclinical Safety Evaluation of *Iṭrīfal Muqawwī-i- Dimāgh*

22.1 Acute Oral Toxicity Study of *Iṭrīfal Muqawwī-i- Dimāgh* in Rats

This study was designed to evaluate acute oral toxicity potential of *Iṭrīfal Muqawwī-i- Dimāgh* in Wistar rats. Considering the low acute toxicity potential, the limit test as per OECD-425 was conducted at the dose of 5000 mg/kg body weight. Animals were individually weighed and orally given a single dose of test drug. Rats were observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with *Iṭrīfal Muqawwī-i- Dimāgh* in three consecutive animals respectively, dosing to further animals was stopped. All the three animals were sacrificed on 15th day and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 5,000 mg/kg bw. Therefore, oral LD_{50} of *Iṭrīfal Muqawwī-i- Dimāgh* in the female Wistar rats was estimated to be greater than 5,000 mg/kg body weight.

22.2 Chronic (Repeated Dose 180-day) Oral Toxicity Study of *Iṭrīfal Muqawwī-i- Dimāgh* in Rats

Chronic toxicity study on *Iṭrīfal Muqawwī-i- Dimāgh* (IMD) has been conducted in rats as per limit test method. 15 Male and 15 Female rats per group were orally treated with vehicle or IMD (2000 mg/kg bw) as an aqueous suspension in water at the maximum volume of 2 mL/100 gm bw, once daily. The control animals were administered with equal amount of vehicle (water) only. Rats were observed for clinical signs, morbidity and mortality twice daily. Detailed clinical observations were made, once before the first exposure and at regular intervals during course of treatment. Body weight and feed consumption were recorded throughout the course of study. After competition of study period of 180-days, animals were sacrificed. Haematological and Biochemistry parameters were performed from the blood samples collected from retro-orbital sinus under anaesthesia. All rats were subjected to gross necropsy

and internal vital organs were stored in formalin. Tissue samples submitted for histopathological investigation.

No abnormal behaviour or clinical signs indicative of systemic toxicity was observed in any of the group. There were four mortalities (04 females and 01 male) in vehicle control group and three mortalities (02 females and 01 male) in IMD-2000 mg/kg treated group during the treatment period (mostly during 5th & 6th month of treatment). Mortality rate in IMD treated groups was less compared to vehicle treated control group and may not be considered treatment related. Body weight and feed consumption pattern in control and IMD-2000 mg/kg treated groups did not show any significant difference throughout the study. No toxicologically significant changes were observed in haematological and biochemical parameters between IMD-2000 mg/kg and vehicle control group except few isolated changes where values of parameters remained within the physiological range and did not exhibit any toxicological relevance. Relative organ weights of organs and tissues were comparable. Gross necropsy of animals survived through 180-day treatment duration revealed no anomalies. However, necropsy of morbid animals during the mid of the study revealed changes related to aspiration pneumonia. Therefore, mortality may not be attributable to the test substance. Changes of histological significance were observed only in the lungs, livers and kidneys of control and treatment groups. In lungs, low grade inflammatory changes were observed in both the groups. However, grade-3 chronic interstitial pneumonitis (CIPn) and abscesses were observed in 30% of treatment group, thus indicating increased inflammation in this group when compared to the control group. In livers and kidneys, as different histopathological changes were observed in both the groups in almost similar frequency, the changes are not considered significant or due to the administration of test drug.

Based on study data, no toxicologically significant changes were observed in behavioural observations, body weight, feed-intake, relative organ weight, and gross necropsy in IMD treated group and control animals. Few changes observed in haematology and clinical biochemistry parameters may be considered incidental as these changes were not consistent and observed values were within normal physiological limits. Considering higher frequency of CIPn grade-3 in IMD-2000 mg/kg group, lungs may be the target organ of toxicity at higher doses on prolonged administration.

23 Preclinical Safety Evaluation of *Jawārish-i-Anārayn*

23.1 Acute Oral Toxicity Study of Jawārish-i-Anārayn in Rats

Jawārish-i-Anārayn (JAN) is a compound Unani formulation considered as *Muqawwī-i-Mi'da wa Jigar* (tonic for stomach and liver) and *Mushtahī* (appetizer). Its indications include *Ḍu'f al- Mi'da*, *Ḍu'f al-kabid*, *Ḍu'f al-Shahwa, Qay', Ghasiyan, Ishāl-i-Ṣafrāwī*. This study was designed to evaluate acute oral toxicity potential of *Jawārish-i-Anārayn* in Wistar rats. Considering the low acute toxicity potential, the limit test as per OECD test guideline number 425 was conducted at the dose of 5000 mg/kg body weight. Animals were weighed and orally administered with a single dose of test drug. Rats were observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no mortality was observed following treatment with *Jawārish-i-Anārayn* in three consecutive animals, dosing to further animals was stopped. All the three animals were sacrificed on 15th day and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 5000 mg/kg bw. Therefore, oral LD50 of the *Jawārish-i-Anārayn* in the female Wistar rats was estimated to be greater than 5,000 mg/kg body weight.

23.2 Sub-acute (Repeated Dose 28-day) Oral Toxicity Study of Jawārish-i-Anārayn in Rats

Repeated dose 28-day oral toxicity of *Jawārish-i-Anārayn* (JAN) was carried out in male and female Wistar rats. Animals were divided into three groups (n=05 per sex per group). JAN was orally administered at the dose of 1000 and 2000 mg/kg bw/day for 28 days. Control animals were administered with vehicle i.e., water. Body weight and feed intake for all animals was measured weekly throughout study duration. Detailed clinical observations were made periodically to detect signs of toxicity. After completion of 28-days, blood samples were collected for haematological and biochemical analysis and animals were sacrificed, organs were harvested for weight determination. Vital organs or tissues were preserved in the neutral buffered formalin and tissues of control and high dose were subjected for histopathological evaluation.

There was no observation of any mortality after administration of JAN at different dose in both male and female rats consecutively for 28 days. Detailed clinical examination in both male and female rats did not reveal any incidence of adverse effect or abnormal clinical sign which was carried out at different time points at different dose levels throughout study duration. No significant changes in terms of body weight and feed intake observed in both male and female animals treated with JAN at all dose levels as compared to control group measured weekly throughout study duration.

No significant changes were observed in terms of haematological parameters in both male and female treated with JAN as compared to control animals except a significant ($p<0.05$) rise in Hb and HCT count in male animals treated with JAN at dose 2000 mg/kg bw as compared to control animals. All haematological parameters of control and treated rats were within normal physiological range. The female animals treated with JAN at dose of 1000 mg/kg bw showed significant ($p<0.05$) reduction in AST and HDL level as compared to vehicle treated control group. The biochemical parameters observed in male animals at both dose level did not indicate significant alteration except significant ($p<0.05$) reduction in AST level at dose 2000 mg/kg bw as compared to control animals. Additionally, male animals treated with JAN showed significant reduction in HDL level at dose 1000 mg/kg bw ($p<0.01$) and 2000 mg/kg bw ($p<0.05$) as compared to vehicle treated control animals. The relative organ weight data analysis has not showed alteration in relative organ weight at dose level of 1000 and 2000 mg/kg bw in the organ/tissue such as brain, thymus, heart, lungs, liver, spleen, adrenals, kidney, testis, epididymis, uterus and ovaries. No dose-related toxic changes were observed in the histology of lungs, liver, spleen, kidney, heart, pancreas, testis or ovaries, stomach and brain of the treatment group when compared with the vehicle control group.

No toxicological significant observation with respect to body weight, feed intake, behavioural pattern, haematology, biochemistry and relative organ weight in JAN treated group at 1000 and 2000 mg/kg bw dose levels or in control animals. Histopathological findings revealed that JAN did not produce any major reactive and toxic changes in any systemic organ up to the highest tested dose i.e., 2000 mg/kg bw. Therefore, JAN may be considered safe based on the above observations up to the highest tested dose of 2000 mg/kg bw in rats.

24 Preclinical Safety Evaluation of *Iṭrīfal Zamānī*

24.1 Acute Oral Toxicity Study of Iṭrīfal Zamānī in Rats

Iṭrīfal Zamānī (ITZ) is a compound Unani formulation indicated for the management of *Mālankhūliyā* (melancholia), *Nazla* (catarrh), *Zukam* (cold), *Qūlanj* (colic), *Ṣudā'* (Headache) and *Qabḍ* (constipation). Presently, no data is available regarding toxicity of this valuable Unani formulation, therefore, this study was designed to evaluate acute oral toxicity potential of *Iṭrīfal Zamānī* in Wistar rats. Considering the low acute toxicity potential, the limit test as per OECD-425 was conducted at the dose of 5000 mg/kg body weight. Animals were orally administered with test drug as aqueous suspension and observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with *Iṭrīfal Zamānī* in three consecutive animals, dosing to further animals was stopped. All the three animals were sacrificed on 15th day and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 5000 mg/kg bw. Therefore, oral LD_{50} of the *Iṭrīfal Zamānī* in the female Wistar rats was estimated to be greater than 5,000 mg/kg body weight.

24.2 Sub-acute (Repeated Dose 28-day) Oral Toxicity Study of Iṭrīfal Zamānī in Rats

Repeated dose 28-day oral toxicity study of *Iṭrīfal Zamānī* (ITZ) was carried out in Wistar rats of both sexes. Animals were divided into three groups (n=5 per sex per group). ITZ was orally administered at the dose of 1000 and 2000 mg/kg bw/day (1X and 2X of therapeutically equivalent dose) for 28 days. Control animals were administered with vehicle. Body weight and feed intake for all animals was measured weekly throughout study duration. Detailed clinical observations were made periodically to detect signs of toxicity. At the end of the treatment period, the overnight fasted (water provided *ad-libitum*) rats

were anaesthetized with isoflurane inhalation (EZ Anaesthesia-1339), blood samples were collected by retro-orbital puncture in the EDTA vacutainers (for haematological) and serum vacutainers (for biochemical analysis). Animals were sacrificed and organs were harvested for weight determination. Vital organs or tissues were preserved in the neutral buffered formalin and tissues of control and high dose were subjected for histopathological evaluation.

There were no post doses adverse effect reported on survival of both male and female rats after oral administration of ITZ for 28 days. There was no report for any abnormal clinical signs based on clinical examination conducted at different time points in ITZ treated animals at 1000 mg/kg bw and 2000 mg/kg bw as compared to animals of control group. No significant difference in body weight and feed intake was observed for ITZ treated animals as compared to control group. ITZ treated animals did not showed any significant alteration in haematological parameters in both male and female rats as compared to control treated animals except a significant increase ($p<0.05$) in platelets count in female group at both tested dose level as compared to control animals. There was significant rise ($p<0.01$) in Hb, RBC and HCT count in male animals treated with ITZ at dose 1000 mg/kg bw as compared to control animals. The observation in female animals treated with ITZ did not show any significant alteration in biochemical parameters except significant reduction ($p<0.05$) in triglyceride and VLDL level at dose level of 1000 mg/kg bw as compared to animals of control group. No significant changes were observed in ITZ treated male animals except significant increase ($p<0.05$) in uric acid level at dose of 2000 mg/kg bw as compared to vehicle treated control animals. The animals treated with ITZ orally for 28 days at dose levels of 1000 and 2000 mg/kg bw did not induce any alteration in relative organ weight in the organ/tissue. No dose-related toxic changes were observed in the histology of lungs, liver, spleen, kidney, heart, pancreas, testis or ovaries, stomach and brain of the treatment group when compared with the vehicle control group.

The finding of the repeated dose 28-day oral toxicity study showed no toxicologically significant observation with respect to body weight, feed intake, behavioural pattern, haematology, biochemistry, organ weight and relative organ weight in ITZ-treated group at both 1000 and 2000 mg/kg bw or in control animals. Histopathological observations revealed that ITZ may not produce any major reactive and toxic changes in any systemic organ up to 2000 mg/kg bw. Therefore, ITZ would be considered safe up to 2000 mg/kg bw in rats.

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25 Preclinical Safety Evaluation of *Sharbat-i-Belgirī*

25.1 Acute Oral Toxicity Study of *Sharbat-i-Belgirī* in Rats

Sharbat-i-Belgirī (SBG) is a compound Unani Pharmacopoeial formulation included in National Formulary of Unani Medicine, Part-V. SBG is mentioned *as Muqawwī-i-Mi'da* (stomachic) and is used for the management of diarrhoea and dysentery. No data was available regarding toxicity of this compound Unani formulation. This study was designed to evaluate acute oral toxicity potential of *Sharbat-i-Belgirī* (SBG) in Sprague Dawley (SD) rats. Considering the low acute toxicity potential of tested drug, a limit test at the dose of 20 mL/kg bw (i.e., maximum feasible dose and about 8X of therapeutically equivalent dose), as per the OECD test guideline number 425, was conducted. A single dose of test substance was administered and animals were observed for lethality and toxic signs & symptoms for 14 days post-treatment. No lethality was observed following treatment with SBG in five consecutive animals, tested sequentially. No mortality was observed at the tested dose of 20 mL/kg bw of SBG. All the five animals were sacrificed on 15th day and necropsy was performed. No treatment related mortality or gross pathological abnormality was observed. Therefore, oral LD_{50} of the SBG in the female SD rats was estimated to be greater than 20 mL/kg body weight.

25.2 Sub-acute (Repeated Dose 28-day) Oral Toxicity Study of *Sharbat-i-Belgirī* in Rats

Repeated dose 28-day oral toxicity study of SBG was conducted in Sprague Dawley (SD) rats. Rats were randomly divided into four groups (n=5 per sex per group). Aqueous suspension of SBG was orally administered at the doses of 2.5, 5 and 10 mL/kg bw/day (i.e., approximately 1X, 2X and 4X of therapeutic equivalent dose, respectively) for 28 days. Control animals were administered with equal volume of vehicle (water). Body weight and feed intake for all animals

was measured weekly throughout study duration. Detailed clinical observations were made periodically to detect signs of toxicity. After completion of 28-days, the overnight fasted (water provided *ad-libitum*) rats were anaesthetized with isoflurane inhalation (EZ Anaesthesia-1339), blood samples were collected by retro-orbital puncture in the EDTA vacutainers (for haematological) and serum vacutainers (for biochemical analysis). Subsequently all animals were euthanized and subjected to gross necropsy. Organs and tissues were examined macroscopically and internal organs / tissues were isolated, trimmed and weighed. Organs or tissues were preserved in the neutral buffered formalin and tissues of control and high dose were subjected to histological examination.

There was no treatment-related adverse effect on survival of both male and female rats after oral administration of SBG for 28 consecutive days. No significant alteration was observed in body weight and feed intake of control and SBG treated rats of either sex. The SBG treated animals did not showed any significant alteration in haematological parameters in both male and female rats as compared to control treated animals. All observed values remained within normal physiological range. No toxicologically significant changes were observed in biochemical parameters except few isolated changes in electrolytes. In male group there was significant ($p<0.01$) decrease in total protein and globulin level in animals treated with SBG 5 mL/kg bw as compared to normal control. SBG did not induce any alteration in relative organ weight in the organs/tissues such as brain, thymus, heart, lungs, liver, spleen, adrenals, kidney, testis, epididymis, uterus and ovaries. No treatment-related toxic changes were observed in the histology of lungs, liver, brain, spleen, kidney, heart, thymus, adrenals, and gonads of the treatment group when compared with the vehicle control group.

The study findings did not indicate any toxicological significant observation in terms of body weight, feed intake, behavioural pattern, haematology, clinical chemistry, organ weight, gross necropsy and histopathology on internal organs in SBG-treated groups at any tested dose levels compared to control animals. Based on the above findings, it may be concluded that NOAEL of SBG may be considered as 10 mL/kg bw/day in rats, and SBG is safe up to the highest tested dose level.

26 Preclinical safety evaluation of *Sharbat-i-Khāksī*

26.1 Acute Oral Toxicity Study of Sharbat-i-Khāksī in Rats

Sharbat-i-Khāksī (SKH) is a compound Unani Pharmacopoeial formulation referred as *Dafa-e-Humma* (antipyretic) and is used for the management of *Ḥummā Judariyya* (smallpox), *Ḥaṣba* (measles), *Ṭayfūdas* (Typhoid) and *Ḥummā Nā'iba* (periodic fever). Presently, no data is available regarding toxicity of this compound Unani formulation. This study was designed to evaluate acute oral toxicity potential of SKH in Sprague Dawley (SD) rats. Considering the low acute toxicity potential of tested drug, a limit test at the dose of 20 mL/kg bw (i.e., maximum feasible dose) as per OECD test guideline number 425 was conducted. Animals were administered with single dose of test formulation and observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with SKH in five consecutive animals, dosing to further animals was stopped. All the five animals were sacrificed on 15th day and necropsy was performed. No treatment related mortality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 20 mL/kg bw. Therefore, oral LD_{50} of the SKH in the female SD rats was estimated to be greater than 20 mL/kg/bw.

26.2 Sub-acute (Repeated Dose 28-day) Oral Toxicity Study of Sharbat-i-Khāksī in Rats

Repeated dose 28-day oral toxicity of SKH was conducted in Sprague Dawley (SD) rats. Animals were randomly divided into four groups (n=5 per sex per group). SKH was orally administered at the dose of 2.5, 5 and 10 mL/kg bw/day (i.e., approximately 1X, 2X and 4X of therapeutic equivalent dose, respectively) for 28 days. Control animals were administered with water as vehicle. Body weight and feed intake for all animals was measured weekly throughout study

duration. Detailed clinical observations were made periodically to detect signs of toxicity. After completion of 28-days, blood samples were collected for haematological and biochemical analysis and animals were sacrificed, organs were harvested for weight determination and histopathological evaluation.

There were no treatment-related adverse effect reported on survival of both male and female rats after oral administration of SKH for 28 consecutive days. There was no significant alteration observed with regard to body weight and feed intake of control and SKH treated rats of either sex. The SKH treated animals did not showed any significant alteration in haematological parameters in both male and female rats at all tested dose level as compared to control treated animals. SKH did not showed any significant alteration in biochemical parameters except significant reduction in triglyceride level at tested dose level 2.5 mL/kg bw ($p<0.05$), 5 mL/kg bw ($p<0.01$) and 10 mL/Kg bw ($p<0.05$) as compared to animals of control group. There was significant reduction in potassium level at dose of 2.5 mL/kg bw ($p<0.01$) and 5 mL/kg bw ($p<0.05$) in female animals as compared to control animals. Significant reduction of calcium level was observed in high dose female group ($p<0.05$) as compared to normal control. In SKH treated male group potassium level was significantly ($p<0.05$) increase in animals treated with high dose (10 mL/kg bw) as compared to control animals. No gross changes were observed during necropsy and relative organ weight of SKH-treated rats and control animals. Heart, brain, lungs, liver, kidneys, spleen, pancreas, adrenals, testes and ovaries of high dose SKH (10 mL/kg bw) and control group did not reveal any toxicologically significant finding following histological investigations.

Based on the findings of the present study, oral administration of SKH at doses 2.5, 5 and 10 mL/kg bw/day for 28 days, it may be concluded that SKH did not show any toxicologically significant adverse effects on clinical observations, body weight and feed intake, haematology, clinical biochemistry, gross necropsy and histopathology. Therefore, no-observed-adverse-effect-level (NOAEL) of SKH may be considered as 10 mL/kg bw in rats.

27 Preclinical safety evaluation of *Khamīra-i-Ābresham Shīrah ‘Unnāb Wālā*

27.1 Acute Oral Toxicity Study of *Khamīra-i-Ābresham Shīrah‘Unnāb Wālā* in Rats

Khamīra-i-Ābresham Shīrah‘Unnāb Wālā (KASU) is a compound Unani Pharmacopoeial formulation included in National Formulary of Unani Medicine-Part-V. KASU is mentioned as *Muqawwī-i-Qalb wa Dimāgh* (tonic for heart and brain). Its therapeutic uses include *Khafaqān* (palpitation), *Ḍu‘f al-Baṣar* (weakness of sight) and dry cough produced by *Sill* (tuberculosis). No data is available regarding toxicity of this compound Unani formulation. This study was performed to evaluate acute oral toxicity potential of KASU in Sprague Dawley (SD) rats. Considering the low acute toxicity potential of tested drug, a limit test at the dose of 2000 mg/kg bw, as per the OECD test guideline number 425 was conducted. Animals were administered with single dose of the test formulation and observed for lethality and toxic signs & symptoms for 14 days post-treatment. No lethality was observed following treatment with KASU in five consecutive female rats, respectively. All the five animals were sacrificed on 15th day and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the tested dose of 2000 mg/kg bw. Therefore, oral LD_{50} of the KASU in the female SD rats was estimated to be greater than 2000 mg/kg body weight.

27.2 Sub-acute (Repeated Dose 28-day) Oral Toxicity Study of *Khamīra-i-Ābresham Shīrah‘Unnāb Wālā* in Rats

Repeated dose 28-day oral toxicity study of *Khamīra-i-Ābresham Shīrah‘Unnāb Wālā* (KASU) was performed in SD rats. Animals were randomly allocated into four groups (n=5 per sex per group). KASU was orally administered at the dose of 500, 1000 and 2000 mg/kg bw/day (i.e., approximately 1X, 2X and 4X of therapeutic equivalent dose, respectively) for 28 days. Animals of control

group were administered with water as vehicle. Body weight and feed intake for all animals was measured weekly throughout the study duration. Detailed clinical observations were made periodically to detect signs of toxicity. After completion of 28-days, blood samples were collected under anaesthesia for haematological and biochemical analysis and animals were sacrificed, organs were harvested for relative organ weight determination and histopathological evaluation.

No mortality and clinical sign of toxicity was observed in both male and female rats after oral administration of KASU for 28 days. Body weight and feed intake of KASU treated rats were comparable to respective vehicle control animals. No significant alteration was observed in relation to haematological parameters in KASU treated animals except a significant increase in WBC count in female at KASU-1000 mg/kg bw ($p<0.05$) and in male at dose 500 mg/kg bw ($p<0.01$) as compared to normal control animals. KASU did not show any significant alteration in biochemical parameters except a significant ($p<0.05$) decrease in BUN level at KASU 500 mg/kg bw (male group) and a significant ($p<0.001$) decrease in cholesterol level was observed at all tested dose levels of KASU (male groups) as compared to control. There was no toxicologically significant alteration in relative organ weights of brain, thymus, heart, lungs, liver, spleen, adrenals, kidney, testis, epididymis, uterus and ovaries in KASU treated rats compared to control group. Gross necropsy did not reveal any overt changes in any KASU-treated or control group. Heart, brain, lungs, liver, kidneys, spleen, pancreas, adrenals, testes or uterus and ovaries of KASU-2000 mg/kg and control group did not reveal any toxicologically significant finding following histological investigations.

The study findings did not indicate any toxicological significant observation in terms of body weight, feed intake, behavioural pattern, haematology, clinical chemistry; relative organ weight, gross necropsy and histopathological findings in KASU treated group at all tested dose level as compared to control animals. Therefore, no-observed-adverse-effect-level (NOAEL) of KASU may be considered greater than 2000 mg/kg bw in rats.

28 Preclinical safety evaluation of Qurṣ-i-Mafāṣil

28.1 Acute Oral Toxicity Study of Qurṣ-i-Mafāṣil in Rats

This study was designed to evaluate acute oral toxicity potential of *Qurṣ-i-Mafāṣil* (QM) in Sprague Dawley rats. Considering the low acute toxicity potential of tested drug, a limit test at the dose of 2000 mg/kg bw (i.e., maximum feasible dose and about 10X of therapeutically equivalent dose) as per OECD guideline number 425 was conducted. Animals were observed for mortality and toxic signs & symptoms for 14 days post-treatment. No mortality was observed following treatment with *Qurṣ-i-Mafāṣil* in five consecutive female rats, respectively. Body weight and feed intake of animals was regularly monitored throughout the study. All the five animals were sacrificed on 15th day and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 2000 mg/kg bw. Therefore, oral LD_{50} of the *Qurṣ-i-Mafāṣil* in the female SD rat was estimated to be greater than 2,000 mg/kg body weight.

