ISSN: 0974-1291

HIPPOCRATIC JOURNAL OF UNANI MEDICINE

Volume 4 • Number 1

January–March 2009

CENTRAL COUNCIL FOR RESEARCH IN UNANI MEDICINE

HIPPOCRATIC JOURNAL OF UNANI MEDICINE

Volume 4, Number 1, January - March 2009

Hippocratic J. Unani Med. 4(1): 1 - 102, 2009

CENTRAL COUNCIL FOR RESEARCH IN UNANI MEDICINE

Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy (AYUSH)

Ministry of Health & Family Welfare, Government of India

Hippocratic Journal of Unani Medicine

Editorial Office

CENTRAL COUNCIL FOR RESEARCH IN UNANI MEDICINE
61 - 65 Institutional Area (Opposite 'D' Block), Janakpuri, New Delhi - 110 058, India
Tel.: +91-11-28521981, 28525982, 28525983, 28525831/52/62/83/97, 28520501, 28522524 • Fax: +91-11-28522965
Web site: www.ccrum.com • E-mail: unanimedicine@gmail.com & ccrum@rediffmail.com

Annual Subscription: Rs. 300/- (India) US$ 100/- (Other countries) **Single Issue:** Rs. 150/- (India) US$ 50/- (Other countries)
Payments in respect of subscription may be sent by bank draft marked payable to Director, CCRUM, New Delhi.

On behalf of Central Council for Research in Unani Medicine (CCRUM) published and printed by Dr. Mohammed Khalid Siddiqui, Director, CCRUM at CCRUM headquarters, 61 - 65 Institutional Area (Opposite 'D' Block), Janakpuri, New Delhi - 110 058 and printed at Rakmo Press Private Limited, C - 59 Okhla Industrial Area Phase - I, New Delhi - 110 020

Editor: Dr. Mohammed Khalid Siddiqui, Director, CCRUM

Contents

EDITORIAL

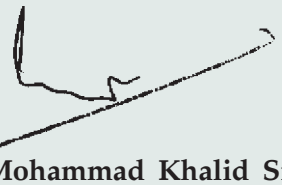
Unani system of Medicine, although originated in Greece, is one of the recognized systems of medicine of the country. Although, the Unani medicine have been in use for centuries and are known for their therapeutic efficacies, there is a need to scientifically establish their efficacy and safety in order to achieve global acceptance. Organized research work in this system was, therefore, a need of the hour. In post independent era, Central Council for Research in Unani Medicine, through its clinical, drug research, literary, survey & cultivation of medicinal plants programme is cultivation of medicinal plants programme is contributing significantly for last three decades. *Vitiligo, sinusitis, filariasis, eczema, malaria, infective hepatitis* are some of the conditions where Unani therapies have earned recognition after scientific validation.

The Council has been publishing the *Hippocratic Journal of Unani Medicine* (HJUM), mainly to bring out fundamental and applied aspects of Unani Medicine. The journal also publishes recent advances in other related sciences and traditional medicines as well as different streams of medical sciences, which have bearing on validation and scientific interpretation of various concepts and strength of Unani medicine.

In view of an overwhelming response, the journal earlier published twice a year, its periodicity has now been changed to quarterly w.e.f. January 2008 to accommodate more articles for quick dissemination of research data among scientific community. The journal has sufficient room for invited articles from luminaries of modern medicine and sciences as well as scholars of Unani medicine. The broad areas being covered include clinical research on single and compound Unani drugs, validation of regimental therapy, clinico-pharmacological studies, standardization of single and compound drugs, development of standard operating procedures, ethnobotanical studies, experimental studies on medicinal plants and development of agro-techniques thereof, and literary research on classics of Unani medicine. The journal is also open for studies on safety evaluation of Unani and other herbo-mineral drugs, nutraceuticals, cosmotherapeutics, aromatics, oral health, life style disorders, sports medicine etc and such other newer areas which are the outcome of modern day living.

The current issue of this journal provides 13 original research and review papers in the areas of clinical research, drug standardization, bio-chemistry, pharmacology and ethnobotanical surveys contributed by eminent scholars in their respective fields. Council acknowledges the authors for their contributions included in this issue and hope for their continued support in this endeavor. We wish to ensure the readers to bring out the future issues of the journal on time.

We at the CCRUM have been constantly striving to reach to higher standard and make HJUM the leading journal of Unani Medicine and related sciences. We sincerely hope and trust that the mission can be accomplished with active partnership of quality-conscious individuals and institutions. Through these lines we seek your cooperation and support in materializing our dreams about the HJUM. In this regard, we request you for your as well as your colleagues' contributions for publication in and subscription to the journal. Further, we will appreciate if the journal is introduced far and wide. We would also welcome esteemed suggestions for achieving the highest standards of quality for the journal.