28.2 Sub-acute (Repeated Dose 28- day) Oral Toxicity Study of Qurṣ-i-Mafāṣil in Rats

Toxicity potential of repeated administration of QM was studied in rats following 28-days repeated oral administration in Sprague Dawley (SD) rats of both sexes. Animals were divided into six groups (n=5 per sex per group). QM was orally administered at the dose levels of 200, 1000 and 2000 mg/kg bw/day (i.e., approximately 1X, 5X and 10X of therapeutic equivalent dose, respectively) for 28 days. Control animals were administered with 0.30% aqueous carboxy methyl cellulose (CMC) suspension. A satellite group (n=5 rats per sex per group) was also included in the control and high dose QM group to monitor the reversibility or persistence of treatment related changes

(if any). Animals in satellite groups received respective treatment for 28-days, followed by maintaining them for two weeks without dosing before euthanasia. Body weight and feed intake for all animals was regularly measured throughout the study. Detailed clinical observations were made periodically to detect signs of toxicity. After completion of respective dosing period, blood samples were collected for haematological and biochemical investigations and animals were euthanized; vital organs were collected for organ weight determination and histopathological evaluation. Tissue or organ samples were submitted to National Institute of Nutrition, Hyderabad for histopathology.

No mortality was observed following administration of QM or vehicle for 28 days or in satellite groups. No behavioural changes indicative of systemic toxicity was observed following QM treatment. Body weight and feed intake of QM treated rats were found almost comparable to their respective control groups of either sex. QM treatment did not result any overt toxicologically significant alteration in haematological and biochemical parameters in either male or female rats at any tested dose level as compared to vehicle control animals except few changes which were mostly observed in satellite group animals (both vehicle and QM sacrificed after two weeks of recovery period). No gross pathological changes/ lesions were observed during necropsy in any group. Relative organ weigh of various treatment groups was found comparable to vehicle control. No toxicologically significant changes were observed during histopathological investigation in any organ except certain changes which were observed in liver of few animals in QM as well as vehicle treated groups.

The study findings did not indicate any toxicological significant alteration in terms of survival, behaviour pattern, body weight, feed intake, haematology, clinical chemistry, relative organ weight and histopathology of organs or tissues in any QM treated group as compared to vehicle control. Based on the data generated, QM may be considered safe at the therapeutically used dosage and liver might be the target organ of toxicity up on repeated administration at high doses.

29 Preclinical safety evaluation of *Capsule Nazla*

29.1 Acute Oral Toxicity Study of Capsule Nazla in Rats

This study was designed to evaluate acute oral toxicity potential of *Capsule Nazla* (CNaz) in female Sprague Dawley rats. Considering the low acute toxicity potential of tested drug, a limit test at the dose of 3000 mg/kg bw (i.e., maximum feasible doses) as per OECD test guideline number 425 was conducted. Animals were administered with single dose of CNaz and observed for lethality and toxic signs & symptoms for 14 days post-treatment. No lethality was observed following treatment with *Capsule Nazla* in five consecutive animals tested. Body weight and feed intake of animals was regularly monitored throughout the study. All the five animals were sacrificed on 15th day and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no mortality was observed at the dose of 3000 mg/kg bw of CNaz. Therefore, oral LD_{50} of the *Capsule Nazla* in the female SD rat is estimated to be greater than 3,000 mg/kg body weight.

29.2 Sub-acute (Repeated Dose 28- day) Oral Toxicity Study of Capsule Nazla in Rats

Toxicity potential of repeated administration of CNaz was studied in rats following 28-days repeated oral administration in Sprague Dawley (SD) rats of both sexes. Animals were divided into six groups (n=5 per sex per group). CNaz was orally administered at the dose levels of 300, 900 and 1800 mg/kg bw/day (i.e., approximately 1X, 3X and 6X of therapeutic equivalent dose, respectively) for 28 days. Control animals were administered with 0.30% aqueous carboxy methyl cellulose (CMC) suspension. A satellite group (n=5 rats per sex per group) was also included in the control and high dose CNaz group to monitor the reversibility or persistence of treatment related changes (if any). Animals in satellite groups received respective treatment for 28-days,

followed by maintaining them for two weeks without dosing before euthanasia. Body weight and feed intake for all animals was regularly measured throughout the study. Detailed clinical observations were made periodically to detect signs of toxicity. After completion of respective dosing period, blood samples were collected for haematological and biochemical investigations and animals were euthanized; vital organs were collected for organ weight determination and histopathological evaluation.

No mortality was observed following administration of CNaz or vehicle for 28 days or in satellite groups. No behavioural changes indicative of systemic toxicity was observed following CNaz treatment. Body weight and feed intake of CNaz treated rats were found almost comparable to their respective control groups of either sex. CNaz treatment did not resulted any overt toxicologically significant alteration in haematological and biochemical parameters in either male or female rats at any tested dose level as compared to vehicle control animals except few changes which were mostly observed in satellite group animals (both vehicle and CNaz; sacrificed after two weeks of recovery period). No gross pathological changes/ lesions were observed during necropsy in any group. Relative organ weigh of various treatment groups was found comparable to vehicle control. No toxicologically significant changes were observed during histopathological investigation in any organ except in the liver of few animals in CNaz as well as vehicle treated group. Observed changes in the liver were fully reversible in the satellite groups after recovery period.

The study findings did not indicate any toxicological significant alteration in terms of survival, behaviour pattern, body weight, feed intake, haematology, clinical chemistry, relative organ weight, and histopathology of organs or tissues in any CNaz treated group as compared to vehicle control. Based on the data generated, CNaz would be considered safe at the therapeutically used dosage and liver might be the target organ for toxicity up on repeated administration at high doses.

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30 Preclinical Safety Evaluation of *Jawārish-i-Āmla Sāda*

30.1 Acute Oral Toxicity Study of Jawārish-i-Āmla Sāda in Rats

Jawārish-i-Āmla Sāda (JAS) is a traditional polyherbal compound Unani Pharmacopoeial formulation. It is mentioned as *Muqawwī* (tonic), *Kāsir-i-Riyāḥ* (carminative), and *Qabiḍ* (induce constipation). It is indicated for the clinical treatment of *Ḍu'f-i-Mi'da* (weakness of the stomach), *Ḍu'f-i-Kabid* (weakness of the liver), *Ḍu'f-i-Qalb* (weakness of the heart), *Khafaqān* (palpitation), *Nafkh-i-Mi'da* (flatulence) and *Ishāl-i- Ṣafrāwī* (bilious diarrhoea).

This study was designed to evaluate acute oral toxicity potential of *Jawārish-i-Āmla Sāda* (JAS) in Sprague Dawley (SD) rats. Considering the low acute toxicity potential of tested drug, a limit test at the dose of 2000 mg/kg bw (i.e., maximum feasible dose) as per OECD test guideline number 425 was conducted. Animals were administered with single dose of formulation and observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with JAS in five consecutive animals respectively, dosing to further animals was stopped. All the five animals were sacrificed on 15th day and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 2000 mg/kg bw. Therefore, oral LD_{50} of the JAS in the female SD rats was estimated to be greater than 2000 mg/kg body weight.

30.2 Sub-acute (Repeated Dose 28-day) Oral Toxicity study of Jawārish-i-Āmla Sāda in Rats

No data is available regarding the repeated dose toxicity of this compound Unani formulation. Therefore, present study was designed to evaluate repeated dose 28-day oral toxicity study of JAS in rats. Study was carried out on SD rats of both sexes. Animals were divided into four groups (n=5 per sex per group). JAS was orally administered at the dose of 500, 1000 and 2000 mg/kg bw/day

(i.e., approximately 1X, 2X and 4X of therapeutic equivalent dose, respectively) for 28 days. Control animals were administered with vehicle. Body weight and feed intake for all animals was measured weekly throughout study duration. Detailed clinical observations were made periodically to detect signs of toxicity. After completion of 28-days, blood samples were collected for haematological and biochemical analysis and animals were sacrificed, organs were harvested for weight determination and histopathological evaluation.

No post-treatment adverse effect was observed on survival of both male and female rats after oral administration of JAS for 28 consecutive days. There was no significant alteration observed in body weight, feed intake and relative organ weights of control and JAS treated rats of either sex. No significant alteration was reported in terms of haematological and biochemical parameters in both male and female rats at all tested dose level as compared to control animals except isolated alteration in total protein and triglyceride level in JAS-500 mg/kg group, which were toxicologically insignificant and remained within normal reference range. No alteration was observed in relative organ weight in the organ or tissue such as brain, thymus, heart, lungs, liver, spleen, adrenals, kidney, testis, epididymis, uterus and ovaries. The histology of brain, heart, thyroid, spleen, adrenals, testes, ovaries, uterus, stomach, and small intestine were found normal. Changes of histological significance were observed only in the lungs, kidneys and livers of vehicle control and JAS -2000 group of animals. However, as the histological changes of varying grades observed in the JAS -2000 mg/kg treated animals were also observed in the vehicle control group, the changes are not considered significant or due to the administration of JAS-2000 mg/kg.

The study findings did not indicate any toxicological significant observation in terms of body weight, feed intake, behavioural pattern, haematology, clinical chemistry, organ weight, and gross necropsy in JAS treated group at all tested dose level as compared to control animals. Both, vehicle control and JAS -2000 mg/kg groups showed normal histology of all vital organs except some histological alterations in lungs, liver and kidneys and these changes were not related JAS-2000 mg/kg treatment as observed in both group with same frequency and pattern. Therefore, JAS would be considered safe based on above observations.

31 Preclinical Safety Evaluation of *Jawārish-i-Ūd Shīrīn*

31.1 Acute Oral Toxicity Study of *Jawārish-i-Ūd* Shīrīn in Rats

Jawārish-i-Ūd Shīrīn (JOS) is a traditional polyherbal compound Unani Pharmacopoeial formulation. It is mentioned as *Qabiḍ (*induces Constipation) and *Muqawwī-i-Mi'da* (Stomachic). It is indicated for the clinical treatment of *Ishāl* (Diarrhoea), *Ḍu'f al-Haḍm* (Indigestion), and *Ḍu'f al-Shahwa* (poor appetite). Considering the low acute toxicity potential of tested drug, a limit test at the dose of 2000 mg/kg bw (i.e., maximum feasible dose) as per OECD test guideline number 425 was conducted. Animals were administered with single dose of formulation and observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no mortality was observed following treatment with JOS in 5 consecutive animals respectively, dosing to further animals was stopped. All the five animals were sacrificed on Day 15 and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 2000 mg/kg bw. Therefore, oral LD_{50} of the JOS in the female SD rat was estimated to be greater than 2000 mg/kg body weight.

31.2 Sub-acute (Repeated Dose 28-day) Oral Toxicity Study of *Jawārish-i-Ūd* Shīrīn in Rats

The present study was designed to evaluate repeated dose 28-day oral toxicity of *Jawārish-i- Ūd Shīrīn* (JOS) in Sprague Dawley (SD) rats. Animals were divided into three groups (n=05 per sex per group). The control group was same for *Jawārish-i-Āmla Sāda* and *Jawārish-i-Ūd Shīrīn* as both studies were conducted simultaneously. JOS was orally administered at the dose of 500, 1000 and 2000 mg/kg bw/day (i.e., approximately 1X, 2X and 4X of therapeutic equivalent dose, respectively) for 28 days. Body weight and feed intake for all animals was measured weekly throughout study duration. Detailed clinical observations were made periodically to detect signs of toxicity, if any. After completion of 28-days, blood samples were collected for haematological and

biochemical analysis and animals were sacrificed, organs were harvested for relative organ weight determination and histopathological evaluation.

Findings of the present study revealed neither mortality nor behavioural changes in 28-day repeated dose oral toxicity assessment of JOS. No significant alteration was observed in body weight and feed intake in HOS treated rats compared to vehicle control rats. The JOS treated animals did not show any significant alteration in haematological parameters in rats at any tested dose level as compared to control animals. JOS treatment did not induce any significant alteration in any biochemical parameters except significant ($p<0.05$) reduction in total bilirubin level at all tested dose levels compared to animals of control group. In JOS treated male group, there was significant ($p<0.05$) increase in BUN at dose 1000 mg/kg bw as compared to control group. Significant reduction (500 mg/kg bw; $p<0.01$) and (1000 & 2000 mg/kg bw; $p<0.001$) of uric acid in male animals was also noted in all JOS-tested dose group as compared to control animals. No statistically significant change was noted in relative organ weights of control and JOS treated rats of either sex. Histology of all vital organs were found normal except lungs, liver and kidneys. Some mild to moderate histological changes were observed in lungs of both vehicle control and JOS -2000 mg/kg groups. Moreover, some mild histological changes were also observed in the livers and kidneys of both vehicle control and JOS -2000 groups. However, as the histological changes of varying grades observed in the JOS -2000 mg/kg treated animals were also observed in the vehicle control group, the changes are not considered significant or due to the administration of JOS -2000 mg/kg.

Findings of the present study revealed neither mortality nor behavioural changes in the acute and 28-day repeated dose oral toxicity assessment of JOS. No toxicological significant difference was observed with respect to body weight, feed intake, haematology, clinical chemistry, relative organ weight and gross necropsy in JOS treated groups or control animals. Histological findings with respect to all vital organs was found normal in both vehicle and JOS 2000 mg/kg bw treated rats. Few alterations were observed in lungs, liver and kidneys and these changes were not attributable to JOS-2000 mg/kg treatment. Hence based on the above observation JOS would be considered safe at therapeutically used level and NOAEL of JOS will be considered as 2000 mg/kg bw in rats.

32 Preclinical Safety Evaluation of *Jawārish-i-Kamūnī*

32.1 Acute Oral Toxicity Study of Jawārish-i-Kamūnī in Rats

Jawārish-i-Kamūnī (JK) is a compound Unani Pharmacopoeial formulation. Its reported actions include *Mujaffif* (drying agent), *Jazib* (absorbefacient), and *Kāsir-i-Riyāḥ* (carminative). It is indicated for the clinical treatment of *Humuzat-e-Meda* (hyperacidity), *Fuwāq* (hiccups), *Qeela Maeeya* (hydrocele), *Nafkh-i-Mi'da* (gastric flatulence), *Fataq Urbi*, (inguinal hernia), *Qabḍ* (constipation). No data is available regarding the toxicity of this compound Unani formulation. This study was designed to evaluate acute oral toxicity potential of *Jawārish-i-Kamūnī* (JK) in Sprague Dawley (SD) rats. Considering the low acute toxicity potential of tested drug, a limit test at the dose of 2000 mg/kg bw (i.e., maximum feasible dose) as per OECD-425 was conducted. Animals were administered with single dose of test formulation and observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with JK in five consecutive animals respectively, dosing to further animals was stopped. All the five animals were sacrificed on 15th day and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 2000 mg/kg bw. Therefore, oral LD_{50} of the JK in the female SD rats was estimated to be greater than 2000 mg/kg body weight.

32.2 Sub-acute (Repeated Dose 28-day) Oral Toxicity Study of Jawārish-i-Kamūnī in Rats.

Repeated dose 28-day oral toxicity study of JK was performed in rats. Animals of both sexes were randomly distributed into three groups (n=05 per sex per group). JK was orally given to rats *via* stainless steel gavage at the dose of 1000, 1500 and 2000 mg/kg bw/day (i.e., approximately 1X, 1.5X and 2X of therapeutic equivalent dose, respectively) for 28 days. Control animals were administered with water as vehicle. Body weight and feed intake for all animals

was measured weekly throughout study duration. Detailed clinical observations were made periodically to detect signs of toxicity. After completion of 28-days, blood samples were collected for haematological and biochemical analysis and animals were sacrificed, organs were harvested for weight determination and histopathological evaluation.

No treatment related adverse effect was observed on survival of both male and female rats after oral administration of JK for 28 consecutive days. There was no significant alteration noted in the body weight, feed intake and relative organ weights of control and JK treated rats of either sex. JK treated animals did not show any significant alteration in haematological parameters except significant reduction in MCHC in female group at all tested dose level and in male animals at dose 1500 mg/kg bw as compared to control treated animals. No significant alterations were reported in terms of biochemical parameters except statistically significant ($p<0.01$) reduction in glucose level at all tested dose levels as compared to animals of control group in both sexes, indicating glucose lowering property of JK. Histology of all vital organs were found normal except lungs and liver. Some mild to moderate histological changes were observed in lungs of both vehicle control and JK-2000 mg/kg groups. Some mild histological changes were also observed in the liver of JK-2000 mg/kg groups (3/10 rats). In the absence of any associated increase in ALT/AST in JK-2000 treated animals, it may conclude that the histological changes in liver were not related to the administration of JK-2000 mg/kg and may be background changes observed in SD rats.

The finding of the study showed no toxicological significant observation with respect to body weight, feed intake, behavioural pattern, haematology, clinical chemistry and relative organ weight in JK treated groups or control animals. JK showed glucose lowering property as it caused reduction in glucose in both sexes at all tested dose as compared to control animals, however obtained values were found within normal physiological range. Histology of all vital organs was found normal except some mild to moderate histological changes were observed in lungs of both vehicle control and JK-2000 mg/kg treated groups and mild changes were observed in the livers of JK-2000 mg/kg treated groups. Based on above findings, JK would be considered safe up to the highest tested dose i.e., 2000 mg/kg bw.

33 Preclinical Safety Evaluation of *Safūf-i-Zaḥīr*

33.1 Acute Oral Toxicity Study of Safūf-i-Zaḥīr in Rats

Safūf-i-Zaḥīr (SZH) also known as *Tiryaq-i-Pechish* is a traditional compound Unani Pharmacopoeial formulation. It is mentioned as *Mufattiḥ Sudad* (deobstruent), and *Kāsir-i-Riyāḥ* (carminative). Its therapeutic uses include *Nafkh-i-Mi'da* (flatulence) and *Zaḥīr* (dysentery). This study was designed to evaluate acute oral toxicity potential of *Safūf-i-Zaḥīr* (SZH) in Sprague Dawley (SD) rats. Considering the low acute toxicity potential of tested drug, a limit test at the dose of 2000 mg/kg bw (i.e., maximum feasible dose) was conducted as per OECD test guideline number 425. Animals were administered with single dose of formulation and observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with SZH in five consecutive animals respectively, dosing to further animals was stopped. All the five animals were sacrificed on 15th day and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 2000 mg/kg bw. Therefore, oral LD_{50} of the SZH in the female SD rats was estimated to be greater than 2000 mg/kg body weight.

33.2 Sub-acute (Repeated Dose 28-day) Oral Toxicity Study of Safūf-i-Zaḥīr in Rats

No data is available regarding the toxicity of this compound Unani formulation. Therefore, present study was designed to evaluate repeated dose 28-day oral toxicity study of *Safūf-i-Zaḥīr* (SZH) in rats.

Rats were randomly divided into 4 groups (n=05 per sex per group). SZH was administered at the dose of 300, 900 and 1500 mg/kg bw/day (i.e., approximately1X, 3X and 5X of therapeutic equivalent dose, respectively) for 28 days. Control group animals were administered with vehicle only (water). Body weight and feed intake for all animals was measured weekly throughout study duration. Detailed clinical observations were made periodically to detect signs of toxicity. After completion of 28 days, blood samples were collected for

haematological and biochemical analysis and animals were sacrificed, organs were harvested for weight determination and histopathological evaluation.

No treatment related adverse effect was observed on survival of both male and female rats after oral administration of SZH for 28 days. There was no significant alteration in body weight, feed intake and relative organ weights of control and SZH treated rats of either sex. The SZH treated animals did not showed any significant alteration in haematological parameters in both male and female rats at any tested dose level as compared to control animals except a significant ($p<0.001$) rise in MCHC values in male animals at all tested dose levels as compared to normal control animals. All tested biochemical parameters were comparable to control animals except few isolated alterations which were toxicologically insignificant and remained within normal reference range such as a significant ($p<0.01$) reduction in bilirubin level at SZH-900 mg/kg bw compared to animals of control group. There was statistically significant reduction in uric acid level in female group at SZH-300 mg/kg bw ($p<0.05$), and SZH-900 mg/kg bw ($p<0.01$) as compared to control animals. In SZH treated male group uric acid was significantly ($p<0.05$) decrease in animals treated with 300 mg/kg bw as compared to control animals. No effect was observed on relative organ weight in the organ/tissue such as brain, thymus, heart, lungs, liver, spleen, adrenals, kidney, testis, epididymis, uterus and ovaries of SZH treated animals.

Histology of all vital organs were found normal except lungs, liver and kidneys. Some mild to moderate histological changes were observed in lungs, livers and kidney of both vehicle control and SZH -1500 mg/kg groups. As the histological changes of varying grades observed in the experimental animals were also noted in the control group, the changes are not considered significant or due to the administration of SZH-1500 mg/kg.

The study findings did not indicate any toxicological significant observation in terms of survival, body weight, feed intake, behavioural pattern, haematology, clinical chemistry, relative organ weight and gross necropsy in SZH treated group at any tested dose level as compared to control animals. Histology of all vital organs was found normal, except some mild to moderate histological changes were observed in lungs, liver and kidneys of both vehicle control and SZH groups and these changes were not related SZH-1500 mg/kg treatment. Therefore, SZH would be considered safe based on above observations.

34 Preclinical Safety Evaluation of Sharbat-i-Zūfā Murakkab

34.1 Acute Oral Toxicity Study of Sharbat-i-Zūfā Murakkab in Rats

Sharbat-i-Zūfā Murakkab (SZM) is a traditional polyherbal compound Unani formulation. It is indicated for the management of *Su'āl Balghami* (productive cough) and *Dama* (asthma). It has been reported as effective and safe in *Su'āl Ratab* (productive cough), No data is available regarding the toxicity of this compound Unani formulation. This study was undertaken to evaluate acute oral toxicity potential of *Sharbat-i-Zūfā Murakkab* (SZM) in Sprague Dawley (SD) rats. Considering the low acute toxicity potential of tested drug, a limit test at the dose of 20 mL/kg bw (i.e., maximum feasible dose) as per OECD test guideline 425 was conducted. Animals were administered with single dose of formulation and observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with SZM in five animals sequentially tested, dosing to further animals was stopped. All the five animals were sacrificed on Day-15 and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 20 mL/kg bw. Therefore, oral LD_{50} of the SZM in the female SD rat was estimated to be greater than 20 mL/kg body weight.

34.2 Sub-acute (Repeated Dose 28-day) Oral Toxicity Study of Sharbat-i-Zūfā Murakkab in Rats

SD rats of both sexes were divided into four groups (n=05 per sex per group). The tested drug SZM was administered orally at the dose of 2, 4 and 10 mL/kg bw/day (approximately 1X, 2X and 5X of therapeutic equivalent dose, respectively) for 28 days. Body weight and feed intake for all animals was measured weekly throughout study duration. Detailed clinical observations were made periodically to detect signs of toxicity. After completion of 28-days, blood

samples were collected for haematological and biochemical analysis and animals were sacrificed, organs were harvested for weight determination and histopathological evaluation.

Findings of the present study revealed neither mortality nor any neuro-behavioural changes in 28-day repeated dose oral toxicity assessment of SZM. There was no significant alteration observed in body weight, feed intake of control and SZM treated rats of either sex compared to control group. The SZM treated animals did not show any significant alteration in haematological parameters in both male and female rats as compared to control treated animals except a significant increase in WBC in male animals at dose 2 mL/kg bw as compared to control (13.50±0.57 *vs.* 10.30±0.38 $x10^3$/µL). All tested biochemical parameters were comparable in SZM treated groups and control animals. No changes were observed during necropsy and relative organ weight of the organ or tissue such as brain, thymus, heart, lungs, liver, spleen, adrenals, kidney, testis, epididymis, uterus and ovaries was comparable to control group. Histology of all vital organs were found normal except lungs and liver. Changes of histological significance were observed only in the lungs and livers of control and SZM-treated group. Similar histological changes were observed in lungs of both SZM-10 mL/kg treated animals as well as animals of control group. Hence the observed changes may not be considered toxicologically significant. Mild change in liver was observed only in one animal (1/10) of SZM-10 mL/kg group therefore may not be considered treatment related effect.

Findings of the present study revealed neither mortality nor behavioural changes in the acute and 28-day repeated dose oral toxicity assessment of SZM. No toxicological significant observation with respect to body weight, feed intake, haematology, clinical chemistry, organ weight and relative organ weight in SZM treated groups or control animals were observed. Histology of all vital organs was found normal, except some mild to moderate histological changes in lungs of both vehicle control and SZM 10 mL/kg treated groups and mild changes in the liver of one animal of SZM 10 mL/kg treated group, which were not considered as treatment related. Therefore, SZM would be considered safe based on above observations and NOAEL of SZM may be considered as 10 mL/kg in rats.

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35 Preclinical Safety Evaluation of *Sharbat-i-I'jāz*

35.1 Acute Oral Toxicity Study of Sharbat-i-I'jāz in Rats

Sharbat-i-I'jāz (SEJ) is a traditional polyherbal compound Unani formulation having action as *Munaffith-i-Balgham* (Expectorant) and *Musakkin-i-Su'āl* (Antitussive). It is indicated for the treatment of *Su'āl* (Cough), *Nazla* (Catarrh) and *Zukam* (Coryza). This study was designed to evaluate acute oral toxicity potential of *Sharbat-i-I'jāz* (SEJ) in Sprague Dawley (SD) rats. Considering the low acute toxicity potential of tested drug, a limit test at the dose of 20 mL/kg bw (i.e., maximum feasible dose) as per OECD-425 was conducted. Animals were administered with single dose of formulation and observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with SEJ in five consecutive animals respectively, dosing to further animals was stopped. All the five animals were sacrificed on 15th day and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 20 mL/kg bw. Therefore, oral LD_{50} of the SEJ in the female SD rats was estimated to be greater than 20 mL/kg body weight.

35.2 Sub-acute (Repeated Dose 28-day) Oral Toxicity Study of Sharbat-i-I'jāz in Rats

No data is available regarding the repeated toxicity potential of this compound Unani formulation. Therefore, present study was designed to evaluate repeated dose 28-day oral toxicity study of SEJ in rats. SD rats were divided into four groups (n=05 per sex per group). The test drug SEJ was given orally at the dose of 2, 4 and 10 mL/kg bw/day (i.e., approximately 1X, 2X and 5X of therapeutic equivalent dose, respectively) for 28 days. Body weight and feed intake for all animals was measured weekly throughout study duration. Detailed

clinical observations were made periodically to detect signs of toxicity. After completion of 28-days, blood samples were collected for haematological and biochemical analysis and animals were euthanised, organs were harvested for organ weight determination and histopathological evaluation.

Findings of the present study revealed neither mortality nor behavioural changes in 28-day repeated dose oral toxicity assessment of SEJ. There was no significant alteration observed in body weight, feed intake of control and SEJ treated rats of either sex. The SEJ treated animals did not reveal any significant alteration in haematological and biochemical parameters in both male and female rats at any tested dose level as compared to control animals except there was significant ($p<0.05$) increase in Blood Urea at dose 4 mL/kg bw only in males as compare to control group. No gross changes were observed during necropsy and relative organ weights of SEJ treated groups were comparable to control animals. Changes of histological significance were observed only in the lungs and livers of vehicle control and SEJ 10 ml/kg group of animals. In lungs as histological changes observed in the experimental animals were also observed in the control group, they are not considered significant. With respect to liver, one animal (10%) in the control group showed a high histological score of 3, and 5 animals (50%) showed a low score of 1. Four animals (40%) of SEJ 10 ml/kg group had a low score of 1 while 50% in SEJ group and showed a moderate score of 2. These histological changes were not related SEJ 10 ml/kg treatment, and may be considered as background changes.

Findings of the present study revealed neither mortality nor behavioural changes in 28-day repeated dose oral toxicity assessment of SEJ. No toxicological significant observation with respect to body weight, feed intake, haematology, clinical biochemistry and relative organ weight in SEJ treated groups or control animals were observed. Both, vehicle control and SEJ 10 ml/kg groups showed normal histology of all vital organs except some histological alterations in lungs and liver and these changes were not related to SEJ-10 ml/kg treatment. Therefore, SEJ can be considered safe based on above observations and NOAEL may be considered as 10 mL/kg bw in rats.

36 Preclinical Safety Evaluation of *Sharbat-i-'Unnāb*

36.1 Acute Oral Toxicity Study of Sharbat-i-'Unnāb in Rats

Sharbat-i-'Unnāb (SUN) is a traditional polyherbal compound Unani formulation having action as *Munaffith-i-Balgham* (Expectorant) and *Musakkin* (calming). It is indicated for the treatment of *Su'āl* (Cough). This study was designed to evaluate acute oral toxicity potential of *Sharbat-i-'Unnāb* (SUN) in Sprague Dawley (SD) rats. Considering the low acute toxicity potential of tested formulation, a limit test was performed at the dose of 20 mL/kg bw (i.e., maximum feasible dose) as per OECD guideline number 425. Animals were administered with single dose of formulation and observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with SUN in five consecutive animals respectively, dosing to further animals was stopped. All the five animals were sacrificed on Day-15 and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 20 mL/kg bw. Therefore, oral LD_{50} of the SUN in the female SD rat was estimated to be greater than 20 mL/kg body weight.

36.2 Sub-acute (Repeated Dose 28-day) Oral Toxicity Study of Sharbat-i-'Unnāb in Rats

As no data is available regarding the repeated dose toxicity of this compound Unani formulation, present study is designed to evaluate repeated dose 28-day oral toxicity study of SUN. SD rats of both sexes were randomly allocated into four groups (n=05 per sex per group). SUN was administered *via* oral route at the dose level of 2, 4 and 10 mL/kg bw/day (i.e., approximately 1X, 2X and 5X of therapeutic equivalent dose, respectively) for 28 days. Control animals were administered with vehicle (water). Body weight and feed intake for all animals was measured weekly throughout study duration. Detailed clinical observations

were made periodically to detect signs of toxicity. After completion of 28-days, blood samples were collected for haematological and biochemical analysis and animals were sacrificed, organs were harvested for weight determination and histopathological evaluation.

No treatment related adverse effect was observed on survival, body weight, feed intake after oral administration of SUN for 28 days. No significant alterations were noted in terms of haematological and biochemical parameters in both male and female rats at any tested dose level as compared to control animals except few isolated alterations (like a significant ($p<0.01$) increase in triglyceride level at SUN-2 mL/kg bw as compared to control group) which were toxicologically insignificant and remained within normal reference range. No change in the relative organ weight was noticed in the organ or tissue such as brain, heart, liver, spleen, adrenals, kidney, testis, epididymis, uterus and ovaries. Histology of all vital organs were found normal except lungs, and liver. In lungs as histological changes observed in the experimental animals were also observed in the control group, they are not considered significant. With respect to liver, one animal (10%) in the control group showed a high histological score of 3, and 5 animals (50%) showed a low score of 1. Among the experimental groups, 30% animals of SUN group had a low score of 1 while 70% animals of SUN group, showed a moderate score of 2. However, these histological changes were not related to SUN-10 ml/kg treatment, and may be considered as background changes as no concomitant increases was noted in the biomarkers of liver injury like ALT. It may conclude that the histological changes were not related to the administration of SUN-10 ml/kg.

The study findings did not indicate any toxicological significant observation in terms of survival, body weight, feed intake, behavioural pattern, haematology, biochemistry, relative organ weight in SUN treated group at any tested dose level as compared to control animals. Both, vehicle control and SUN 10 ml/kg groups showed normal histology of all vital organs except some histological alterations in lungs and liver and these changes were not related SUN 10 ml/kg treatment. Therefore, SUN may be considered safe at the therapeutically used dose level.

37 Preclinical Safety Evaluation of Iṭrīfal Shāhitara

37.1 Acute Oral Toxicity Study of Iṭrīfal Shāhitara in Rats

Iṭrīfal Shāhitara (ITS) is a traditional polyherbal compound Unani Pharmacopoeial formulation having action as *Muṣaffī-i-Dam* (Blood Purifier), *Mulayyin (*Laxative). It is indicated for *Taṣfiya al-Dam* (purification of Blood), *Ātshak* (Syphilis), *Ṣudā'* (Headache), *Ḥikka* (Pruritus). This study was designed to evaluate acute oral toxicity potential of *Iṭrīfal Shāhitara* (ITS) in Sprague Dawley (SD) rats. Considering the low acute toxicity potential of tested drug, a limit test at the dose of 2000 mg/kg bw (i.e., maximum feasible dose) as per OECD guideline number 425 was conducted. Rats were administered with single dose of formulation and observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with ITS in five consecutive rats, dosing to further animals was stopped. All the five rats were sacrificed on 15th day and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 2000 mg/kg bw. Therefore, oral LD_{50} of the ITS in the female SD rat was estimated to be greater than 2000 mg/kg body weight.

37.2 Sub-acute (Repeated Dose 28-day) Oral Toxicity Study of Iṭrīfal Shāhitara in Rats

In the absence of data on safety of this compound Unani formulation, a study was conducted to evaluate repeated dose 28-day oral toxicity study of ITS in rats. Study was carried out on SD rats of both sexes. Animals were divided into three groups (n=05 per sex per group). ITS was orally administered as aqueous suspension at the dose of 500, 1,000 and 1,500 mg/kg bw/day (i.e., approximately 1X, 2X and 3X of therapeutic equivalent dose, respectively) for 28 days. Body weight and feed intake for all animals was measured weekly throughout study duration. Detailed clinical observations were made periodically

to detect signs of toxicity. After completion of 28-days, blood samples were collected for haematological and biochemical analysis and animals were euthanised, organs were harvested for weight determination and preserved for histopathological evaluation.

No treatment related adverse effect was observed on the survival, body weight gain, and feed consumption of both male and female rats after oral administration of ITS for 28 days. No significant alterations were observed in terms of haematological and biochemical parameters in both male and female rats at any tested dose level as compared to control animals except a significant ($p<0.05$) increase in AST level in females at ITS-1500 mg/kg bw, as compared to control group. There was no significant alteration observed in body weight and feed intake of control and ITS treated rats of either sex. No gross changes were visible during necropsy and relative organ weights of ITS treated groups were comparable to control animals. Histology of all vital organs were found normal except lungs and liver of control and ITS 1500 mg/kg group. In lungs, as histological changes observed in the experimental animals were also observed in the control group, they are not considered significant. With respect to liver, one animal (10%) in the control group showed a high histological score of 3 and 5 animals (50%) showed a low score of 1. Among the experimental groups, 30% animals of ITS group had a low score of 1 while, 40% of the ITS group showed a moderate score of 2.

The study findings did not indicate any toxicological significant observation in terms of body weight, feed intake, behavioural pattern, haematology, clinical biochemistry parameters, organ weight in ITS treated group at any tested dose level as compared to control animals. Therefore, ITS can be considered safe at the therapeutically used concentration while liver might be the target organ of toxicity upon repeated administration of high doses.

38 Preclinical Safety Evaluation of *Sharbat-i-Tūt Siyāh*

38.1 Acute Oral Toxicity Study of Sharbat-i-Tūt Siyāh in Rats

Sharbat-i-Tūt Siyāh (STS) is a traditional polyherbal compound Unani formulation having action as *Muḥallil* (Resolvent) and *Mulaṭṭif* (attenuant). It is indicated for the treatment of *Waram-i-Lawzatayn* (Tonsilitis), *Waram-i-Ḥanjara* (Laryngitis), *Nazla* (Catarrh), *Su'āl* (Cough). This study was designed to evaluate acute oral toxicity potential of *Sharbat-i-Tūt Siyāh* (STS) in Sprague Dawley (SD) rats. Considering the low acute toxicity potential of tested drug, a limit test at the dose of 20 mL/kg bw (i.e., maximum feasible dose) as per OECD-425 was conducted. Animals were administered with single dose of formulation and observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with STS in five consecutive animals, dosing to further animals was stopped. All the five animals were sacrificed on 15th day and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 20 mL/kg bw. Therefore, oral LD_{50} of the STS in the female SD rat was estimated to be greater than 20 mL/kg body weight.

38.2 Sub-acute (Repeated Dose 28-day) oral Toxicity Study of Sharbat-i-Tūt Siyāh in Rats

In the absence of safety data on repeated administration, a study was conducted to evaluate repeated dose 28-day oral toxicity study of STS in rats. Study was carried out on SD rats of both sexes. Animals were divided into four groups (n=05 per sex per group). STS was orally administered at the dose of 2, 4 and 10 mL/kg bw/day (i.e., approximately 1X, 2X and 5X of therapeutic equivalent dose, respectively) for 28 days. Control animals were administered with water as vehicle. Body weight and feed intake of all the animals were measured weekly throughout study duration. Detailed clinical observations were made periodically to detect signs of toxicity. After completion of 28-days, blood samples were collected for haematological and biochemical analysis

and animals were sacrificed, organs were harvested for weight determination and histopathological evaluation.

No treatment related adverse effect was observed on survival, neuro behaviour, body weight, feed intake of both male and female rats after oral administration of STS for 28 days. No significant alterations were reported in terms of haematological and biochemical parameters in both male and female rats at any tested dose levels as compared to control animals. However, a significant ($p<0.05$) decrease in AST level was noted in females at STS-4 and STS-10 mL/kg bw as compared to respective control group. No significant findings were noted during necropsy and relative organ weights of treated groups were comparable to vehicle control. Changes of histological significance were observed only in the lungs and livers of control and STS 10 ml/kg group. In lungs, as histological changes observed in the STS 10 ml/kg animals were also observed in the control group, they are not considered significant. With respect to liver, while one animal (10%) in the control group had normal liver, 3 animals (30%) in STS-10 mL/kg group had normal livers. 4 (40%) animals of control group showed a low score of 1, 10% animals in the STS group showed score 1. 40% animals of the control group and 60% animals of the STS group had score 2. Hence, although the experimental STS group showed a marginally higher number of animals with a higher score of 2, however, the control group also showed almost similar high scores, thus indicating that the histological changes may not be attributed to the STS administration.

The study findings did not indicate any toxicological significant observation in terms of body weight, feed intake, behavioural pattern, haematology, biochemistry, relative organ weight in STS treated group at all tested dose levels as compared to control animals. Both, vehicle control and STS 10 ml/kg groups showed normal histology of all vital organs except some histological alterations in lungs and liver and these changes were not related STS 10 ml/kg treatment. Therefore, STS may be considered safe based on above observations.

Publications (Oral Presentation)

Urooj M, Husain GM, Munshi YI, Ahmed NZ. Preclinical Safety Evaluation of *Sharbat-e-Toot* Siyah: A *Morus nigra* L. based Unani formulation. 44th Annual Conference of Society of Toxicology (STOX) India, 14-10-2025, PGIMER, Chandigarh.

2023-2024

39 Preclinical Safety Evaluation of *Ma'jūn Muṣaffī-i-ʿAẓam*

39.1 Acute Oral Toxicity Study of *Ma'jūn Muṣaffī-i-ʿAẓam* in Rats

Ma'jūn Muṣaffī-i-ʿAẓam (MMA) is polyherbal compound Unani Pharmacopoeial formulation having an action as *Muṣaffī-i-Dam* (Blood Purifier), *Mukhrij-i-Mawād-i-Fasida* (Expels morbid matter). It is indicated for the treatment of *Buthūr Labaniyya* (Acne, Pimples), *Dummal* (Furuncles, Boil), *Ātshak* (Syphilis) and *Waja'al-Mafāṣil* (Arthralgia). The aim of this study was to assess the acute oral toxicity potential of *Ma'jūn Muṣaffī-i-ʿAẓam* in female Sprague Dawley rats. A limit test at the dose of 5000 mg/kg bw was conducted as per the OECD test guideline number 425. After receiving a single dosage of MMA, the animals were monitored for 14 days for signs and symptoms of toxicity and mortality. No lethality was observed following treatment with MMA in three consecutive animals tested. Body weight and feed intake of animals were regularly monitored throughout the study. On 15th day, all three animals were euthanised, and necropsy was carried out. There were no gross pathological abnormalities related to the treatment. Under the given conditions, no mortality was observed at the dose of 5000 mg/kg bw of MMA. Therefore, oral LD_{50} of MMA in the female SD rat is estimated to be greater than 5,000 mg/kg body weight.

39.2 Sub-acute (Repeated Dose 28-day) Oral Toxicity Study of *Ma'jūn Muṣaffī-i-ʿAẓam* in Rats

Toxicity potential of repeated administration of MMA was studied in rats following 28-days repeated oral administration in Sprague Dawley (SD) rats of both sexes. Animals were divided into four groups (n=05 per sex per group). MMA was orally administered at the dose levels of 1200, 1800 and 2400 mg/kg bw/day (i.e., approximately 1X, 1.5X and 2X of therapeutic equivalent

dose, respectively) for 28 days. Control animals were administered with drinking water as vehicle. Body weight and feed intake for all animals were regularly measured throughout the study. Detailed clinical observations were made periodically to detect any signs of toxicity. After completion of respective dosing period, motor co-ordination of all the animals was tested using Rotarod apparatus. Blood samples were collected for haematological and biochemical investigations and animals were euthanized, vital organs were collected for organ weight determination and preserved for histopathological evaluation.

No mortality was observed following administration of MMA or vehicle for 28 days. No behavioural changes indicative of systemic toxicity were observed following MMA treatment. Body weight, water intake and feed intake of MMA treated rats were found almost comparable to their respective control groups of either sex. MMA treatment did not result in any overt toxicologically significant alteration in haematological and biochemical parameters in either male or female rats at any tested dose level as compared to vehicle control animals. No gross pathological changes/ lesions were observed during necropsy in any group. Relative organ weight of various treatment groups was found comparable to vehicle control. No significant alterations were observed in histology of organs like brain, thyroid, adrenals, heart, sternum, spleen, stomach, small intestine, pancreas, and gonads which retained normal histological architecture. Few histopathological changes observed in the MMA-2400 mg/kg treated group were comparable to those seen in the vehicle control group and are not considered to be treatment-related or of pathological significance.

The study findings did not indicate any toxicological significant alteration in terms of survival, behaviour pattern, body weight, feed intake, haematology, clinical chemistry, and relative organ weight and histopathology in any MMA treated group as compared to vehicle control. Based on the data generated, MMA would be considered safe at the therapeutically used dosage, and NOAEL may be considered as 2400 mg/kg bw in rats.

40 Preclinical Safety Evaluation of *Safūf-i-Makhānā*

40.1 Acute Oral Toxicity Study of Safūf-i-Makhānā in Rats

Safūf-i-Makhānā (SM) is a traditional polyherbal compound Unani Pharmacopoeial formulation having an action as *Muqawwī-i-Bāh* (Aphrodisiac), *Mughalliẓ-i-Manī* (semen inspissant/ increases viscosity of semen). It is indicated for the treatment of *Kathra al-Iḥtilām* (excessive nocturnal emission), *Jarayān* (semenorrhoea), *Sur'a al-Inzāl* (Premature ejaculation), *Sayalān al-Raḥim* (leucorrhoea). The aim of this study was to assess the acute oral toxicity potential of *Safūf-i-Makhānā* (SM) in female Sprague Dawley rats. As per the OECD test guideline number 425, a limit test at a dosage of 2000 mg/kg bw was conducted. After receiving a single dosage of SM, the animals were monitored for 14 days for signs and symptoms of toxicity and mortality. No lethality was observed following treatment with SM in five consecutive animals tested. Body weight and feed intake of animals were regularly monitored throughout the study. On Day-15, all five rats were sacrificed, and a necropsy was carried out. There were no gross pathological abnormalities related to the treatment. Under the given conditions, no mortality was observed at the dose of 2000 mg/kg bw of SM. Therefore, oral LD_{50} of SM in the female SD rat is estimated to be greater than 2,000 mg/kg body weight.

40.2 Sub-acute (Repeated Dose 28-day) Oral Toxicity Study of Safūf-i-Makhānā in Rats

Toxicity potential of repeated administration of SM was studied in rats following 28-days repeated oral administration in Sprague Dawley (SD) rats of both sexes. Animals were divided into four groups (n=05 per sex per group). SM was orally administered as aqueous suspension at the dose levels of 600, 1200 and 1800 mg/kg bw/day (i.e., approximately 1X, 2X and 3X of therapeutic equivalent dose, respectively) for 28 days. Control animals were administered with animal drinking water. Body weight and feed intake for all animals were regularly measured throughout the study. Detailed clinical observations were

made periodically to detect any signs of toxicity. After completion of respective dosing period, motor co-ordination of all the animals was tested using Rotarod apparatus. Blood samples were collected for haematological and biochemical investigations and animals were euthanized; vital organs were collected for organ weight determination and histopathological evaluation.

No mortality was observed following administration of SM or vehicle for 28 days. No neuro-behavioural changes indicative of systemic toxicity were observed following SM treatment. Body weight, water intake and feed intake of SM treated rats were found almost comparable to the respective control groups of either sex. SM treatment did not result in any overt toxicologically significant alteration in haematological parameters. Haemoglobin was significantly ($p<0.01$) increased in male rats treated with SM-1800 mg/kg bw compared to vehicle control group. MCHC was significantly ($p<0.001$) increased in male rats treated with SM at all three tested dose levels, compared to vehicle control group. No overt changes were noted in biochemical parameters in SM-treated group compared to vehicle control animals. Serum creatinine level was significantly ($p<0.05$) decreased in female rats treated with SM at tested dose levels 1200 mg/kg bw & 1800 mg/kg bw, compared to vehicle control group. Uric Acid was significantly ($p<0.05$) decreased in female rats treated with SM at tested dose level 600 mg/kg bw, compared to vehicle control group. No gross pathological changes/ lesions were observed during necropsy in any group. Relative organ weight of various treatment groups was found comparable to vehicle control. Histological analysis revealed that organs/tissues maintained normal structural integrity in both the control and SM-1800 mg/kg treated groups. Some pathological changes were observed in the liver, lungs, and kidneys across both the groups. The histopathological changes observed in the SM-treated group closely resembled those in the vehicle control group and are not indicative of treatment-related effects or pathological significance.

The study findings did not indicate any toxicological significant alteration in terms of survival, behaviour pattern, body weight, feed intake, haematology, clinical chemistry, relative organ weight, and histopathology in any SM treated group as compared to vehicle control. Based on the data generated, SM would be considered safe up to the highest tested dose level of 1800 mg/kg bw and NOAEL may be considered as 1800 mg/kg bw in rats.

41 Preclinical Safety Evaluation of *Mufarriḥ Shaikh al-Ra'īs*

41.1 Acute Oral Toxicity Study of Mufarriḥ Shaikh al-Ra'īs in Rats

Mufarriḥ Shaikh al-Ra'īs (MSUR) is a traditional herbo-mineral compound Unani Pharmacopoeial formulation having an action as *Muqawwī-i-Qalb* (Cardiotonic). It is indicated for the treatment of *Ḍu'f al- Qalb* (Weakness of the heart) and *Khafaqān* (Palpitation). This study was designed to evaluate the acute oral toxicity potential of *Mufarriḥ Shaikh al-Ra'īs* in female Sprague Dawley rats. Considering the low acute toxicity potential of tested drug, a limit test at the dose of 2000 mg/kg bw was carried out as per OECD test guideline number 425. Animals were administered with a single dose of MSUR and observed for signs & symptoms of toxicity and lethality for 14 days post-treatment. Five consecutive animals were tested and no lethality was observed following treatment with MSUR. Body weight and feed intake of animals were regularly monitored throughout the study. All the five animals were sacrificed on 15th day and necropsy was performed. No treatment related gross pathological abnormalities were observed. Under the experimental condition as no mortality was observed at the dose of 2000 mg/kg bw of MSUR, the oral LD_{50} of the MSUR in the female SD rat is estimated to be more than 2,000 mg/kg body weight.

41.2 Sub-acute (Repeated Dose 28-day) Oral Toxicity Study of Mufarriḥ Shaikh al-Ra'īs in Rats

The toxicity potential of MSUR was evaluated in Sprague Dawley (SD) rats by administering repeated oral dose for 28-days in both sexes. Animals were randomly divided into four groups of 5 animals each per sex per group (n=05 per group). MSUR was administered orally by gavage to the treatment groups at the dose levels of 500, 1000 and 2000 mg/kg bw/day (i.e., approximately 1X, 2X and 4X of therapeutic equivalent dose, respectively along with control animals which received water *via* oral gavage for 28 days. Body weight and feed intake of all animals was recorded on a regular basis throughout the study.

Thorough clinical observations were made throughout the study to detect signs of toxicity. After completion of respective dosing period, blood samples were collected for haematological and biochemical investigations and animals were euthanized; vital organs were collected for organ weight determination and histopathological evaluation.

No mortality was observed following administration of MSUR or vehicle for 28 days. No behavioural changes indicative of systemic toxicity was observed following MSUR treatment. Body weight and feed intake of MSUR treated rats were found almost comparable to their respective control groups of either sex. Treatment with MSUR had not resulted in any significant toxicological alteration in haematological or biochemical parameters in either male or female rats at any tested dose level as compared to vehicle control animals except for few isolated changes. Total bilirubin was significantly ($p<0.001$) decreased in female rats treated with MSUR at all three tested dose levels compared to vehicle control group. Total cholesterol was significantly ($p<0.05$) decreased in male rats treated with MSUR at tested dose levels 500 mg/kg bw and 2000 mg/kg bw, compared to vehicle control group. No gross pathological changes/ lesions were observed during necropsy in any group. Relative organ weight of various treatment groups was found comparable to vehicle control. However, relative organ weight of liver of male rats treated with MSUR-1000 mg/kg bw showed significant ($p<0.001$) increase compared to vehicle control group. Histological analysis revealed that most of organs/tissues like brain, liver. lungs, kidneys, thyroid, heart, spleen, stomach, pancreas, small intestine, adrenals, sternum, gonads, maintained normal structural integrity in both the control and MSUR-2000 mg/kg treated groups. Few pathological changes were observed in the liver and lungs across both the groups with much lesser frequency in the MSUR-2000 mg/kg treated group compared with vehicle control group and are not indicative of treatment-related effects or pathological significance.

The study findings have not shown any toxicological significant alteration in terms of survival, behaviour pattern, body weight, feed intake, haematology, clinical chemistry, relative organ weight, and histology in any MSUR treated group as compared to vehicle control. Based on the findings of present study, MSUR would be considered safe up to the highest tested dose level and NOAEL may be considered as 2000 mg/kg bw in rats.

42 Preclinical Safety Evaluation of *Ḥabb-i-Gul-i-Āk.*

42.1 Acute Oral Toxicity Study of *Ḥabb-i-Gul-i-Āk* in Rats

Ḥabb-i-Gul-i-Āk (HGA) is a traditional polyherbal compound Unani Pharmacopoeial formulation having an action as *Musakkin* (calming), *Muḥallil* (Resolvent). It is indicated for the treatment of *Waja'al-Mafāṣil* (Arthralgia/ arthritis). This study was designed to evaluate the acute oral toxicity potential of *Ḥabb-i-Gul-i-Āk* in female Sprague Dawley rats. Considering the low acute toxicity potential of tested drug, a limit test at the dose of 2000 mg/kg bw was carried out as per OECD-425. Animals were administered with a single dose of HGA and observed for signs & symptoms of toxicity and lethality for 14 days post-treatment. Five consecutive animals were tested and no lethality was observed following treatment with HGA. Body weight and feed intake of animals were regularly monitored throughout the study. All the five animals were sacrificed on 15th day and necropsy was performed. No treatment related gross pathological abnormalities were observed. As no mortality was observed at the dose of 2000 mg/kg bw of HGA, the oral LD_{50} of HGA in the female SD rat is estimated to be more than 2000 mg/kg body weight.

42.2 Sub-chronic (Repeated Dose 90-day) Oral Toxicity Study of *Ḥabb-i-Gul-i-Āk* in Rats

Repeated dose toxicity potential of HGA was evaluated in Sprague Dawley (SD) rats by administering repeated oral doses for 90 days in both sexes as per OECD-408. Animals were randomly divided into four groups of 10 animals, each per sex per group (n=10 per group). HGA was administered orally by gavage to the treatment groups at the dose levels of 25, 125, and 250 mg/kg bw/day (i.e., approximately 1X, 5X, and 10X of therapeutic equivalent dose),

respectively, along with control animals which received water for 90 days. Body weight and feed intake were recorded regularly throughout the study. Thorough clinical observations were made throughout the study to detect signs of toxicity. After the respective dosing period was completed, blood samples were collected for haematological and biochemical investigations, animals were euthanized, vital organs were collected for organ weight determination and histopathological evaluation.

No mortality was observed following the administration of HGA or vehicle for 90 days. No behavioural changes indicative of systemic toxicity were observed following HGA treatment. The body weight and feed intake of HGA-treated rats were almost comparable to their respective control groups of either sex. Treatment with HGA had not resulted in any significant toxicological alteration in haematological or biochemical parameters in either male or female rats at any tested dose level as compared to vehicle control animals except for few changes such as significant ($p<0.05$) increase in HDL (mg/dL) in female rats treated with HGA-25 mg/kg bw compared to vehicle control group. No gross pathological changes/lesions were observed during necropsy in any group. The relative organ weight of various treatment groups was found to be comparable to vehicle control. No significant alterations were observed in histology of organs like brain, thyroid, adrenals, heart, sternum, spleen, stomach, small intestine, pancreas, and gonads which retained normal histological architecture. The histopathological changes observed in the liver, lung, and kidney of the HGA-treated group were similar in nature and distribution to those seen in the vehicle control group. Therefore, these findings are not considered to be treatment-related or of pathological significance.

The study findings have not shown any significant toxicological alteration in terms of survival, behaviour pattern, body weight, feed intake, haematology, clinical chemistry, relative organ weight, histopathology in any HGA-treated group as compared to vehicle control suggesting HGA is safe up to the highest tested dose of 250 mg/kg bw and NOAEL of HGA may be considered as 250 mg/kg in rats.

IMR Projects

43 Preclinical Safety Evaluation of Coded Unani Formulation *UNIM-004*.

Title of IMR Project: Effect of Unani formulation(s) used for treatment of Bars (Vitiligo) on melanocytes - an *in-vitro* and *in-vivo* study.

PI: Dr. Alokananda Chakraborty, R.O. (Physiology, S-IV)/ Dr. Towseef Amin Rafeeqi, R.O. (Biochemistry)

Co-I: Dr. Gulam Mohammed Husain, R.O. (Pharmacology)

43.1 *Acute Oral Toxicity Study of Coded Unani Formulation UNIM-004 in Rats*

This study was conducted to evaluate acute oral toxicity potential of *UNIM-004* in Wistar rats. Considering the low acute toxicity potential, the limit test as per OECD-425 was conducted at the dose of 2000 mg/kg body weight. Animals were weighed, observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with *UNIM-004* in three consecutive animals, dosing to further animals was stopped. All the three animals were sacrificed on Day 15 and necropsy was performed. No treatment related mortality, morbidity or gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 2000 mg/kg bw of *UNIM-004*. Therefore, oral LD_{50} of the *UNIM-004* in the female Wistar rats was estimated to be greater than 2,000 mg/kg body weight.

43.2 *Sub-chronic (Repeated Dose 90-day) Oral Toxicity Study of Coded Unani Formulation UNIM-004 in Rats*

UNIM-004 is a coded Unani formulation of CCRUM being used for vitiligo. Though *UNIM-004* is used clinically in Unani system of medicine, there is no availability of scientific data to support the safety of this coded formulation on prolonged use as envisaged based on its indication in vitiligo. Hence, the present study is designed to investigate repeated dose oral toxicity study in rats.

Repeated dose 90-day oral toxicity study was conducted as per the OECD test guideline number 408 in Wistar rats. *UNIM-004* was orally administered at the limit dose of 1,000 mg/kg bw/day (i.e., about 5 times of therapeutically equivalent dose). Animals were regularly observed for clinical sign of toxicity, mortality, morbidity, body weight gain and feed consumption. After completion of treatment duration of 90-day, rats were fasted overnight (water provided *ad libitum*) and blood samples were collected from retro-orbital sinus under isoflurane anaesthesia (EZ Anaesthesia-1339) for haematological and biochemical analysis. Haemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), haematocrit (HCT) and platelet (PLT) were analysed using automated haematology analyser. Biochemical parameters such as glucose, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin, creatinine, blood urea nitrogen (BUN), total cholesterol (TC), triglycerides (TG), total protein (TP) and albumin were analysed using automatic analyser (Erba-EM200). Serum electrolytes such as sodium, potassium, and chloride were also estimated using automatic electrolyte analyser. Animals were immediately euthanised under carbon dioxide, gross necropsy was performed, organs were collected and relative organ weight was calculated. Vital organs were subjected to histological examination.

Treatment with *UNIM-004* showed no significant differences in body weight gain, feed consumption, haematology and biochemistry profile except minor changes in AST and slight increase in WBC in males which are toxicologically not significant as the values remained in the normal physiological range. No changes were observed in the gross necropsy though there is an increase in relative organ weight of spleen and adrenal glands in females and decrease in relative organ weight of kidney and testes in males treated with *UNIM-004* compared to vehicle control. No toxicologically significant changes were observed in *UNIM-004* treated rats in any organ during histological examination.

There was no treatment related change in survival, body weight and feed consumption following *UNIM-004* treatment in rats. No toxicologically relevant alterations were found in the clinical signs of toxicity, haematology and biochemistry profile of *UNIM-004* treated animals compared with control. No treatment related changes were observed in vital organs during histological investigation. Based on present findings, *UNIM-004* may be considered safe at the dose level of 1,000 mg/kg bw/day in Wistar rats.

Publications

1. Ghali SK, Chakraborty A, Pallapolu P, Rafeeqi TA, Husain GM, Javed G, Waheed MA, Kazmi MH. Expression of MITF Gene in Melanogenesis with *Psoralea corylifolia, Zingiber officinale, Terminalia chebula, Punica granatum* and *Eclipta alba* Based on Poly-Herbal Formulation. Journal of Biologically Active Products from Nature. 2021;11(2):82-96. https://doi.org/10.1080/22311866.2021.1908164

2. Ghali SK, Rafeeqi TA, Husain GM, Javed G, Waheed MA, Kazmi MH, Chakraborty A. The effect of Polyherbal Unani formulation on melanogenesis mechanism in the treatment of hypopigmentation disorder. Phytomedicine Plus. 2022;2(4):100333. https://doi.org/10.1016/j.phyplu.2022.100333

44 Preclinical Safety and Efficacy Evaluation of Coded Unani Formulation *UNIM-301*.

Project Title: Evaluation of Anti-inflammatory Activity of Unani Formulation(s) – An *in-vitro* and *in-vivo* Study.

PI: Dr. Alokananda Chakraborty, R.O. (Physiology, S-IV)/ Dr. Towseef Amin Rafeeqi, R.O. (Biochemistry)

Co-I: Dr. Gulam Mohammed Husain, R.O. (Pharmacology)

44.1 Acute Oral Toxicity Study of Coded Unani Formulation UNIM-301 in Rats

This study was conducted to evaluate acute oral toxicity potential of *UNIM-301* (a coded Unani formulation of CCRUM) in Sprague Dawley rats. Considering the low acute toxicity potential, the limit test as per OECD-425 was conducted at the dose of 2000 mg/kg body weight. Animals were weighed, observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with *UNIM-301* in three consecutive animals, dosing to further animals was stopped. All the three animals were sacrificed on Day 15 and necropsy was performed. No treatment related gross pathological abnormality was observed following visual observation. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 2000 mg/kg bw of UNIM-301. Therefore, oral LD_{50} of the *UNIM-301* in the female Sprague-Dawley rats was estimated to be greater than 2,000 mg/kg body weight.

44.2 Sub-chronic (Repeated Dose 90-day) Oral Toxicity Study of Coded Unani Formulation UNIM-301 in Rats

UNIM-301 is a coded Unani formulation of CCRUM indicated for pathological conditions involving inflammation. *UNIM-301* is used clinically in Unani system of medicine. No scientific data is available to support the safety of this coded formulation on prolonged use as envisaged based on its indications such as arthritis. Hence, the present study was performed to investigate repeated dose toxicity potential of *UNIM-301* in rats.

Repeated dose 90-day oral toxicity study was performed as per the OECD test guideline 408 in SD rats. *UNIM-301* was orally administered at the limit dose of 2,000 mg/kg bw/day (i.e., approximately 10 times of Therapeutically Equivalent Dose). Animals were regularly observed for clinical sign of toxicity, mortality, morbidity, body weight gain and feed consumption. At the end of study, haematology, clinical biochemistry, electrolytes, gross pathology, relative organ weight and histological examination were conducted.

Treatment with *UNIM-301* showed no significant differences in body weight gain, feed consumption, haematology and biochemistry profile except certain minor changes in ALP, ALT, BUN and Cholesterol level (mostly observed only in one sex) which are clinically not significant as the values lies within the normal physiological range. ALT was significantly raised in male rats while ALP was significantly increased in both male and female rats treated with *UNIM-301*. No changes were observed in the gross necropsy and relative organ weight data of control and UNIM-301 treated rats. Histological investigation of liver revealed that only 5% animals in the control group showed micro-vacuolation in the liver, whereas 40% animals in *UNIM-301* treated group showed micro-vacuolation changes of varying grades which could be attributed to the administration of the test compound. All the other organs evaluated were found to be normal histologically.

There were no toxicologically relevant treatment related changes in body weight, feed intake, haematological, biochemical (except alteration in ALT and ALP), and gross pathological examination following oral administration with UNIM-301 at the dose of 2,000 mg/kg bw/day. Observed biochemical changes and histological evidence indicates that liver might be the target organ of toxicity following repeated administration of UNIM-301 at the dose of 2000 mg/kg bw/day (i.e., approximately 10 times of Therapeutically Equivalent Dose) in rats.

44.3 Evaluation of Anti-inflammatory Activity of a Coded Unani Formulation UNIM-301 in Rats

Anti-inflammatory activity of *UNIM-301* was evaluated using carrageenan induced paw oedema model. Protocol of the study was approved by the Institutional Animals Ethics Committee vide protocol no. CRIUM/IAEC/2015/01/P03. Animals were provided with standard feed pellets (SAFE diet, France) and water *ad libitum*, unless stated otherwise. Animals were acclimatized to the laboratory conditions for one week before using them for experiment.

Male and female Sprague Dawley rats were used for the study. The recommended therapeutic dose of UNIM-301 in Unani system of medicine is 2 gm/day. As per body surface area conversion, Therapeutic Equivalent Dose (TED) for 2 gm human dose is 200 mg/kg bw/day in rats. Therefore, present anti-inflammatory study was carried out at three dose levels i.e., 100 mg/kg bw (half of therapeutic equivalent dose), 200 mg/kg bw (i.e., therapeutic equivalent dose) and 400 mg/kg bw (two times of therapeutic equivalent dose).

Rats were fasted overnight and were administered with 5 mL of 0.3% Carboxymethyl cellulose (CMC) suspension by stomach tube (vehicle controls) or *UNIM-301* suspended in the same volume of 0.3% CMC to insure uniform hydration. One hour after oral administration of test formulation or vehicle, rats were challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the plantar region of the left hind paw. The paw was marked with permanent marker at the level of the lateral malleolus and immersed in plethysmograph cell filled with water up to this mark. The paw volume was measured using digital plethysmometer (Make: Labomed, India) immediately after injection, and again at 3h, 6h, and eventually 24 h after challenge.

The increase of paw volume at each time interval was calculated as percentage compared with the volume measured immediately after injection of the carrageenan for each animal and compared statistically using one way ANOVA. The difference of average values between treated animals and control groups were calculated for each time interval and percentage inhibition of mean paw volume was calculated.

Oral administration of *UNIM-301* at the dose of 100 mg/kg, 60 min before carrageenan, significantly ($p<0.01$) reduced the oedema only at 6h after carrageenan injection. At higher doses (200 and 400 mg/kg) *UNIM-301* was able to significantly reduce ($p<0.01$ or $p<0.001$) the oedema at 3, 6 and 24 h after the carrageenan injection and anti-inflammatory effect of *UNIM-301* was persisted even at 24 h after the carrageenan injection. The oedema was strongly inhibited by oral administration of indomethacin at 10 mg/kg bw ($p<0.001$ vs. vehicle control group) at 3, 6 and 24 h after the carrageenan injection. Percent inhibition of mean paw volume by indomethacin was always higher compared to any tested dose of UNIM-301.

Based on the observed findings, it may be concluded that *UNIM-301* possesses strong anti-inflammatory potential only at the doses of 200 and 400 mg/kg which validate its use in inflammatory conditions. Further work is required to be conducted to identify the mechanism of action and role of various active constituents responsible for the observed anti-inflammatory activity.

Publications

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2. Lahari K, Ghali SK, Rafeeqi TA, Husain GM, Waheed MA, Javed G, Kazmi MH, Chakraborty A. Effect of Anti-inflammatory Activity of Aqueous, Hydro-ethanol and Methanol extracts of two Unani formulations. Research Journal of Pharmacy and Technology. 2022; 15(4):1560-6. DOI: https://doi.org/10.52711/0974-360X.2022.00260

45 Evaluation of a Classical Unani Formulation for Putative Anticancer Activity.

PI: Dr. Gulam Mohammed Husain, Research Officer (Pharmacology)

Co-I: Dr. Tasleem Ahmad, Research Officer (Biochemistry)

Cancer is a major public health burden in both developed and developing countries. Several types of cancer are preventable and treatable, provided that cancer is diagnosed at an early stage. Worldwide, cancer incidence and cancer related deaths are rising. Plant derived compounds have been an important source of several clinically useful anti-cancer agents. These include camptothecin derivatives such as irinotecan, topotecan, podophyllotoxin derivatives such as etoposide and teniposide, and vinca alkaloids such as vinblastine, vincristine and, taxanes derivatives such as docetaxel, paclitaxel.

A compound polyherbal Unani formulation (PUF) mentioned in classical textbook *Kamil-Us-Sana* for the purpose of evacuation of *Sawdā'* in the chapter of *Sartān* (Cancer) was selected for screening of its anti-cancer potential. Aqueous, hydroethanolic (1:1) and methanolic extract of PUF was prepared. Qualitative tests were performed for the presence of phytoconstituents like alkaloids, total phenols, flavonoids, and glycosides in aqueous, hydro-ethanolic and methanolic extracts of polyherbal Unani formulation (PUF). Further, total phenolic content (TPC) and total flavonoid content (TFC) were quantitatively estimated. Antioxidant potential of PUF was determined using DPPH, ABTS and FRAP assay. All three extracts of the selected Unani formulation were used for the cytotoxicity experiments. MTT assay was performed to measure the cytotoxicity activity of PUF in A-549 (human alveolar basal epithelial cell adenocarcinoma), HeLa (human cervical cancer), and MCF-7 (Michigan Cancer Foundation-7; breast adenocarcinoma), and B16F10 (mouse melanoma) cell lines. Further, individual herbal ingredients of the PUF were also tested for antioxidant potential and cytotoxicity activity in MCF-7 cell lines.

The results showed that PUF had significant amount of total phenolic contents as well as flavonoid contents. PUF as well as its individual ingredients showed remarkable anti-oxidant activity in DPPH, ABTS and FRAP assay. Methanolic extract of PUF was found to be most potent in term of cytotoxicity with IC_{50} value of 25.84 µg/mL in B16F10 cells which is almost equal to positive control doxorubicin (IC_{50} 24.63 µg/mL). PUF also increased the caspase-3 activity in HeLa and A-549 cells suggesting induction of apoptosis in these cancer cells. Observed cytotoxic activity of PUF may be attributed to the cytotoxicity potential of its individual ingredients in MCF-7 cells as evident from present study as well as abundant reports of cytotoxicity of these ingredients in various cancer cell lines. Findings of the present investigation validate the traditional claim mentioned in Unani text and open new avenue for further research to explore molecular mechanism and development this Unani formulation for effective management for cancer.

Publications

Husain GM, Raja SS, Vinay E, Urooj M, Dwivedi DK, Anjum N, Munshi YI, Ahmad T. Exploring Anticancer Potential of Aqueous, Hydroethanolic and Methanolic Extract of an Age-old Unani Formulation. Journal of Natural Remedies. 2025;25(3):551-565. DOI: https://doi.org/10.18311/jnr/2025/44283

46 Evaluation of Anti-pyretic, Analgesic, Anti-inflammatory, Anti-thrombocytopenic and Immunomodulatory Activity of a Polyherbal Unani Formulation for Dengue.

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An Intra Mural Research project was conducted at NRIUMSD Hyderabad with the objective of evaluation of anti-pyretic, analgesic, anti-inflammatory, hepatoprotective, anti-thrombocytopenic and immunomodulatory potential of a coded polyherbal Unani formulation for dengue (PUFD) in animal models. 50% Hydroethanolic extract of PUFD, supplied by DSRU of NRIUMSD Hyderabad was used in the present study. Preliminary phytochemical screening (Qualitative analysis) of PUFD was performed for the presence of different phytochemical constituents. Further, quantitative analysis was performed for total flavonoids using aluminium chloride colorimetric assay and total phenols were analysed by Folin-Ciocalteu method. HPTLC fingerprint of PUFD was also developed. Antioxidant assay of PUFD was performed using DPPH and ABTS free radical scavenging and FRAP assay. Based on the data of dose range finding study on PUFD, it was decided to conduct efficacy studies at three dose levels i.e., PUFD-250, 500 and 1000 mg/kg, p.o., in rats and PUFD-500, 1,000 and 2,000 mg/kg, p.o., in mice. Acute and repeated dose 28-day oral toxicity study was also conducted in rats to ascertain the safety of PUFD on repeated administration.

Qualitative analysis confirmed the presence of alkaloids, phenols, flavonoids, and glycosides in PUFD. Quantitative analysis revealed that PUFD contains 87.20±0.74 µg of GAE/mg of total phenolic content and 50.66±6.83 µg of QAE/mg of total flavonoid content. HPTLC chemical fingerprint of PUFD was

developed and it will be used as a quality benchmarks for PUFD. In in vitro anti-oxidant assays, IC_{50} of PUFD was found to be 123.16±6.47 µg/mL and 115.79±0.19 µg/mL in DPPH and ABTS assay, respectively. PUFD has capacity to reduce 1.19±0.03 mM FeSO4/mg of extract in FRAP assay.

Acute and repeated dose toxicity potential of PUFD was assessed in rats to ascertain its safety. No mortality was observed in acute oral toxicity test conducted at 2000 mg/kg bw and LD50 may be considered more than 2000 mg/kg bw in rats. In repeated dose 28-day oral toxicity study conducted in rats, no toxicologically significant effect was observed with respect to body weight, feed intake, behavioural parameters, haematology, biochemical parameters, gross pathology, relative organ weight and histopathology in any treatment group (PUFD-250, 500 or 2000 mg/kg bw) compared to vehicle control group. Considering lack of any treatment related toxic changes, the NOAEL may be considered as 2,000 mg/kg bw.

Antipyretic activity of PUFD was evaluated in brewer's yeast induced pyrexia in rats using paracetamol as positive control. PUFD treatment at doses of 500 and 1000 mg/kg decreased the rectal temperature in dose and time-dependent manner. PUFD-1000 mg/kg showed the best anti-pyretic activity which was observed 30 min (after oral administration) onwards and continued till 4-hr. Paracetamol showed more pronounced effect than any dose of PUFD which was observed 30 min onwards and maintained till 4-hr. Analgesic effect of PUFD was evaluated using formalin induced pain model in rats with indomethacin as positive control. Time spent in licking/flicking the formalin injected paw by each rat was observed during early phase (0-10 min, post injection) and late phase (15-45 min, post injection). Results indicate that indomethacin and PUFD have inhibitory effect only in the late inflammatory pain phase indicating peripheral analgesic effect while none of the treatment attenuated early phase, suggesting lack of central analgesic activity in this model. Further, none of the tested dose of PUFD showed any significant analgesic effect in Randall Sellitto Analgesiometer test conducted on same animals subjected to formalin induced pain, suggesting lack of any potent analgesic effect in this test.

Anti-inflammatory activity of PUFD was studied in a sub-acute inflammation model i.e., cotton wool granuloma in rats. Indomethacin (positive control) has moderate effect (45.43 % inhibition) while PUFD-500 and PUFD-1000 have

demonstrated mild anti-transudative effect (13.71 and 18.27 % inhibition, respectively) in this sub-acute inflammation setup. PUFD was also tested in mice model of acetic acid induced vascular permeability test which is a pathological condition involving acute phase inflammation and vascular damage. This method correlates to effect of PUFD on blood vascular permeability which is common pathology in dengue leading to fluid leakage and vascular complications. Mice treated with PUFD mid (1000 mg/kg) and high dose (2000 mg/kg) significantly and dose dependently decreased acute inflammation and vascular permeability challenged by acetic acid. Indomethacin used as standard in this model has profound inhibitory effect on vascular leak and inflammation.

Hepatoprotective activity of PUFD was performed using paracetamol induced hepatotoxicity in rats. PUFD-500 and PUFD-1000 treated animals showed significant decrease in ALT and ALP levels compared to toxic control group. All the three dose levels of PUFD treated rats showed significant decrease in AST levels. Significant reversal of relative liver weight in PUFD-500 and PUFD-1000 treated animals was detected compared to toxic control. Histopathological findings also corroborate the protective effect of PUFD in paracetamol induced hepatotoxicity which may be beneficial in cases of dengue.

Anti-thrombocytopenic activity of PUFD was performed using Busulfan induced thrombocytopenia in rats. Rats were treated with PUFD 250, 500 and 1000 mg/kg, p.o., continuously for 20 days. Thrombocytopenia was induced by subcutaneous injection of busulfan at the dose of 5 mg/kg on day 1, 5, 10, and day-15. Platelet count was measured before and after treatment. Rats were scarified, liver and spleen were removed, weighed and subjected to histopathological examination. Platelet count ($x10^3$/µl) was significantly reduced in toxic control group (51.83±12.93) when compared to normal control group (1008.00±15.70). Treatment with PUFD-500 (101.20±4.91) and PUFD-1000 mg/kg (169.30±9.91) significantly increased platelet count when compared to toxic control group though the values remained considerably low compared to normal range. PUFD-250 did not resulted statistically significant increase in platelet count (64.42±2.53). PUFD-1000 mg/kg showed best protection against busulfan induced injury with least degenerative changes in spleen and liver histology. Overall, PUFD moderately attenuated thrombocytopenia which may be a positive effect in dengue.

Immunomodulatory potential of PUFD was evaluated in Swiss Albino mice at the dose of 2000 mg/kg p.o. Immunomodulatory activity was assessed by two methods i.e., Haemagglutination Titre Assay (HIA) and Delayed Type Hypersensitivity (DTH) method. In both methods cyclophosphamide was used for immune suppression and Sheep Red Blood Cells (SRBC) were used as antigen. PUFD treated group had higher antibody titre than levamisol (used as control) treated group in HA titre assay whereas DTH assay results demonstrate that PUFD group has lesser effect on mean percentage inflammation compared to levamisol group. This indicates PUFD has prominent effect on humoral immunity while moderate effect on delayed type hypersensitivity.

Based on the data of present study, PUFD may be considered safe without any overt toxicological consequences. Observed anti-pyretic, anti-inflammatory, hepatoprotective, moderate anti-thrombocytopenic activity and humoral immunity boosting potential advocate further testing of this formulation as adjuvant therapy to available treatment options in mild to moderate cases of dengue fever or similar pathological conditions. Further, anti-dengue viral inhibition potential of this valuable formulation using *in vitro* and *in vivo* approaches is worth testing considering reported DENV inhibition potential of its ingredients.

Publications (Conference Presentations)

1. Vinay E, Urooj M, Anjum N, Minhajuddin A, Husain GM. Antipyretic and anti-inflammatory activity of Polyherbal Unani Formulation proposed for the management of Dengue fever. 10th International Conference of Laboratory Animal Scientists' Association (LASA), India, on "Animal Models for One Health Programme: Challenges and Future Perspectives" jointly organized by NIAB, NARFBR and LASA India in Hyderabad during 3rd & 4th June 2022.

2. Dwivedi DK, Vinay E, Urooj M, Anjum N, Minhajuddin A, Husain GM. Hepatoprotective, anti-thrombocytopenic and immunomodulatory activity of Polyherbal Unani Formulation proposed for the management of Dengue fever. Conference on "Innovative approaches in Laboratory Animal Research" Organized by ICMR-National Animal Resource Facility for Biomedical Research, Telangana, 24th April 2023.

Pharmacology and Toxicology Laboratory

क्षेत्रीय यूनानी चिकित्सा अनुसंधान संस्थान, श्रीनगर

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BACKGROUND

Pre-clinical studies are conducting since last twenty years at RRIUM, Srinagar with good experience and expertise to design and conduct the toxicity studies and efficacy studies with various routes of administration in accordance with international guidelines such as OECD, Schedule Y and Ayush guidelines.

The preclinical facility is having Clinical Pathology and Histopathology laboratory. The Clinical pathology carries out haematology, clinical chemistry, coagulation and urine analysis which are mandatory in general toxicity evaluation. The histopathology laboratory deals with the necropsy, tissue weighing, grossing, tissue processing, embedding or impregnation and section cutting and in the end pathologist evaluation of microscopic section.

Animal Holding Facility

GLP standard animal holding facility is equipped for small animal experimentation with separate arrangements for quarantine, experimentation rooms, dosing room and necropsy room. Animal experimentation is under the control of the Institutional Animal Ethics Committee (IAEC) of RRIUM, Srinagar which is registered with CCSEA, Govt. of India with registration No. 927/GO/Re/S/2006/CPCSEA, dated 14/03/2016.

The laboratory is also associated with PG programme of the institute where the MD (Ilmul Advia) students carry out the research project on preclinical studies.

Prior to 2013

1 Preclinical Safety Evaluation of *Capsule Shaqīqa*

1.1 Acute oral toxicity study of Capsule Shaqīqa in Wistar rats

Acute oral toxicity study of *Capsule Shaqīqa* was studied at the dose level of 2000 mg/kg of body weight in both male and female Wistar rats. In both groups of males and females a single oral dose of the drug was given. Animals were weighed initially and at weekly intervals, water intake and feed intake were monitored on daily basis. Animals were observed carefully for any behavioural and neurological changes for 24 hours after the administration of the drug and sacrificed after 14 days of the drug administration. Blood was collected for haematological and biochemical parameter analysis. Animals were dissected and gross examination of the tissues and organs was carried out. In male rats the feed intake values are more or less unaffected by the drug while the water intake level was increased significantly in comparison to the controls. Body weight gain of treated group of animals remained comparable to the controls and their blood biochemical and haematological parameters were also similar except the enzymatic activity of Alkaline phosphatise (ALP) was found to be elevated. Gross examination of the organs and tissues of male rats did not reveal any treatment-related differences in the treated and control groups. In treated female rats there was a slight decrease in the values of feed intake when compared to their respective controls, while a slight increase was detected in the water intake value. Body weight of animals was normal throughout the study period. SGPT was increased in the treated female rats and their serum triglycerides were also increased in comparison to the control female rats. Biochemical parameters and the haematological parameters were comparable among the treated and control female rats. Gross examination of tissues also did not reveal any significant differences among the treated and control female rats.

1.2 Sub-acute oral toxicity study of Capsule Shaqīqa in Wistar rats

Sub-acute oral toxicity study of *Capsule Shaqīqa* was studied at the dose level of 90 mg/kg of body weight in both male and female Wistar rats. In each group (N=7) of males and females daily oral dose of the drug was given for 28 days. The rats were observed carefully for any behavioural and neurological changes for 24 hours after the administration of the drug. The rats were sacrificed after 28 days of the drug administration. Blood was collected for haematological and biochemical parameter analysis. The rats were kept on fasting for overnight prior to sacrifice. The rats were dissected and organs were observed for any morphological changes. Gross examination of the tissues and organs was carried out. The physiological parameters such as change in body weight, water intake and feed intake values were also monitored. The rats were weighed weekly with monitoring of water and feed intake values. There was a slight decrease in average feed consumption per day in the drug treated male rats as compared with control and a slight increase in average water consumption when compared with control male rats. Gross examination of the tissues revealed the normal appearance of the tissues. The biochemical parameters are also similar to the normal male rats except an increase in triglyceride values in the drug treated rats. The haematological parameters remained unaffected when compared to normal male rats. There was also a gradual increase in body weight in drug treated female rats. The values of biochemical parameters are closely related to control female rats except an increase in triglyceride level in the drug administered females. The haematological parameters are within the normal range as compared with control. On gross examination, tissues were found to be in normal architecture.

1.3 Sub-chronic oral toxicity study of Capsule Shaqīqa in Wistar rats

A sub-chronic oral toxicity study of *Capsule Shaqīqa* was conducted at a dose level of 90 mg/kg body weight in both male and female Wistar rats. Each group consisted of five animals per sex per group (N = 5). The test drug was administered orally once daily for 90 consecutive days.

The animals were observed for behavioural and neurological changes immediately after dosing and for 24 hours thereafter. After completion of the treatment period, the rats were fasted overnight and sacrificed on day 90. Blood samples were collected for haematological and biochemical analyses. A complete necropsy was performed and major organs were examined for gross morphological changes. Tissue and organ samples were collected for histopathological examination. Physiological parameters including body weight, feed consumption and water intake were monitored throughout the study. Body weights were recorded weekly and feed and water intake were assessed on a weekly basis.

In male rats, there was no significant effect on average daily feed consumption in the drug-treated group compared to normal controls. A slight increase in average water intake was observed. Body weight gain followed a normal growth pattern. Gross examination of tissues and organs revealed no abnormal findings. The biochemical and haematological parameters remained within the normal physiological range and were comparable to those of control male rats.

In female rats, average feed and water consumption in the drug-treated group was higher than that of normal controls. A gradual increase in body weight was observed throughout the treatment period. The biochemical parameters were comparable to those of normal female rats and haematological values remained within normal limits. Gross examination of tissues and organs revealed a normal appearance with no treatment-related changes. The study indicated that *Capsule Shaqīqa* did not produce any treatment-related toxic effects at the tested dose level in either male or female Wistar rats following repeated oral administration for 90 days.

Publications

Ghazanfar K, Ahmad Dar S, Akbar S, Nazir T, Hamdani M, Siddiqui KM, Kumar P, Masood A. Safety evaluation of Unani formulation: capsule Shaqeeqa in albino Wistar rats. Scientifica. 2016; 2016(1):2683403

2 Preclinical Safety Evaluation of *Capsule Dīdān*.

2.1 Acute oral toxicity study of Capsule Dīdān in Wistar rats

An acute oral toxicity study of *Capsule Dīdān* was conducted at a dose level of 2000 mg/kg body weight in both male and female Wistar rats. In each sex group, a single oral dose of the test drug was administered.

Animals were weighed prior to dosing and at weekly intervals thereafter. Feed and water intake were monitored on a daily basis throughout the study period. Animals were carefully observed for behavioural and neurological changes for 24 hours following drug administration. The animals were sacrificed on day 14 after dosing. At termination, blood samples were collected for haematological and biochemical analyses. A complete necropsy was performed and gross examination of major organs and tissues was carried out.

In treated male rats, feed intake was found to be increased, while water intake remained largely unaffected compared to controls. Body weight gain in treated males was comparable to that of the control group. Biochemical analysis showed an increase in liver enzyme activity, particularly SGPT (ALT) and ALP. Haematological parameters remained largely unaffected by the treatment. Gross examination of organs and tissues revealed no treatment-related abnormalities in most animals however, in one male rat, the liver and kidneys were unusually enlarged and darker in colour.

In treated female rats, average feed and water intake were comparable to control animals. Body weight gain followed a normal pattern during the study period. Biochemical parameters remained largely unaffected, except for an increase in ALP activity and elevated triglyceride levels in the treated group. Haematological parameters were also within normal limits. Gross examination of tissues and organs did not reveal any significant differences between treated and control female rats. The acute oral administration of *Capsule Dīdān* at 2000 mg/kg body weight was well tolerated in both male and female Wistar rats with no mortality and minimal treatment-related effects.

2.2 Sub-acute oral toxicity study of Capsule Dīdān in Wistar rats

A sub-acute oral toxicity study of *Capsule Dīdān* was conducted at a dose level of 90 mg/kg body weight in both male and female Wistar rats. Each treatment group consisted of seven animals per sex (N = 7). The test drug was administered orally once daily for 28 consecutive days.

The animals were observed for behavioural and neurological changes for 24 hours following drug administration. At the end of the treatment period, the rats were sacrificed on day 28. Blood samples were collected for haematological and biochemical analyses. A complete necropsy was performed and major organs were examined for gross morphological changes.

Physiological parameters including body weight, feed consumption and water intake were monitored throughout the study. Body weights were recorded weekly while feed and water intake were monitored on a daily basis. In male rats, a slight decrease in average daily feed consumption was observed in the drug-treated group compared to normal controls along with a slight increase in average water intake. Body weight gain followed a normal growth pattern. Gross examination of tissues and organs revealed a normal appearance. Biochemical parameters were comparable to those of normal male rats except for an increase in fasting blood glucose levels in the treated group. Haematological parameters remained within normal physiological ranges and were unaffected by the treatment.

In female rats, average feed and water consumption in the drug-treated group was comparable to that of normal controls. A gradual and normal increase in body weight was observed throughout the study period. Biochemical parameters were similar to those of normal female rats except for an elevation in fasting blood glucose levels in treated animals. Haematological parameters remained within normal limits. Gross examination of tissues and organs revealed no treatment-related abnormalities. The repeated oral administration of *Capsule Dīdān* at 90 mg/kg body weight for 28 days did not produce any significant treatment-related toxic effects in male or female Wistar rats.

2.3 *Sub-chronic oral toxicity study of Capsule Dīdān in Wistar rats*

A sub-chronic oral toxicity study of *Capsule Dīdān* was conducted at a dose level of 90 mg/kg body weight in both male and female Wistar rats. Each treatment group consisted of five animals per sex (N = 5). The test drug was administered orally once daily for 90 consecutive days.

The animals were carefully observed for behavioural and neurological changes for 24 hours following drug administration. At the end of the treatment period, the rats were fasted overnight and sacrificed on day 90. Blood samples were collected for haematological and biochemical analyses. Necropsy was performed and major organs were examined for gross morphological changes. Tissue and organ samples were collected for histopathological examination. Physiological parameters including body weight, feed consumption and water intake were monitored throughout the study. Body weights were recorded weekly and feed and water intake were assessed on a weekly basis.

In male rats, body weight gain followed a normal growth pattern throughout the study period. Average feed and water consumption remained unaffected by the drug. Gross examination of tissues and organs revealed a normal appearance with respect to shape, size and texture. Biochemical and haematological parameters in treated males were comparable to those of control animals. In female rats, body weight gain was also normal. Average feed and water consumption in drug-treated females was comparable to that of control females. The drug did not produce any significant changes in biochemical or haematological parameters in treated female rats. The repeated oral administration of *Capsule Dīdān* at 90 mg/kg body weight for 90 days did not produce any treatment-related toxic effects in male or female Wistar rats

Publications

Dar SA, Ghazanfar K, Akbar S, Masood A, Nazir T, Siddiqui KM, Kumar P. Acute and Sub-acute oral toxicity studies of Deedan-A Unani drug in Albino rats. Journal of Applied Pharmaceutical Science. 2015 27; 5(4):107-14.

2013-2014

3 Preclinical Safety Evaluation of *Capsule Siras*

3.1 Acute oral toxicity study of Capsule Siras in Wistar rats

Acute oral toxicity study of *Capsule Siras* was studied at the dose level of 2000 mg/kg of body weight in both male and female Wistar rats. In both groups (N=7) of male and female a single oral dose of the drug was given. Animals were weighed initially and at weekly intervals, and water intake and feed intake were monitored on daily basis. Animals were observed carefully for any behavioural and neurological changes for 24 hours after the administration of the drug, and sacrificed after 14 days of the drug administration. Blood was collected for haematological and biochemical parameter analysis. Animals were dissected and gross examination of the tissues and organs was carried out. In male rats the feed intake values are more or less unaffected by the drug while the water intake level was increased slightly in comparison to the controls. Body weight gain of treated group of animals remained comparable to the controls and their blood biochemical and haematological parameters were also similar. Gross examination of the organs and tissues of male rats did not reveal any treatment-related differences in the treated and control groups. In treated female rats there was no change in the values of feed intake when compared to their respective controls, while a slight increase of negligible significance was found in the water intake value. Animals gained body weight normally during the study period. The blood biochemical parameters were normal when compared with the normal females except the increased activity of ALP was determined. The haematological parameters were also similarly comparable to control. Gross examination of tissues also did not reveal any significant differences among the treated and control female rats.

3.2 Sub-acute oral toxicity study of Capsule Siras in Wistar rats

Sub-acute oral toxicity study of *Capsule Siras* was studied at the dose level of 90 mg/kg, of body weight in both male and female Wistar rats. In each group (N=5) of males and females daily oral dose of the drug was given for 28 days. The rats were observed carefully for any behavioural and neurological changes for 24 hours after the administration of the drug and then regularly on daily basis. The rats were sacrificed after 28 days of the drug administration. Blood was collected for haematological and biochemical analysis. The rats were kept on fasting for overnight prior to sacrifice. The rats were dissected and organs were observed for any morphological changes. Gross examination of tissues and organs was carried out. The physiological parameters such as change in body weight, water intake, feed intake values were also monitored. The physiological parameters such as gain in body weight, feed and water consumption in both male and female treated groups was found to be unaffected by the drug administration. There was no significant change found in various biochemical and haematological parameters of both drug-treated male and female groups when compared with the control male and female animals respectively.

3.3 Sub-chronic oral toxicity study of Capsule Siras in Wistar rats

A sub-chronic oral toxicity study of *Capsule Siras* was conducted at a dose of 90 mg/kg body weight in male and female Wistar rats (N = 5 per sex). The test drug was administered orally once daily for 90 days. Animals were monitored for behavioural and neurological changes, body weight, feed consumption and water intake throughout the study. At the end of the treatment period, rats were fasted overnight, sacrificed and blood samples were collected for haematological and biochemical analyses. Gross examination and organ weight assessment revealed no treatment-related morphological changes, and tissues appeared normal. Male rats showed normal body weight gain with increased water intake but unchanged feed consumption, while female rats exhibited normal body weight gain, comparable water intake and slightly reduced feed consumption. Biochemical, haematological, and organ weight parameters remained within normal limits in both sexes, indicating no sub-chronic toxicity of Capsule Siras at the tested dose.

4 Preclinical Safety Evaluation of *Capsule Ḥābis*

4.1 Acute oral toxicity study of Capsule Ḥābis

An acute oral toxicity study of *Capsule Ḥābis* was conducted at a dose level of 2000 mg/kg body weight in both male and female Wistar rats (N = 5 per sex). A single oral dose of the test drug was administered and animals were monitored for behavioural and neurological changes for 24 hours post-dosing. Body weight, feed consumption and water intake were recorded at baseline and at weekly intervals. The rats were sacrificed on day 14 and blood samples were collected for haematological and biochemical analyses followed by gross examination of organs and tissues. In both male and female rats, body weight gain was normal, feed intake remained unchanged and only a slight increase in water consumption was observed. Biochemical and haematological parameters were comparable to controls and no treatment-related abnormalities were observed on gross examination indicating that *Capsule Ḥābis* was well tolerated at the tested dose.

4.2 Sub-acute oral toxicity study of Capsule Ḥābis

A sub-acute oral toxicity study of *Capsule Ḥābis* was conducted at a dose level of 150 mg/kg body weight in both male and female Wistar rats (N = 5 per sex). The test drug was administered orally once daily for 28 consecutive days. Animals were carefully observed for behavioural and neurological changes for 24 hours following each administration.

At the end of the treatment period, the rats were sacrificed on day 28, and blood samples were collected for haematological and biochemical analyses. A complete necropsy was performed and major organs were examined for gross morphological changes. Representative tissue samples were collected, processed, embedded in paraffin and preserved for histopathological examination. Physiological parameters including body weight, feed intake and water intake, were monitored throughout the study. Body weights were recorded weekly and feed and water consumption were assessed on a weekly basis.

The average feed and water consumption in both male and female treated rats remained within normal limits and animals exhibited normal growth and body weight gain. No mortality, behavioural abnormalities or signs of systemic toxicity were observed during the study period. Biochemical and haematological parameters in treated animals were comparable to those of control groups. Gross examination revealed no treatment-related abnormalities and tissues appeared normal in size, shape, and texture. The repeated oral administration of *Capsule Ḥābis* at 150 mg/kg body weight for 28 days did not produce any treatment-related toxic effects in male or female Wistar rats

4.3 Sub-chronic oral toxicity study of Capsule Ḥābis

A sub-chronic oral toxicity study of *Capsule Ḥābis* was conducted at a dose level of 150 mg/kg body weight in male and female Wistar rats. The animals were randomly divided into four groups with five rats per group (N = 5). Group I and Group II served as male and female control groups, respectively, and received the vehicle (water) orally. Group III and Group IV consisted of drug-treated male and female rats respectively and were administered *Capsule Ḥābis* orally at a dose of 150 mg/kg body weight once daily for 90 consecutive days.

All animals were observed carefully for behavioural and neurological changes for 24 hours after dosing and twice daily thereafter until the completion of the study. At the end of the treatment period, rats were fasted overnight and sacrificed on day 90. Blood samples were collected from the dorsal vena cava for haematological and biochemical analyses. A complete necropsy was performed and major organs were examined for gross morphological changes. Representative organ and tissue samples were collected for histopathological examination. Physiological parameters including body weight, feed consumption and water consumption were monitored throughout the study. Body weights were recorded weekly and feed and water intake were assessed on a weekly basis.

The mean body weight gain in drug-treated male and female rats was comparable to that of their respective control groups and animals exhibited normal growth throughout the study period. The average feed consumption in control and treated males (16.7 g and 17.2 g, respectively) and in control and treated females (17.2 g and 16.5 g, respectively) showed only minor variations. Similarly, average water consumption in treated male and female rats remained comparable to controls indicating that *Capsule Ḥābis* did not significantly affect feed or water intake.

Biochemical parameters including liver and kidney function tests (LFT and KFT) showed no significant differences between treated and control groups. Haematological parameters also remained within normal physiological limits, with no treatment-related adverse changes observed. Gross examination revealed that the organs and tissues of treated rats were normal in shape, size, texture, and weight, with no drug-related abnormalities, deformities or lesions. The oral administration of *Capsule Ḥābis* at a dose of 150 mg/kg body weight for 90 days did not produce any treatment-related adverse effects in male or female Wistar rats.

5 Preclinical safety evaluation of *Ḥabb-i-Shifā*

5.1 Acute oral toxicity study of *Ḥabb-i-Shifā* in Wistar rats

Acute oral toxicity study of *Ḥabb-i-Shifā* was studied at the dose level of 2000 mg/kg of body weight in both male and female Wistar rats. In both groups (N=5) of males and females a single oral dose of the drug was given. Animals were weighed initially and at weekly intervals and water intake and feed intake were also monitored on weekly basis. Animals were observed carefully for any behavioural and neurological changes for 24 hours after the administration of the drug and sacrificed after 14 days of the drug administration. Blood was collected for haematological and biochemical analysis. Rats were sacrificed and gross examination of the tissues and organs was carried out. In male rats the average feed water consumption was increased slightly in comparison to the controls. The treated males gained body weight normally and their blood biochemical and haematological parameters were also similar to the control males, however the activity of liver enzyme like ALP in male drug treated rats was found to be increased. Gross examination of the organs and tissues of male rats did not reveal any treatment-related differences in the treated and control groups. In treated female rats there was no change in the values of feed intake when compared to their respective controls while a slight increase of negligible significance was found in the water intake value. Rats gained body weight normally during the study period. The blood biochemical and haematological parameters were also similar comparable to the control ones except an increase in activity of ALP was found in treated females. Gross examination of tissues also did not reveal any significant differences among the treated and control female rats.

5.2 Sub-acute oral toxicity study of Ḥabb-i-Shifā in Wistar rats

A sub-acute oral toxicity study of *Ḥabb-i-Shifā* was conducted in Wistar rats at dose levels of 150 mg/kg body weight in females and 500 mg/kg body weight in males (N = 5 per sex). The test drug was administered orally once daily for 28 consecutive days. Animals were observed for behavioural and neurological changes for 24 hours following dosing. At the end of the treatment period, rats were sacrificed on day 28 and blood samples were collected for haematological and biochemical analyses. A complete necropsy was performed and major organs were examined for gross morphological changes with representative tissues processed for histopathological evaluation. Body weight, feed intake and water intake were monitored weekly. The treated rats exhibited normal growth and body weight gain with no mortality or treatment-related behavioural changes. Feed and water consumption remained within normal limits and biochemical and haematological parameters showed no significant deviations from control values. Gross examination revealed normal organ morphology indicating no sub-acute toxic effects of *Ḥabb-i-Shifā* at the tested dose levels.

5.3 Sub-chronic oral toxicity study of Ḥabb-i-Shifā in Wistar rats

Sub-chronic oral toxicity study of *Ḥabb-i-Shifā* was studied at the dose levels of 120 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 5 rats. The Group I and II being the male and female controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered with *Ḥabb-i-Shifā* at the dose of 120 mg/kg body weight for 90 days daily. The rats were observed carefully for any behavioural and neurological changes for next 24 hours after the administration of the drug and daily twice thereafter till the completion of experimentation. The rats were sacrificed after 90 days of the daily oral drug administration. The rats were kept on fasting for overnight prior to sacrifice. Blood was collected for haematological and biochemical parameter analysis. The rats were dissected and organs were observed for any morphological changes. Blood was collected from dorsal vena cava upon cutting the abdomen by scissor. Gross examination of the tissues and organs was carried out. Tissue and organ samples were collected for histological studies. The physiological parameters like body weight change, water consumption and feed consumption were also monitored. The rats

were weighed regularly after a week time and water consumption and feed consumption were also monitored on weekly basis. There was no effect of the drug on average feed consumption per day in the drug treated male and female rats as compared with the control. The rats gained weight in a normal fashion, the body weight gain of treated groups did not vary from those of respective controls. Gross examination of the tissues or organs revealed the normal appearance of the tissues. There were no deformity or lesions found in any organ or tissue. The biochemical parameters of treated males and females were also similar to the control male and female rats. The haematological parameters of treated rats were found to be non-significant when compared with respective controls. The collected tissues or organs were found to be normal in appearance, size, shape and texture when compared with the normal controls. The drug *Ḥabb-i-Shifā* when administered daily for a period of 90 days to albino Wistar rats at the dose of 120 mg/kg of body weight was found to be safe.

2014-2015

6 Preclinical Safety Evaluation of *Ma'jūn IQ* in Wistar Rats

6.1 Acute oral toxicity study of Ma'jūn IQ in Rats

Acute oral toxicity study of *Ma'jūn IQ* was studied at the dose level of 5000 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 5 rats. The Group I and II being the male and female controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered once with *Ma'jūn IQ* at the dose of 5000 mg/kg body weight. Animals were weighed initially and at weekly intervals. The effect of the drug *Ma'jūn IQ* on water consumption and feed consumption was monitored and recorded on weekly basis. Animals were observed carefully for any behavioural and neurological changes for 24 hours after the administration of the drug and sacrificed after 14 days of the drug administration. The rats were kept on fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava upon cutting the abdomen by scissor. Blood was collected for haematological and biochemical parameter analysis. The gross examination of the tissues and organs was carried out. The drug *Ma'jūn IQ* was found to have no effect on the body weight gain of the treated male and female rats. There were no significant changes in body weight when compared with the respective male and female controls. There was no significant change in the feed and water consumption of the treated male and female rats when compared with respective controls. Body weight gain of treated group of animals remained comparable to the control and their blood biochemical and haematological parameters were also similar. Gross examination of the organs and tissues did not reveal any treatment-related differences in the treated and control groups.

6.2 Sub-acute oral toxicity study of Ma'jūn IQ in Rats

Sub-acute oral toxicity study of *Ma'jūn IQ* was studied at the dose levels of 4800 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into four groups each consisted of five rats. The Group I and II being the male and female controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats were orally administered with *Ma'jūn IQ* at the dose of 4800 mg/kg body weight for 28 days daily. The rats were observed carefully for any behavioural and neurological changes for next 24 hours after the administration of the drug and twice daily thereafter till the completion of experimentation. The rats were sacrificed after 28 days of the daily drug administration. The rats were kept on fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava upon cutting the abdomen by scissor. Blood was collected for haematological and biochemical parameter analysis. The rats were dissected and organs were observed for any morphological changes. Tissue and organ samples were collected for histological studies. The physiological parameters such as change in body weight, water consumption and feed consumption were also monitored on weekly basis. The male and female treated rats were found to have a normal weight gain and there were no signs of abnormal behaviour in the treated rats. There was a slight decrease (statistically insignificant) in average water consumption per day in the drug treated male and female rats as compared with the respective controls. The drug was found to have no effect in altering the average feed consumption in drug treated rats. Gross examination of the tissues revealed the normal appearance of the tissues or organs. The results of biochemical parameters did not show any significant change in the values when compared with the controls. The liver and kidney function test parameters were found to be normal in the drug treated groups. The lipid profiles of the drug treated male and female rats was found to be unaffected by the drug administration when compared with respective controls. The haematological parameters remained unaffected by the drug as the values of drug treated rats are within the range of control rats. There was no treatment related morphological changes found in the vital organs of the rats such as brain, heart, lung, liver, kidney, spleen, adrenal, testes and ovaries of the rats at the dose level tested. There is no significant difference in the organ weights of male and female treated rats when compared with respective controls.

6.3 Sub-chronic oral toxicity study of Ma'jūn IQ in Rats

Sub-chronic oral toxicity study of *Ma'jūn IQ* was studied at the dose levels of 2000 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 5 rats. The Group I and II being the male and female Controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered with *Ma'jūn IQ* at the dose of 4800 mg/kg body weight for 90 days daily. The rats were observed carefully for any behavioural and neurological changes for next 24 hours after the administration of the drug and daily twice thereafter till the completion of experimentation. The rats were sacrificed after 90 days of the daily oral drug administration. The rats were kept on fasting for overnight prior to sacrifice. Blood was collected for haematological and biochemical parameter analysis. The rats were dissected and organs were observed for any morphological changes. Blood was collected from dorsal vena cava upon cutting the abdomen by scissor. Gross examination of the tissues and organs was carried out. Tissue and organ samples were collected for histological studies. The physiological parameters like body weight change, water consumption and feed consumption were also monitored on weekly basis. There was no mortality or morbidity found in any of the rats administered with the drug *Ma'jūn IQ.* The drug was found to have no effect on the feed and water consumption when compared with the respective controls. The biochemical and haematological parameters of the drug treated male and female rats were found to be unaffected as the results were statistical non-significant. The gross examination of the tissues or organs of both male and female drug treated rats revealed the normal appearance in size, shape and texture. There were no significant changes in the organ weight of male and female treated rats from respective controls. The drug *Ma'jūn IQ* was found to be safe in acute, sub-acute and sub-chronic oral toxicity studies at the selected dose levels in male and female Wistar albino rats. There were no significant change observed in biochemical, haematological and histopathological examination.

Publications

Showkat A. Dar, Mariya Hamdani, Khalid Ghazanfar, Tazeen Nazir, Akbar Masood, Khalid M. Siddiqui, and Seema Akbar. Acute and Sub-acute Oral Toxicity Studies of Majoon-IQ – A Unani Brain Tonic. Hippocratic Journal of Unani Medicine. 2017;12(2): 51-64.

7 Preclinical Safety Evaluation of *Safūf-i-Chutkī*

7.1 Acute oral toxicity study of Safūf-i-Chutkī in rats

Acute oral toxicity study (single dose – 14 days study) of the drug *Safūf-i-Chutkī* was studied at the dose level of 2000 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 5 rats. The Group I and II being the male and female controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered once with *Safūf-i-Chutkī* at the dose of 2000 mg/kg body weight. The dose 2000 mg/kg of body weight was selected as it corresponds to highest limit dose. Animals were weighed initially and at weekly intervals. The effect of the drug *Safūf-i-Chutkī* on water consumption and feed consumption was monitored and recorded on weekly basis. Animals were observed carefully for any behavioural and neurological changes for 24 hours after the administration of the drug, thereafter twice daily and sacrificed after 14 days of the drug administration. The effect of the drug was observed on the gross general parameters like salivation, lacrimation, lethargy, sleep, herd activity and fur loss. The rats were kept on fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava. Blood was collected for haematological and biochemical parameter analysis. The gross examination of the tissues and organs was carried out. The drug *Safūf-i-Chutkī* was found to have no effect on the body weight gain of the treated male and female rats. There were no significant changes on body weight when compared with the respective male and female controls. There were no significant change in the feed and water consumption of the treated male and female rats. The gross behaviour of rats was not changed by the drug administration as no significant change was found in the parameters observed. Body weight gain of treated group of animals remained comparable to the controls and their biochemical and haematological parameters were also similar. Gross examination of the organs and tissues did not reveal any treatment-related differences in the treated and control groups.

7.2 Sub-acute oral toxicity study of Safūf-i-Chutī in rats

Sub-acute oral toxicity study (repeated dose – 28 days study) of the drug *Safūf-i-Chutkī* was studied at the dose levels of 1000 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 5 rats. The Group I and II being the male and female Controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered with *Safūf-i-Chutkī* at the dose of 1000 mg/kg body weight for 28 days daily. The rats were observed carefully for any behavioural and neurological changes for next 24 hours after the administration of the drug and twice daily thereafter till the completion of experimentation. The effect of the drug was observed on the gross general parameters like salivation, lacrimation, lethargy, sleep, herd activity and fur loss. The rats were sacrificed after 28 days of the daily drug administration. The rats were kept on fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava. Blood was collected for haematological and biochemical analysis. The rats were dissected and organs were observed for any morphological changes. Gross examination of the tissues and organs was carried out. Tissue and organ samples were collected for histological studies. The physiological parameters such as change in body weight, water consumption and feed consumption were also monitored on weekly basis. The male and female treated rats were found to have a normal weight gain, there were no signs of abnormal behaviour in the treated rats. There was no statistically significant change in average water consumption per day in the drug treated male and female rats as compared with the respective controls. The drug was found to have no effect in altering the average feed consumption in drug treated rats. Gross examination of the tissues revealed the normal appearance of the tissues or organs. The results of biochemical parameters did not show any significant change in the values when compared with the controls. The liver and kidney function test parameters were found to be normal in the drug treated groups. The lipid profiles of the drug treated male and female rats was found to be unaffected by the drug administration when compared with respective controls. The haematological parameters also remained unaffected by the drug as the values of drug treated rats are within the range of control rats. There was no treatment related morphological changes found in the vital organs of the rats such as brain, heart, lung, liver, kidney, spleen, adrenal, testes and ovaries of the rats at the dose level tested.

7.3 Sub-chronic oral toxicity study of Safūf-i-Chutkī in rats

Sub-chronic oral toxicity study of *Safūf-i-Chutkī* was studied at the dose levels of 1000 mg/kg, of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 5 rats. The Group I and II being the male and female Controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered with *Safūf-i-Chutkī* at the dose of 1000 mg/kg body weight for 90 days daily. The rats were observed carefully for any behavioural and neurological changes for next 24 hours after the administration of the drug and daily twice thereafter till the completion of experimentation. The rats were sacrificed after 90 days of the daily oral drug administration. The physiological parameters such as body weight change, water consumption and feed consumption were also monitored on weekly basis. The rats were kept on fasting for overnight prior to sacrifice. Blood was collected for haematological and biochemical parameter analysis. The rats were dissected and organs were observed for any morphological changes. Blood was collected from dorsal vena cava. Gross examination of the tissues and organs was carried out. Tissue and organ samples were collected for histological studies. There was no mortality or morbidity found in any of the rats administered with the drug *Safūf-i-Chutkī.* The drug was found to have no effect on the feed and water consumption when compared with the respective controls. The biochemical and haematological parameters of the drug treated male and female rats were found to be unaffected as the results were statistically non-significant. The gross examination of the tissues or organs of both male and female drug treated rats revealed the normal appearance in size, shape and texture. There were no significant changes in the organ weights of male and female treated rats from respective controls.

The drug *Safūf-i-Chutkī* was found to be safe in acute, sub-acute and sub-chronic oral toxicity studies at the selected dose levels in male and female Wistar albino rats. There were no significant changes found in physiological, biochemical, haematological and histopathological examination.

8 Preclinical Safety Evaluation of *Chutkī (Drops)*

8.1 Acute oral toxicity study of Chutkī (Drops) in rats

Acute oral toxicity study (single dose – 14 days study) of the drug *Sharbat-i-Chutkī* was studied at the dose level of 2000 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 5 rats. The Group I and II being the male and female Controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered once with *Sharbat-i-Chutkī* at the dose of 2000 mg/kg body weight. The dose 2000 mg/kg of body weight was selected as it corresponds to highest limit dose. The effect of the drug *Sharbat-i-Chutkī* on water consumption and feed consumption was monitored and recorded on weekly basis. Animals were observed carefully for any behavioural and neurological changes for 24 hours after the administration of the drug, thereafter twice daily and sacrificed after 14 days of the drug administration. The effect of the drug was observed on the gross general parameters like salivation, lacrymation, lethargy, sleep, herd activity and fur loss. The rats were kept on fasting for overnight prior to sacrifice. Blood was collected for haematological and biochemical analysis. The gross examination of the tissues and organs was carried out. There was no mortality or morbidity induced by the drug *Sharbat-i-Chutkī* in rats. The physiological parameters such as feed and water consumption remained unaffected by the drug as there were no significant changes found. The biochemical and haematological parameters of both male and female drug treated rats were also found to be non-significant when compared with respective controls.

8.2 Sub-acute oral toxicity study of Chutkī (Drops) in rats

Sub-acute oral toxicity study (repeated dose – 28 days study) of the drug *Sharbat-i-Chutkī* was studied at the dose levels of 2000 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 5 rats. The Group I and II being the male and female Controls were orally treated with water (Vehicle). The Group III and IV

being the drug treated male and female rats, were orally administered with *Sharbat-i- Chutkī* at the dose of 2000 mg/kg body weight for 28 days daily. The rats were observed carefully for any behavioural and neurological changes for next 24 hours after the administration of the drug and twice daily thereafter till the completion of experimentation. The effect of the drug was observed on the gross general parameters such as salivation, lacrymation, lethargy, sleep, herd activity and fur loss. The rats were sacrificed after 28 days of the daily drug administration. The rats were kept on fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava. Blood was collected for haematological and biochemical analysis. The rats were dissected and organs were observed for any morphological changes. Gross examination of the tissues and organs was carried out. Tissue and organ samples were collected for histological studies. The physiological parameters such as body weight change, water consumption and feed consumption were also monitored on weekly basis. The drug *Sharbat-i-Chutkī* was found to be safe under these circumstances as no signs of toxicity was found in any drug treated male or female rat. There was no significant change in the physiological parameters of drug treated rats from the respective controls. The biochemical and haematological parameters of drug treated rats were also found to be unaffected as no statistical significant difference was observed. The organ weight of drug treated and control rats were found to be in same range.

8.3 Sub-chronic oral toxicity study of Chutkī (Drops) in rats

Sub-chronic oral toxicity study of *Sharbat-i-Chutkī* was studied at the dose levels of 2000 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 5 rats. The Group I and II being the male and female Controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats were orally administered with *Sharbat-i-Chutkī* at the dose of 2000 mg/kg body weight for 90 days daily. The rats were observed carefully for behavioural and neurological changes for next 24 hours after the administration of the drug and daily twice thereafter till the completion of experimentation. The rats were sacrificed after 90 days of the daily oral drug administration. The rats were kept on fasting for overnight prior to sacrifice. Blood was collected for haematological and biochemical parameter analysis. The rats were dissected and organs were observed for any morphological changes. Blood was collected

from dorsal vena cava. Gross examination of the tissues and organs was carried out. Tissue and organ samples were collected for histological studies. The physiological parameters such as body weight change, water consumption and feed consumption were also monitored on weekly basis. There was no mortality or morbidity found in any of the rats administered with the drug *Sharbat-i-Chutkī.* The drug was found to have no effect on the feed and water consumption when compared with the respective controls. The biochemical and haematological parameters of the drug treated male and female rats were found to be unaffected as the results were statistical non-significant. The gross examination of the tissues or organs of both male and female drug treated rats revealed the normal appearance in size, shape and texture. There were no significant changes in the organ weights of male and female treated rats from respective controls. The histopathological slides of vital organs or tissues are yet to be interpreted. The drug *Sharbat-i-Chutkī* was found to be safe in acute, sub-acute and sub-chronic oral toxicity studies at the selected dose levels in male and female Wistar albino rats. There were no significant changes found in parameters of behaviour, physiological, biochemical and haematological examination.

2015-2016

9 Preclinical Safety Evaluation of *'Araq-i-Harā Bharā*

9.1 Acute oral toxicity study of *'Araq-i-Harā Bharā* in rats

Acute oral toxicity study (single dose–14 days study) of the drug *'Araq-i-Harā Bharā* was studied at the dose level of 80 ml/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 5 rats. The Group I and II being the male and female controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered once with *'Araq-i-Harā Bharā* at the dose of 80 ml/kg body weight. The dose 80 ml/kg of body weight was selected as it corresponds to highest limit dose. Animals were weighed initially and at weekly intervals. The rats were orally given *'Araq-i-Harā Bharā* at doses of 4 ml/100g twice of rat weight. The effect of the drug *'Araq-i-Harā Bharā* on water consumption and feed consumption was monitored and recorded on weekly basis. Animals were observed carefully for behavioural and neurological changes for 24 hours after the administration of the drug, thereafter twice daily and sacrificed after 14 days of the drug administration. The effect of the drug was observed on the gross general parameters such as salivation, lacrymation, lethargy, sleep, herd activity and fur loss. The rats were kept on fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava. Blood was collected for haematological and biochemical parameter analysis. The gross examination of the tissues and organs was carried out. There was no mortality or morbidity induced by the drug *'Araq-i-Harā Bharā* in rats. The physiological parameters like feed and water consumption remained unaffected by the drug as there were no significant changes found. There were no significant changes on body weights when compared with the respective male and female controls. The biochemical and haematological parameters of both male and female drug treated rats were found to be non-significant different from respective controls.

9.2 Sub-acute oral toxicity study of 'Araq-i-Harā Bharā in rats

Sub-acute oral toxicity study (repeated dose – 28 days study) of the drug *'Araq-i-Harā Bharā* was studied at the dose levels of 40 ml/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 5 rats. The Group I and II being the male and female Controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered with *'Araq-i-Harā Bharā* at the dose of 40 ml/kg body weight for 28 days daily. The rats were administered twice oral doses of 2ml/100g of rat weight. The rats were observed carefully for behavioural and neurological changes for next 24 hours after the administration of the drug and twice daily thereafter till the completion of experimentation. The effect of the drug was observed on the gross general parameters like salivation, lacrymation, lethargy, sleep, herd activity and fur loss. The rats were sacrificed after 28 days of the daily drug administration. The rats were kept on fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava. Blood was collected for haematological and biochemical parameter analysis. The rats were dissected and organs were observed for any morphological changes. Gross examination of the tissues and organs was carried out. Tissue and organ samples were collected for histological studies. The physiological parameters like body weight change, water consumption and feed consumption were also monitored on weekly basis. The male and female treated rats were found to have a normal weight gain; there were no signs of abnormal behaviour in the treated rats. There was a statistical insignificant change in average water and feed consumption per day in the drug treated male and female rats as compared with the respective controls. Gross examination of the tissues revealed the normal appearance of the tissues or organs. The results of biochemical parameters did not show any significant change in the values when compared with the controls. The liver and kidney function test parameters were found to be normal in the drug treated groups. The lipid profiles of the drug treated male and female rats was found to be unaffected by the drug administration when compared with respective controls. The haematological parameters also remained unaffected by the drug as the values of drug treated rats are within the range of control rats. There was no treatment related morphological changes found in the vital organs of the rats such as brain, heart, lung, liver, kidney, spleen, adrenal, testes and ovaries of the rats at the dose level tested. There is no significant difference in the organ weights of male and female treated rats when compared with respective controls. The histopathological slides of the vital organs are yet to be interpreted.

10 Preclinical Safety Evaluation of *Qurṣ-i-Ṭabāshīr Saraṭāni*

10.1 Acute oral toxicity study of Qurṣ-i-Ṭabāshīr Saraṭāni in rats

Acute oral toxicity study (single dose-14 days study) of the drug *Qurṣ-i-Ṭabāshīr Saraṭāni* was studied at the dose level of 3000 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 5 rats. The Group I and II being the male and female Controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered once with *Qurṣ-i-Ṭabāshīr Saraṭāni* at the dose of 3000 mg/kg body weight. The dose 3000 mg/kg of body weight was selected as it corresponds to highest limit dose. The effect of the drug *Qurṣ-i-Ṭabāshīr Saraṭāni* on water consumption and feed consumption was monitored and recorded on weekly basis. Animals were observed carefully for any behavioural and neurological changes for 24 hours after the administration of the drug, thereafter twice daily and sacrificed after 14 days of the drug administration. The effect of the drug was observed on the gross general parameters like salivation, lacrymation, lethargy, sleep, herd activity and fur loss. The rats were kept on fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava upon cutting the abdomen by scissor. Blood was collected for haematological and biochemical parameter analysis. The gross examination of the tissues and organs was carried out. There was no mortality or morbidity induced by the drug *Qurṣ-i-Ṭabāshīr Saraṭāni* in rats. The physiological parameters like feed and water consumption remained unaffected by the drug as there were no significant changes found. There were no significant changes on body weights when compared with the respective male and female controls respectively.

10.2 Sub-acute oral toxicity study of Qurṣ-i-Ṭabāshīr Saraṭāni in rats

Sub-acute oral toxicity study (repeated dose – 28 days study) of the drug *Qurṣ-i-Ṭabāshīr Saraṭāni* was studied at the dose levels of 1500 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 5 rats. The Group I and II being the male and female controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered with *Qurṣ-i-Ṭabāshīr Saraṭāni* at the dose of 1500 mg/kg body weight for 28 days daily. The rats were observed carefully for any behavioural and neurological changes for next 24 hours after the administration of the drug and twice daily thereafter till the completion of experimentation. The effect of the drug was observed on the gross general parameters like salivation, lacrymation, lethargy, sleep, herd activity and fur loss. The rats were sacrificed after 28 days of the daily drug administration. The rats were kept on fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava upon cutting the abdomen by scissor. Blood was collected for haematological and biochemical analysis. The rats were dissected and organs were observed for any morphological changes. Gross examination of the tissues and organs was carried out. Tissue and organ samples were collected for histological studies. The physiological parameters like body weight change, water consumption and feed consumption were also monitored on weekly basis. There was no mortality or morbidity induced by the drug in the rats. The gross behaviour was normal in drug treated rats.

11 Effect of Co-administration of Unani Pharmacopoeial formulations (UPF); *Qurṣ-i-Ṭabāshīr Saraṭāni* (QTS) & *'Araq-i-Harā Bharā* (AHB) with anti-tuberculosis (CAT-I) drugs in adult Wistar albino rats

Collaborative study between CCRUM and NIRT Chennai (ICMR)

The ATT study was conducted to determine the hepatoprotective effect of two Unani Pharmacopoeial formulations: *Qurṣ-i-Ṭabāshīr Saraṭāni* and *'Araq-i-Harā Bharā* co-administered with known anti-tubercular Drugs (ATT) in Albino Wistar rats. The study was conducted for 14, 60 and 180 days, respectively. The rats were randomly divided into four groups (sex ratio 50%). Group I served as the control while Group II received only UPF. Group III received only CAT-I and Group IV received CAT-I and UPF in combination, respectively. The 14, 60 and 180 days studies stands completed. The body weight of rats were recorded after every two days and the feed and water consumption were recorded alternately for 180 days study. The calculation for dosage of drugs was carried out as per the newly recorded body weights and fresh stocks of drug suspensions was made accordingly. The data pertaining to the body weight, feed consumption, water consumption and oral dosing of all the groups are recorded on Data Recording Sheets (DRS).

Tissues of 60 and 180 days ATT study were fixed in 10% formalin followed by tissue processing which was carried out on automatic tissue processor. Tissue Blocks were prepared and labelled. Finally, all the tissue block as well as fixed tissues were sent to RRIUM, Chennai. All the study data pertaining to this study was sent to RRIUM Chennai as laid down in the protocol.

Publications

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2016-2017

12 Preclinical Safety Evaluation of *Ḥabb-i-Tinkār*

Sub-acute oral toxicity study of Ḥabb-i-Tinkār in rats

Sub-acute oral toxicity study of *Ḥabb-i-Tinkār* was carried out at the dose levels of 1000 mg/kg of body weight in both male and female Wistar rats. The animals were randomly divided into 4 groups. Each group consisted of 5 rats. The Group I and II being the male and female Controls were orally treated with distilled water (Vehicle only). The Group III and IV being the drug treated male and female rats, were orally administered *Ḥabb-i-Tinkār* at the dose of 1000 mg/kg body weight for 28 days daily. Cage side Observation of rats was carried out for any behavioural and neurological changes for next 24 hours after the administration of the drug and daily twice thereafter till the completion of experimentation. The physiological parameters such as body weight change, water and feed consumptions were recorded on weekly basis. The animals were weighed regularly after a week and water and feed consumption were also monitored. They were sacrificed after 28 days of the daily oral drug administration. The animals were fasted overnight prior to sacrifice. Blood was collected for haematological and biochemical analysis from dorsal vena cava after opening the abdomen. The rats were dissected, organs collected and observed for any macroscopic morphological changes and the individual organ weight was recorded. Tissue samples were collected for histological studies. There was no effect of the drug on average feed and water consumption per day in the drug treated male and female rats as compared with the Control. The rats gained weight in a normal fashion, the body weight gain of treated groups did not vary from those of respective controls. The biochemical parameters of treated males and females were also similar to that of control male and female rats. The haematological parameters of treated rats were also found to be non-significant when compared with respective controls. Gross examination of the tissues or organs revealed the normal appearance of the tissues. There were no deformity or lesions found in any organ or tissue. The tissues collected from drug treated rats were found to be normal in appearance, size, shape and texture when compared with the normal controls. The drug *Ḥabb-i-Tinkār* when administered daily for a period of 28 days to albino Wistar rats at the dose of 1000 mg/kg body weight showed no adverse effect and was found to be safe for oral consumption at the therapeutic dose level.

13 Preclinical Safety Evaluation of *Ḥabb-i-Sūranjān*

Sub-chronic oral toxicity study of Ḥabb-i-Sūranjān in rats

Sub-chronic oral toxicity study of *Ḥabb-i-Sūranjān* carried out at the dose levels of 2440 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups. Each group consisted of 5 rats. The Group I and II being the male and female Controls were orally given distilled water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered with *Ḥabb-i-Sūranjān* for 90 days daily. The rats were observed carefully for any behavioural and neurological changes for next 24 hours after the administration of the drug and daily twice thereafter till the completion of experimentation. The physiological parameters water or feed consumption and body weight gain were recorded on weekly basis. The rats were sacrificed on 91st day after overnight fasting. Blood was collected for haematological and biochemical analysis from dorsal vena cava after opening the abdomen. The rats were dissected and organs were observed for any morphological changes. Tissue and organ samples were collected for histological studies. The mean body weight gain of all the drug treated male and female rats remain unchanged when compared with respective male and female controls. The rat groups treated with *Ḥabb-i-Sūranjān* (at the dose level of 2440 mg/kg of bw daily for 90 days) were found to gain body weight in a normal fashion. The average feed consumption in control male and female rats was found to be 18.5 and 14.6 gm whereas the drug treated groups were recorded as 19.7 and 15.3 gm.

The average water consumption of control male & female rats was found as 29.0 and 25 ml, while as the drug treated male & female rats was found to be 30.5 and 25 ml respectively. The average water consumption of drug treated male & female group was found to be in the close range of control group. It was found that the drug *Ḥabb-i-Sūranjān* did not cause any major change in feed and water intake of treated rats.

The biochemical parameters of treated groups were found to have no significant change from the biochemical parameters of respective controls. The organ function tests like liver function test (LFT) and kidney function test (KFT) were found to be normal in treated male and female groups. *Ḥabb-i-Sūranjān* was found to have no adverse effect in changing any haematological parameter. There was a non-significant change in the haematological parameters of treated male and female groups when compared with respective controls. The collected organs or tissues of treated male and female rats were found to be normal in shape, size and texture. There were no drug related abnormality, deformity or lesions found in the organs or tissues of *Ḥabb-i-Sūranjān* treated male and female rats. The organ or tissue weights of *Ḥabb-i-Sūranjān* treated male and female rats were comparable to those of respective controls.

The drug *Ḥabb-i-Sūranjān* when administered daily for a period of 90 days to albino Wistar rats at the dose of 2440 mg/kg of bodyweight was found to be have no drug related adverse effects. Therefore, the drug may be considered safe for oral consumption at the therapeutic dose level.

Publications

Ghazanfar K, Dar SA, Nazir T, Akbar S. Sub-chronic oral toxicity study of Habb-e-Suranjan in albino Wistar rats. *Journal of Complementary and Integrative Medicine.* 2018; 15(3).

2018-2019

14 Preclinical Safety Evaluation of *Ma'jūn-i-Piyāz*

14.1 Acute oral toxicity study of *Ma'jūn-i-Piyāz* in rats

Acute oral toxicity study (single dose – 14 days study) of the drug *Ma'jūn-i-Piyāz* was studied at the dose level of 2000 mg/kg of body weight in both male and female Albino Wistar rats. The rats were randomly divided into 4 groups each group consisted of 4 rats. The Group I and II being the male and female controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered once with *Ma'jūn-i-Piyāz*, at the dose of 2000 mg/kg body weight. The dose 2000 mg/kg of body weight was selected as it corresponds to highest limit dose. Body Weight, Feed and Water consumption of rats was recorded initially and there after weekly. Animals were also monitored for any behavioural and neurological changes for 24 hours after the administration of the drug, thereafter twice daily and sacrificed after 14 days of the drug administration. The rats were kept fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava upon cutting the abdomen by scissor for haematological and biochemical parameter analysis. The gross examination of the tissues and organs was carried out and the organ weight was recorded. The drugs *Ma'jūn-i-Piyāz* was found to have no adverse effect on the body weight gain of the treated male and female rats, as the treated rats were found to grow up in a normal pattern. There were no significant changes on body weights when compared with the respective male and female controls. There were no significant changes in the feed and water consumption of the treated male and female rats when compared with respective controls. Body weight gain of treated group of animals remained comparable to the controls and their blood biochemical and haematological parameters were also similar. The drug was observed to have no adverse effect on biochemical and haematological parameters. Gross examination of the organs and tissues did not reveal any treatment-related differences in the treated and control groups.

14.2 Sub-chronic oral toxicity study of Ma'jūn-i-Piyāz in rats

Sub-chronic oral toxicity study of the drug, *Ma'jūn-i-Piyāz* was carried at the dose concentration of 1000 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 7 rats. The Group I and II being the male and female controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered once with *Ma'jūn-i-Piyāz*, at the dose of 1000 mg/kg body weight. The dose 1000 mg/kg of body weight was selected as it corresponds to highest limit dose. Body Weight, Feed and Water consumption of rats was recorded initially and there after weekly. Animals were also monitored for any behavioural and neurological changes for 24 hours after the administration of the drug, there after twice daily and sacrificed after 90 days of the drug administration. The rats were kept fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava upon cutting the abdomen by scissor for haematological and biochemical parameter analysis. The gross examination of the tissues and organs was carried out and the organ weight was recorded. The male and female treated rats were found to have a normal weight gain. There were no significant changes in the feed and water consumption of the treated male and female rats when compared with respective controls. There was no treatment related behavioural changes. Gross examination of the tissues revealed the normal appearance of the tissues or organs. The results of biochemical parameters and haematological parameters did not show any significant change in the values when compared with the controls. The liver and kidney function test parameters were found to be normal in the drug treated groups. The lipid profiles of the drug treated male and female rats was found to be unaffected by the drug administration when compared with respective controls. There was no treatment related morphological changes found in the vital organs of the rats such as brain, heart, lung, liver, kidney, spleen, adrenal, testes and ovaries of the rats at the dose level tested.

15 Preclinical Safety Evaluation of 50% hydroalcoholic extract of *Ma'jūn-i-Piyāz*

15.1 Acute oral toxicity study of 50% hydroalcoholic extract of *Ma'jūn-i-Piyāz* in rats

Acute oral toxicity study (single dose – 14 days study) of the 50% hydroalcoholic Extract of *Ma'jūn-i-Piyāz* was studied at the dose level of 2000 mg/kg of body weight in both male and female Albino Wistar rats. The rats were randomly divided into 4 groups each group consisted of 4 rats. The Group I and II being the male and female controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered once with 50% hydroalcoholic Extract of *Ma'jūn-i-Piyāz*, at the dose of 2000 mg/kg body weight. The dose 2000 mg/kg of body weight was selected as it corresponds to highest limit dose. Body weight, feed and water consumption of rats was recorded initially and there after weekly. Animals were also monitored for any behavioural and neurological changes for 24 hours after the administration of the drug, thereafter twice daily and sacrificed after 14 days of the drug administration. The rats were kept fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava upon cutting the abdomen by scissor for haematological and biochemical parameter analysis. The gross examination of the tissues and organs was carried out and the organ weight was recorded. The 50% hydroalcoholic Extract of *Ma'jūn-i-Piyāz* was found to have no adverse effect on the body weight gain of the treated male and female rats, as the treated rats were found to grow up in a normal pattern. There were no significant changes on body weights when compared with the respective male and female controls. There were no significant changes in the feed and water consumption of the treated male and female rats when compared with respective controls. The gross behaviour of rats was not changed by the drug administration as no significant change was found in the parameters observed. Body weight gain of treated group of animals remained comparable to the

controls and their blood biochemical and haematological parameters were also similar. The drug was observed to have no adverse effect on biochemical and haematological parameters. Gross examination of the organs and tissues did not reveal any treatment-related differences in the treated and control groups.

15.2 Sub-chronic oral toxicity study of 50% hydroalcoholic extract of Ma'jūn-i-Piyāz in rats

Sub-chronic oral toxicity studies of the drug, 50% extract of *Ma'jūn-i-Piyāz*, was carried at the dose concentration of 1000 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 7 rats. The Group I and II being the male and female controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered once with *Ma'jūn-i-Piyāz,* at the dose of 1000 mg/kg body weight. The dose 1000 mg/kg of body weight was selected as it corresponds to highest limit dose. Body Weight, Feed and Water consumption of rats was recorded initially and there after weekly. Animals were also monitored for any behavioural and neurological changes for 24 hours after the administration of the drug, there after twice daily and sacrificed after 90 days of the drug administration. The rats were kept fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava upon cutting the abdomen by scissor for haematological and biochemical parameter analysis. The gross examination of the tissues and organs was carried out and the organ weight was recorded. The male and female treated rats were found to have a normal weight gain; There were no significant changes in the feed and water consumption of the treated male and female rats when compared with respective controls. There was no treatment related behavioural changes. Gross examination of the tissues revealed the normal appearance of the tissues/organs. The results of biochemical parameters and haematological parameters did not show any significant change in the values when compared with the controls. The liver and kidney function test parameters were found to be normal in the drug treated groups. The lipid profiles of the drug treated male and female rats was found to be unaffected by the drug administration when compared with respective controls. There was no treatment related morphological changes found in the vital organs of the rats such as brain, heart, lung, liver, kidney, spleen, adrenal, testes and ovaries of the rats at the dose level tested

16 Preclinical Safety Evaluation of *Tiryāq-i- Afaʿī*

Chronic oral toxicity study of Tiryāq-i- Afaʿī in Wistar rats

Chronic oral toxicity studies of the drug, *Tiryāq-i- Afaʿī ,* was carried at the dose concentration of 1000 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 7 rats. The Group I and II being the male and female controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered once with *Tiryāq-i- Afaʿī* at the dose of 1000 mg/kg body weight. The dose 1000 mg/kg of body weight was selected as it corresponds to highest limit dose. Animals were weighed initially and at weekly intervals. Body Weight, Feed and Water consumption of rats was recorded initially and there after weekly. Animals were also monitored for any behavioural and neurological changes for 24 hours after the administration of the drug, there after twice daily and sacrificed after 180 days of the drug administration. The rats were kept fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava upon cutting the abdomen by scissor for haematological and biochemical parameter analysis. The gross examination of the tissues and organs was carried out and the organ weight was recorded. The male and female treated rats were found to have a normal weight gain. There were no significant changes in the feed and water consumption of the treated male and female rats when compared with respective controls. There was no treatment related behavioural changes. Gross examination of the tissues revealed the normal appearance of the tissues or organs. The results of biochemical parameters and haematological parameters did not show any significant change in the values when compared with the controls. The liver and kidney function test parameters were found to be normal in the drug treated groups. The lipid profiles of the drug treated male and female rats was found to be unaffected by the drug administration when compared with respective controls. There was no treatment related morphological changes found in the vital organs of the rats such as brain, heart, lung, liver, kidney, spleen, adrenal, testes and ovaries of the rats at the dose level tested.

17 Preclinical Safety Evaluation of 50% hydro alcoholic extract of *Tiryāq-i- Afaʿī*

Chronic oral toxicity study of 50% hydro alcoholic extract of Tiryāq-i- Afaʿī in rats

The chronic oral toxicity study of the drug, 50% extract of *Tiryāq-i- Afaʿī* was carried at the dose concentration of 1000 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 7 rats. The Group I and II being the male and female controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered once with 50% extract of *Tiryāq-i- Afaʿī* at the dose of 1000 mg/kg body weight. The dose 1000 mg/kg of body weight was selected as it corresponds to highest limit dose. Animals were weighed initially and at weekly intervals. Body weight, feed and water consumption of rats was recorded initially and there after weekly. Animals were also monitored for any behavioural and neurological changes for 24 hours after the administration of the drug, there after twice daily and sacrificed after 180 days of the drug administration. The rats were kept fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava upon cutting the abdomen by scissor for hematological and biochemical parameter analysis. The gross examination of the tissues and organs was carried out and the organ weight was recorded. The male and female treated rats were found to have a normal weight gain; There were no significant changes in the feed and water consumption of the treated male and female rats when compared with respective controls. There was no treatment related behavioural changes. Gross examination of the tissues revealed the normal appearance of the tissues or organs. The results of biochemical parameters and hematological parameters did not show any significant change in the values when compared with the controls. The liver and kidney function test parameters were found to be normal in the drug treated groups. The lipid profiles of the drug treated male and female rats was found to be unaffected by the drug administration when compared with respective controls. There was no treatment related morphological changes found in the vital organs of the rats such as brain, heart, lung, liver, kidney, spleen, adrenal, testes and ovaries of the rats at the dose level tested.

2019-2020

18 Preclinical Safety Evalauation of *Khamīra-i- Gā'ozabān Sāda*

18.1 Acute oral toxicity of Khamīra-i- Gā'ozabān Sāda *in rats*

Acute oral toxicity study (single dose – 14 days study) of the drug *Khamīra-i-Gā'ozabān Sāda* (*KG Sāda)* was studied at the dose level of 2000 mg/kg of body weight in both male and female Albino Wistar rats. The rats were randomly divided into 4 groups each group consisted of 4 rats. The Group I and II being the male and female controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered once with *KG Sada*, at the dose of 2000 mg/kg body weight. The dose 2000 mg/kg of body weight was selected as it corresponds to highest limit dose. Body weight, feed and water consumption of rats was recorded initially and there after weekly. Animals were also monitored for any behavioural and neurological changes for 24 hours after the administration of the drug, thereafter twice daily and sacrificed after 14 days of the drug administration. The rats were kept fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava upon cutting the abdomen by scissor for hematological and biochemical parameter analysis. The gross examination of the tissues and organs was carried out and the organ weight was recorded. The drugs *KG Sāda*_was found to have no adverse effect on the body weight gain of the treated male and female rats. There were no significant changes in body weights when compared with the respective male and female controls. There were no significant changes in the feed and water consumption of the treated male and female rats when compared with respective controls. Body weight gain of treated group of animals remained comparable to the controls and their blood biochemical and haematological parameters were also similar. The drug was observed to have no adverse effect on biochemical and haematological parameters. Gross examination of the organs and tissues did not reveal any treatment-related differences in the treated and control groups.

18.2 Chronic oral toxicity study of Khamīra-i- Gā'ozabāṉ Sāda *in rats*

Chronic oral toxicity study of the drug, *Khamīra-i- Gā'ozabāṉ Sāda*, was carried at the dose concentration of 1000 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 7 rats. The Group I and II being the male and female controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered once with *Khamīra-i-Gā'ozabāṉ Sāda* at the dose of 1000 mg/kg body weight. The dose 1000 mg/kg of body weight was selected as it corresponds to highest limit dose. Animals were weighed initially and at weekly intervals. Body Weight, Feed and Water consumption of rats was recorded initially and there after weekly. Animals were also monitored for any behavioural and neurological changes for 24 hours after the administration of the drug, there after twice daily and sacrificed after 180 days of the drug administration. The rats were kept fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava upon cutting the abdomen by scissor for haematological and biochemical parameter analysis. The gross examination of the tissues and organs was carried out and the organ weight was recorded. The male and female treated rats were found to have a normal weight gain and there were no significant changes in the feed and water consumption of the treated male and female rats when compared with respective controls. There was no treatment related behavioural changes. Gross examination of the tissues revealed the normal appearance of the tissues/organs. The results of biochemical parameters and haematological parameters did not show any significant change in the values when compared with the controls. The liver and kidney function test parameters were found to be normal in the drug treated groups. The lipid profiles of the drug treated male and female rats was found to be unaffected by the drug administration when compared with respective controls. There was no treatment related morphological changes found in the vital organs of the rats such as brain, heart, lung, liver, kidney, spleen, adrenal, testes and ovaries of the rats at the dose level tested.

19 Preclinical Safety Evaluation of *Ma'jūn-i-'Ushba*

19.1 Acute toxicity study of *Ma'jūn-i-'Ushba* in rats

Acute oral toxicity study (single dose – 14 days study) of the drug *Ma'jūn-i-'Ushba* was studied at the dose level of 2000 mg/kg of body weight in both male and female Albino Wistar rats. The rats were randomly divided into 4 groups each group consisted of 4 rats. The Group I and II being the male and female controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered once with *Ma'jūn-i-'Ushba*, at the dose of 2000 mg/kg body weight. The dose 2000 mg/kg of body weight was selected as it corresponds to highest limit dose. Body weight, feed and water consumption of rats was recorded initially and there after weekly. Animals were also monitored for any behavioural and neurological changes for 24 hours after the administration of the drug, thereafter twice daily and sacrificed after 14 days of the drug administration. The rats were kept fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava upon cutting the abdomen by scissor for haematological and biochemical parameter analysis. The gross examination of the tissues and organs was carried out and the organ weight was recorded. The drug *Ma'jūn-i-'Ushba* was found to have no adverse effect on the body weight gain of the treated male and female rats, as the treated rats were found to grow up in a normal pattern. There were no significant changes on body weights when compared with the respective male and female controls. There were no significant changes in the feed and water consumption of the treated male and female rats when compared with respective controls. The gross behaviour of rats was not changed by the drug administration as no significant change was found in the parameters observed. Body weight gain of treated group of animals remained comparable to the controls and their blood biochemical and haematological parameters were also similar. The drug was observed to have no adverse effect on biochemical and haematological parameters. Gross examination of the organs and tissues did not reveal any treatment-related differences in the treated and control groups.

19.2 Sub-acute toxicity study of Ma'jūn-i-'Ushba in rats

Sub-acute oral toxicity study of the *Ma'jūn-i-'Ushba*, was carried at the dose concentration of 1000 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 5 rats. The Group I and II being the male and female controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered once with *Ma'jūn-i-'Ushba*, at the dose of 2000 mg/kg body weight. The dose 1000 mg/kg of body weight was selected as it corresponds to highest limit dose. Animals were weighed initially and at weekly intervals. Body weight, feed and water consumption of rats was recorded initially and there after weekly. Animals were also monitored for any behavioural and neurological changes for 24 hours after the administration of the drug, there after twice daily and sacrificed after 28 days of the drug administration. The rats were kept fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava upon cutting the abdomen by scissor for haematological and biochemical parameter analysis. The gross examination of the tissues and organs was carried out and the organ weight was recorded. The male and female treated rats were found to have a normal weight gain. There were no significant changes in the feed and water consumption of the treated male and female rats when compared with respective controls. There was no treatment related behavioural changes. Gross examination of the tissues revealed the normal appearance of the tissues or organs. The results of biochemical parameters and haematological parameters did not show any significant change in the values when compared with the controls. The liver and kidney function test parameters were found to be normal in the drug treated groups. The lipid profiles of the drug treated male and female rats was found to be unaffected by the drug administration when compared with respective controls. There was no treatment related morphological changes found in the vital organs of the rats at the dose level tested.

2020-2021

20 Preclinical Safety Evaluation of *Ḥabb-i-Surfa*

20.1 Acute oral toxicity study of Ḥabb-i-Surfa in rats

Acute oral toxicity study (single dose – 14 days study) of the drug *Ḥabb-i-Surfa* was studied at the dose level of 2000 mg/kg of body weight in both male and female Albino Wistar rats. The rats were randomly divided into 4 groups each group consisted of 6 rats. The Group I and II being the male and female controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered once with *Ḥabb-i-Surfa*, at the dose of 2000 mg/kg body weight. The dose 2000 mg/kg of body weight was selected as it corresponds to highest limit dose. Body Weight, Feed and Water consumption of rats was recorded initially and there after weekly. Animals were also monitored for any behavioural and neurological changes for 24 hours after the administration of the drug, thereafter twice daily and sacrificed after 14 days of the drug administration. The rats were kept fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava upon cutting the abdomen by scissor for haematological and biochemical parameter analysis. The gross examination of the tissues and organs was carried out and the organ weight was recorded. The drug *Ḥabb-i-Surfa* was found to have no adverse effect on the body weight gain of the treated male and female rats and there were no significant changes in body weights when compared with the respective male and female controls. There were no significant changes in the feed and water consumption of the treated male and female rats when compared with respective controls. The gross behaviour of rats was not changed by the drug administration as no significant change was found in the parameters observed. Body weight gain of treated group of animals remained comparable to the control and the blood biochemical and haematological parameters were also similar. The drug was observed to have no adverse effect on biochemical and haematological parameters. Gross examination of the organs and tissues did not reveal any treatment-related differences in the treated and control groups.

20.2 Sub-acute oral toxicity study of Ḥabb-i-Surfa in rats

Sub-acute oral toxicity study of *Ḥabb-i-Surfa* was carried at the dose concentration of 1000 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 7 rats. The Group I and II being the male and female controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered once with *Ḥabb-i-Surfa* at the dose of 2000 mg/kg body weight. The dose 1000 mg/kg of body weight was selected as it corresponds to highest limit dose. Animals were weighed initially and at weekly intervals. Body weight, feed and water consumption of rats was recorded initially and there after weekly. Animals were also monitored for any behavioural and neurological changes for 24 hours after the administration of the drug, there after twice daily and sacrificed after 28 days of the drug administration. The rats were kept fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava upon cutting the abdomen by scissor for haematological and biochemical parameter analysis. The gross examination of the tissues and organs was carried out and the organ weight was recorded. There were no significant changes in the feed and water consumption of the treated male and female rats when compared with respective controls. There was no treatment related behavioural changes. Gross examination of the tissues revealed the normal appearance of the tissues or organs. The results of biochemical parameters and haematological parameters did not show any significant change in the values when compared with the controls. The liver and kidney function test parameters were found to be normal in the drug treated groups. The lipid profiles of the drug treated male and female rats was found to be unaffected by the drug administration when compared with respective controls. There was no treatment related morphological changes found in the vital organs of the rats of the rats at the dose level tested.

21 Preclinical Safety Evaluation of *Ma'jūn Dabīd al-Ward*

21.1 Acute oral toxicity study of Ma'jūn Dabīd al-Ward in rats

Acute oral toxicity study (single dose – 14 days study) of the drug *Ma'jūn Dabīd al-Ward* was studied at the dose level of 2000 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 6 rats. The Group I and II being the male and female controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered once with *Ma'jūn Dabīd al-Ward* at the dose of 2000 mg/kg body weight The dose 2000 mg/kg of body weight was selected as it corresponds to highest limit dose. Body weight, feed and water consumption of rats was recorded initially and there after weekly. Animals were also monitored for any behavioural and neurological changes for 24 hours after the administration of the drug, thereafter twice daily and sacrificed after 14 days of the drug administration. The rats were kept fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava upon cutting the abdomen by scissor for haematological and biochemical parameter analysis. The gross examination of the tissues and organs was carried out and the organ weight was recorded. The drug *Ma'jūn Dabīd al-Ward* was found to have no adverse effect on the body weight gain of the treated male and female rats. There were no significant changes on body weights when compared with the respective male and female controls. There were no significant changes in the feed and water consumption of the treated male and female rats when compared with respective controls. The gross behaviour of rats was not changed by the drug administration as no significant change was found in the parameters observed. Body weight gain of treated group of animals remained comparable to the controls and their blood biochemical and haematological parameters were also similar. The drug was observed to have no adverse effect on biochemical and haematological parameters. Gross examination of the organs and tissues did not reveal any treatment-related differences in the treated and control groups.

21.1 Sub-acute oral toxicity study of Ma'jūn Dabīd al-Ward in rats

Sub-acute oral toxicity study of *Ma'jūn Dabīd al-Ward*, was carried at the dose concentration of 1000 mg/kg of body weight in both male and female Wistar rats. The rats were randomly divided into 4 groups each group consisted of 7 rats. The Group I and II being the male and female controls were orally treated with water (Vehicle). The Group III and IV being the drug treated male and female rats, were orally administered once with *Ma'jūn Dabīd al-Ward*, at the dose of 1000 mg/kg body weight. The dose 1000 mg/kg of body weight was selected as it corresponds to highest limit dose. Animals were weighed initially and at weekly intervals. Body weight, feed and water consumption of rats was recorded initially and there after weekly. Animals were also monitored for any behavioural and neurological changes for 24 hours after the administration of the drug, there after twice daily and sacrificed after 28 days of the drug administration. The rats were kept fasting for overnight prior to sacrifice. Blood was collected from dorsal vena cava upon cutting the abdomen by scissor for hematological and biochemical parameter analysis. The gross examination of the tissues and organs was carried out and the organ weight was recorded. The male and female treated rats were found to have a normal weight gain. There were no significant changes in the feed and water consumption of the treated male and female rats when compared with respective controls. There was no treatment related behavioural changes. Gross examination of the tissues revealed the normal appearance of the tissues or organs. The results of biochemical parameters and haematological parameters did not show any significant change in the values when compared with the controls. The liver and kidney function test parameters were found to be normal in the drug treated groups. The lipid profiles of the drug treated male and female rats was found to be unaffected by the drug administration when compared with respective controls. There was no treatment related morphological changes found in the vital organs of the rats at the dose level tested.

22 Preclinical Safety Evaluation of *Capsule Mubārak*

Acute and sub-acute oral toxicity study of Capsule Mubārak in rats

In acute oral toxicity study, a single dose of the drug (2000 mg/kg of body weight) was given orally to the rats. In sub-acute toxicity study, the drug was administered daily to rats of different groups at dose levels of 1000 mg/kg of body weight for duration of 28 days. After every time period of experimentation i.e., 14 days and 28 days respectively of acute and subacute study, the rats were sacrificed and organs were collected. Blood was collected for haematological and biochemical parameter analysis. The rats were dissected and organs were observed for any morphological changes. Gross examination of the tissues and organs was carried out. The physiological parameters like weight change, water intake, feed intake values were also monitored. Animals who received the treatment acquired the body weight normally and no unusual change in the behaviour was observed. The physiological parameters were found to be unaffected as the rats fed and consumed water normally. The other blood biochemical parameters and all the haematological parameters were found to be normal and within physiological range. The organs or tissues collected from the treated group of rats of subacute study appeared to be in normal shape, size and texture; no lesion or tissue deformity was observed. No drug related adversities were observed in major organ function test like kidney function test, liver function and lipid profile or glucose. Gross examination of tissues also did not reveal any significant differences among the treated rats.

23 Preclinical Safety Evaluation of *Qurṣ-i-Mulayyin*

Acute and sub-acute oral toxicity study of Qurṣ-i-Mulayyin in rats

In acute oral toxicity study, a single dose of the drug (2000 mg/kg of body weight) was given orally to the rats. In sub-acute toxicity study, the drug was administered daily to rats of different groups at dose levels of 1000 mg/kg of body weight for duration of 28 days. After every time period of experimentation i.e. 14 days and 28 days respectively of acute and sub-acute study, the rats were sacrificed and organs were collected. Blood was collected for haematological and biochemical parameter analysis. The rats were dissected and organs were observed for any morphological changes. Gross examination of the tissues and organs was carried out. The physiological parameters like weight change, water intake, feed intake values were also monitored. Animals who received the treatment acquired the body weight normally and no unusual change in the behavior was observed. The other blood biochemical parameters, and all the haematological parameters were found to be normal and within physiological range. The organs/tissues collected from the treated group of rats of subacute study appeared to be in normal shape, size and texture; no lesion or tissue deformity was observed. No drug related adversities were observed in major organ function test like kidney function test, liver function and lipid profile or glucose. Gross examination of tissues also did not reveal any significant differences among the treated rats.

2022-2023

24 Preclinical Safety Evaluation of *Safūf-i- Damā (Ḥaldī Wālā)*

24.1 Acute oral toxicity study of Safūf-i- Damā (Ḥaldī Wālā) in rats

Acute oral toxicity study of the drug *Safūf-i- Damā (Ḥaldī Wālā)* was studied at the dose level of 2000 mg/kg of body weight in both male and female Wistar rats. In both groups (N=7) of males and females a single oral dose of the drug was given. Animals were weighed initially and at weekly intervals, and water intake and feed intake were monitored on daily basis. Animals were observed carefully for any behavioural and neurological changes for 24 hours after the administration of the drug, and sacrificed after 14 days of the drug administration. Blood was collected for haematological and biochemical parameter analysis. Animals were dissected and gross examination of the tissues and organs was carried out. The feed and water intake in both male and female rats was unaffected and was comparable with control groups. Body weight gain of treated group of animals remained comparable to the controls, and their blood biochemical and haematological parameters were also similar. Gross examination of the organs and tissues of male rats did not reveal any treatment-related differences in the treated and control groups. Rats gained body weight normally during the study period. The activity of liver enzymes, namely, SGOT and SGPT was increased in the treated female rats and their serum triglycerides were also increased in comparison to the control female rats. The biochemical parameters and haematological parameters were comparable among the treated and control female rats. Gross examination of tissues also did not reveal any significant differences among the treated and control female rats.

24.2 Sub-acute oral toxicity study of Safūf-i- Damā (Ḥaldī Wālā) in rats

Sub-acute oral toxicity study of the drug *Safūf-i- Damā (Ḥaldī Wālā)* was studied at the dose levels of 1000 mg/kg of body weight in both male and female Wistar rats. In each group (N=6) of males and females daily oral dose of the drug was given for 28 days. The rats were observed carefully for any behavioural and neurological changes for 24 hours after the administration of the drug. The rats were sacrificed after 28 days of the drug administration. Blood was collected for haematological and biochemical parameter analysis. The rats were dissected and organs were observed for any morphological changes. Gross examination of the tissues and organs was carried out. The physiological parameters like weight change, water intake, feed intake values were also monitored. The rats were weighed after a week time and water intake and feed intake values were monitored on daily basis. The feed and water intake in both male and female rats was unaffected and was comparable with control groups. Body weight gain of treated group of animals remained comparable to the controls and their blood biochemical and haematological parameters were also similar. Gross examination of the organs and tissues of male rats did not reveal any treatment-related differences in the treated and control groups. The activity of liver enzymes namely SGOT and SGPT was increased in the treated female rats and their serum triglycerides were also increased in comparison to the control female rats. Gross examination of tissues also did not reveal any significant differences among the treated and control female rats.

25 Preclinical Safety Evalaution of *Qurṣ-i-Aṣfar*

25.1 Acute oral toxicity study of Qurṣ-i-Aṣfar in Wistar rats

Acute oral toxicity study of the drug *Qurṣ-i-Aṣfar* was studied at the dose level of 2000 mg/kg of body weight in both male and female Wistar rats. In both groups (N=6) of males and females a single oral dose of the drug was given. Animals were weighed initially and at weekly intervals, and water intake and feed intake were monitored on daily basis. Animals were observed carefully for any behavioural and neurological changes for 24 hours after the administration of the drug, and sacrificed after 14 days of the drug administration. Blood was collected for haematological and biochemical parameter analysis. Animal were dissected and gross examination of the tissues and organs was carried out. The feed and water intake in both male and female rats was unaffected and was comparable with control groups. Body weight gain of treated group of animals remained comparable to the controls, and their blood biochemical and haematological parameters were also similar. Gross examination of the organs and tissues of male rats did not reveal any treatment-related differences in the treated and control groups. The activity of liver enzymes SGOT and SGPT was increased in the treated female rats, and their serum triglycerides were also increased in comparison to the control female rats. The biochemical parameters and haematological parameters were comparable among the treated and control female rats. Gross examination of tissues also did not reveal any significant differences among the treated and control female rats.

25.2 Sub-acute oral toxicity study of Qurṣ-i-Aṣfar in Wistar rats

Sub-acute oral toxicity study of the drug *Qurṣ-i-Aṣfar* was studied at the dose levels of 1000 mg of body weight in both male and female Wistar rats. In each group (N=6) of males and females daily oral dose of the drug was given for 28 days. The rats were observed carefully for any behavioural and neurological changes for 24 hours after the administration of the drug. The rats were sacrificed after 28 days of the drug administration. Blood was collected for haematological and biochemical parameter analysis. The rats were dissected and organs were observed for any morphological changes. Gross examination of the tissues and organs was carried out. The physiological parameters like weight change, water intake, feed intake values were also monitored. The rats were weighed after a week time and water intake and feed intake values were monitored on daily basis. The feed and water intake in both male and female rats was unaffected and was comparable with control groups. Body weight gain of treated group of animals remained comparable to the controls and their blood biochemical and haematological parameters were also similar. Gross examination of the organs and tissues of male rats did not reveal any treatment-related differences in the treated and control groups. SGOT and SGPT was increased in the treated female rats and their serum triglycerides were also increased in comparison to the control female rats. The biochemical parameters and haematological parameters were comparable among the treated and control female rats. Gross examination of tissues also did not reveal any significant differences among the treated and control female rats.

2023-2024

26 Preclinical Safety Evalaution of *Ḥabb-i-Khardal*

Acute and sub-acute oral toxicity study of Ḥabb-i-Khardal in rats

Ḥabb-i-Khardal (HK) is a traditional polyherbal compound Unani Pharmacopoeial formulation having an action as *Munaffith-i-Balgham* (expectorant) and *Muḥallil* (resolvent) effect. It is indicated for the treatment of *Buḥḥ a al-Ṣawt* (hoarseness), *Waram-i-Ḥalq* (Pharyngitis), *Waram-i-Ḥanjara* (laryngitis), *Nazla-i-Muzmin* (chronic catarrh) and *Su'āl Balghami* (phlegmatic cough). No data is available regarding the toxicity of this compound Unani formulation. Therefore, present study was designed to evaluate acute and repeated dose 28-day oral toxicity study of HK in Wistar rats.

An acute oral toxicity study was conducted, administering a single dose of 2000 mg/kg body weight, in accordance with OECD-425 guidelines. Subsequently, a repeated dose oral toxicity study was performed using Wistar rats at dosage levels of 1000 mg/kg body weight for 28 days. The initial finding based on haematology, biochemistry, bodyweight and feed intake indicated that *Ḥabb-i-Khardal* can be considered safe for its intended use. The histopathological examination of vital organs such as liver, kidney, lungs, pancreas, spleen, heart, adrenal gland and testes or ovaries did not reveal any morphological changes after oral administration of drug *Ḥabb-i-Khardal* at the dose level of 1000 mg /kg body weight for 28 days. Thus, the study showed that the drug *Ḥabb-i-Khardal* is safe for oral consumption as there was no significant changes seen in the vital organs of the rats.

27 Preclinical Safety Evaluation of *La'ūq-i-'Unsul*

27.1 Acute oral toxicity of La'ūq-i-'Unsul in rats

La'ūq-i-'Unsul (LU) is a traditional polyherbal compound Unani Pharmacopoeial formulation having an action as *Munaffith-i-Balgham* (expectorant). It is indicated for the treatment of *Su'āl Muzmin* (Chronic Cough). No safety data is available regarding the toxicity of this compound Unani formulation. Therefore, present study was designed to evaluate acute and repeated dose 28-day oral toxicity study of LU in Wistar rats.

An acute oral toxicity study was conducted following OECD-425 guidelines with single dose of 2000 mg/kg body weight. No treatment related mortality or gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 2000 mg/kg bw.

27.2 Repeated dose 28-day (sub-acute) oral toxicity of La'ūq-i-'Unsul in rats

The repeated dose 28-day oral toxicity study was carried out using Wistar rats at dose levels of 1000 mg/kg body weight. The study findings did not indicate any toxicological significant observation in terms of body weight, feed intake, behavioural pattern, haematology, biochemistry; relative organ weight in *La'ūq-i-'Unsul* (LU) treated group at tested dose level as compared to control animals. Hence the LU may be considered safe based on above findings for intended use. The histopathological examination of vital organs such as Liver, Kidney, Lungs, Pancreas, Spleen, Heart, Adrenal gland and Testes or Ovaries shows normal architecture and did not reveal any morphological changes after oral administration of drug *La'ūq-i-'Unsul* at the dose level of 1000 mg /kg body weight for 28 days.

2024-2025

28 Preclinical Safety Evaluation of *Jawārish-i-Ūd Mulayyin*

Acute and repeated dose 28-day oral toxicity of Jawārish-i-Ūd Mulayyin in rats

Jawārish-i-Ūd Mulayyin (JOM) is a traditional polyherbal compound Unani Pharmacopoeial formulation having an action as *Mulayyin* (laxative). It is indicated for the treatment of *Qabḍ* (constipation) and *Ḍu'f al-Shahwa* (poor appetite). It helps in improving digestion and relieving bloating. So far, no data is available regarding the toxicity of this compound Unani formulation. Therefore, the present study is designed to evaluate acute and repeated dose 28-days oral dose toxicity in rats to determine safety profile of the drug.

An acute toxicity study was conducted by administering a single dose of JOM (2000 mg/kg bw) as per OECD-425 guidelines. Subsequently, JOM was administered at the dose 1000 mg/kg bw/day p.o. for 28 days to conduct 28-day repeated dose oral toxicity. The findings of the present study revealed neither mortality nor behavioural changes in both acute and 28-day repeated dose oral toxicity assessment of JOM. No toxicological significant observation with respect to body weight, feed intake, haematology, clinical biochemistry and relative organ weight in JOM treated rats. Therefore, JOM would be considered safe for its intended use based on above observations.

The drug *Jawārish-i-Ūd Mulayyin* was administered to the Wistar albino rats repeatedly for 28 days at the dose level of 1000 mg /kg body weight. The rats were sacrificed on 29th day of the study after overnight fasting. The vital organs such as liver, kidney, lungs, pancreas, spleen, heart, adrenal gland and testes or ovaries were collected and processed for histopathological examination. The macroscopic examination of the vital organs shows normal tissue or organ shape, size and texture. The histopathological examination of vital organs of drug treated rats did not reveal any pathological or morphological changes with no evidence of degenerative or necrotic changes or congestion, no cellular degeneration was seen when compared with the control rats. However, a few emphysematous areas were noted which might be the result of agonal struggle at the time of administration of anaesthesia. Since no pathological changes were evident, thus this study shows that the drug *Jawārish-i-Ūd Mulayyin* appears to be safe for oral consumption in Wistar albino rats.

29 Preclinical Safety Evaluation of *Iṭrīfal-i-Khabath al-Ḥadīd*

Acute and repeated dose 28-day oral toxicity of Iṭrīfal-i-Khabath al-Ḥadīd in rats

Iṭrīfal-i-Khabath al-Ḥadīd (IKH) is a traditional herbo-mineral compound Unani Pharmacopoeial formulation having an action as *Kāsir-i-Riyāḥ* (Carminative), *Muqawwī-i-Miʿda* (stomachic) and *Muqawwī-i- Aʿṣāb* (nerve tonic). It is indicated for the treatment of *Iḥtibās al-Ṭamth* (amenorrhoea), *Bawāsīr* (haemorrhoids), *Qabḍ* (constipation), *Ḍuʿf al-Shahwa* (poor appetite), *Nafkh-i-Miʿda* (flatulence) and *Wajaʿal-Khāṣira* (low backache). So far, no data is available regarding the toxicity of this compound Unani formulation. Therefore, the present study is designed to evaluate acute and repeated dose 28-days oral dose toxicity in rats to determine safety profile of the drug.

An acute toxicity study was conducted by administering a single dose of IKH (2000 mg/kg bw) as per OECD-425 guidelines. Subsequently, IKH was administered at the dose 1000 mg/kg bw/day p.o. for 28 days to evaluate 28-day repeated dose oral toxicity. The findings of the present study revealed neither mortality nor behavioural changes in the acute and 28-day repeated dose oral toxicity assessment of IKH. No toxicological significant observation with respect to body weight, feed intake, haematology, clinical biochemistry and relative organ weight in IKH treated groups or control animals were observed. Therefore, IKH would be considered safe based on above observations.

The drug *Iṭrīfal-i-Khabath al-Ḥadīd* was administered to the Wistar albino rats repeatedly for 28 days at the dose level of 1000 mg /kg body weight. The rats were sacrificed on 29th day of the study after overnight fasting. The vital organs such as liver, kidney, lungs, pancreas, spleen, heart, adrenal gland and testes or ovaries were collected and processed for histopathological examination. The macroscopic examination of the vital organs did not show any pathological changes in tissue/ organ shape, size and texture. The histopathological examination of vital organs of drug treated rats did not reveal any pathological or morphological changes with no evidence of degenerative or necrotic changes or congestion was seen when compared with the control rats. Since no pathological changes were evident, thus this study shows that the drug *Iṭrīfal-i-Khabath al-Ḥadīd* appears to be safe for oral consumption in Wistar albino rats.

30 Acute toxicity study on Unani drugs used in Sub Clinical projects under Centre of excellence in *'Ilāj bi'l Tadbīr* (Regimental Therapy)

Acute Oral Toxicity Study of following drugs under Centre of Excellence in Wistar albino rats:-

1. *Dawā' al-Misk Motadil*
2. *Sufūf-i- Ghatiya*
3. *Sufūf-i-Mohazzil + Araq-i Zīrā*
4. *Tukhm-i-Khashkhāsh*

Acute toxicity study: This study was designed to evaluate acute oral toxicity potential of *Dawā' al-Misk Motadil, Sufūf-i-Ghatiya, Sufūf-i-Mohazzil + Araq-i Zīrā* and *Tukhm-i-Khashkhāsh* under COE in Wistar rats. Considering the low acute toxicity potential of tested drug, a limit test at the dose of 2000 mg/kg bw (i.e., maximum dose) as per OECD-425 was conducted. Animals were administered with single dose of formulation and were observed for lethality and toxic signs & symptoms for 14 days post-treatment. As no lethality was observed following treatment with test drug in five consecutive animals per drug respectively, dosing to further animals was stopped. All the five animals per test drug were sacrificed on Day 15 and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 2000 mg/kg bw. Therefore, oral LD_{50} of the above test drugs in the female Wistar rat was estimated to be greater than 2000 mg/kg body weight.

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