(Dr. Mohammad Khalid Siddiqui)
Editor-in-Chief

Prevalence of Type-2 Diabetes Mellitus in Kashmir Valley of India

[1]Arsheed Iqbal,
[2]Ab. Ahad Wani,
[2]Riyaz-u-saif Andrabi
and
[2]Imran R. Rangrez

[1]Regional Research Institute of Unani Medicine (RRIUM), Srinagar-196006 (J&K), India

[2]Sheri Kashmir Institute of Medical Sciences (SKIMS), Srinagar-196006 (J&K), India

Abstract

This study was conducted to assess the prevalence of known type-2 D.M. in Kashmir valley of north India. This was a Multicentric study conducted at Regional Research Centre of Unani Medicine and Sheri Kashmir Institute of Medical Sciences (Tertiary Care Institute) in Kashmir Valley of India between 2006-2007. After proper randomization, 12110 subjects belonging to all age groups and all parts of valley were interviewed to ascertain the prevalence of type-2 D.M in Kashmir Valley. Prevalence of type 2 DM is very high in Kashmir Valley of north India. It increases with age The prevalence was found 0%, 0.47%, 0.96%, 2.51% and 8.1%, respectively, for age groups of <20 years, 20-30 years, 31-40 years, 41-50 years and above 50 year. In conclusion type-2 D.M is very common in Kashmir Valley and is witnessing the epidemic of type-2 D.M like rest of the world.

Key Words: Type 2 Diabetes Mellitus, Kashmir, Epidemiology.

Introduction

Two-third of world's population with diabetes mellitus lives in developing countries Aronymous 2001. India and China alone are projected to account for 1/3 of all diabetics in world by the year 2025 (King and Aubert, 1998). The developing countries like India, Pakistan, Bangladesh and Srilanka are also witnessing rapid increase in the prevalence of Type-2 D.M. The prevalence of T2DM in India has risen from 1.2% to 11% over the last three decades (Tripathy *et. al.,* 1971).

The present epidemological study was designed to estimate the burden of Diabetes Mellitus (known) in all age groups in Kashmir Valley (North India).

Material and Methods

Kashmir Valley is in the northern region of Indian subcontinent. It is surrounded by Himalayas and borders of China and Pakistan. The Valley has a population of 5.4 million as per last official census (2001). The capital city Srinagar is the only urban district while the other five districts are semi urban or rural. The population is homogenous with respect to food habits and consumption of alcohol is insignificant. The valley has six districts (Administrative units) comprising of tehsils which in turn are made up of villages and Mohallas.

This study was designed to know the prevalence of known diabetes in Kashmir valley.

In this study 12110 eligible subjects belonging to all age groups belonging to all the districts were intervived for presence of type-2 D.M. defined as physician diagnosed D.M, as ascertained by medical records.

Statistical analysis was done by using statistical package for social sciences (SPSS inc. 1992). A two tailed P-value was used for calculating statistical significance. A P-Value of <0.05 was considered significant.

Results

Of the 12110 subjects enrolled in our study. Prevalence of diabetes was 1.54% and 2.1% in male and female group respectively with total prevalence of 1.77% in total population: (Table 1).

Of the 12110 subjects belonging to all age groups, no body was found to have diabetes below 20 years age. The prevalence was found to increase with age: 0.47%, 0.96%, 2.51% and 8.1% for age groups of 20-30 years, 31-40 years 41-50 years and >50 years, respectively (Table 2).

Conclusion

Type-2 D.M. is very common in Kashmir Valley (North India) like other parts of India and world (Zargar et al., 2000; Shera et al., 1995; Ramachandran et al., 1998; 1997, 2001, 2003).

Table-1. Showing prevalence of type-2 diabetes mellitus in Kashmir valley. Prevalence of known Diabetes Mellitus in studied population

Gender	Number	Yes	No
Total	12110	215 1.77%	12110-215 98.33%
Male	7110	110 1.54%	7000 98.46%
Female	5000	105 2.1%	4895 97.9%

Table-2. Age-wise Prevalene of type-2 Diabetes Mellitus

Age	No	Yes	No
<20 Years	569	0	569100%
20-30 Years	1895	9 0.47	188699.53%
31-40 Years	2500	240.96%	247699.04%
41-50 Years	4215	1062.51%	410997.49%
>50 Years	2904	2358.1%	266991.1%

(P < 0.001)

Acknowledgements

We thank Director, Central Council for Research in Unani Medicine, New Delhi, Regional Research Institute of Unani Medicine, and Sheri Kashmir Institute of Medical Sciences, (SKIMS) Srinagar (J&K), for providing all the facilities for the present work; and the Medical officers of all the districts for providing their support in completing this study.

References

Anonymous, 2001. Diabetes and cardiovascular disease: Time to act: Brussels: International Diabetes Federation.

King, H. and Aubert, R.E.,1998. Global burden of diabetes 1995-2005: Prevalence, Numerical estimates and projections. *Diabetes Care,* 21: 1414-31.

Ramachandra, A., Snehalata, C. and Vishwanathan, V., 2003. Explosion of type-2 diabetes in Indian subcontinent. *International Diabetes Monitor;* 15: 1.

Ramachandran, A., Jali, M.V. and Mohan, V. *et al.,* 1998. High Prevalence of diabetes in urban population in South India. *Br. Med. J.,* 297-587-90.

Ramachandran, A., Snehalatha, C. and Latha, E. *et al.,* 1997. Rising Prevalence of NIDDM in urban population in India. *Diabetalogia;* 40: 232-37.

Ramachandran, A., Snehalatha, C. and Kapur, A. et al., 2001. High Prevalence of diabetes and impaired glucose tolerance in India: National Urban Diabetes Survey. *Diabetologia;* 44: 1094-1101.

Shera, A.S., Rafique, G. and Khawaja, I.A. *et al.,*1995. Pakistan National Diabetes Survey. Prevalence of glucose intolerance and associated factors in Shikarpur, Sindh province. *Diabetes Med;* 12: 1116-21.

Tripathy, B.B., Panda, N.C. and Tej, S.C. *et al.,*1971. Survey for detection of glycosuria hyperglycemia and Diabetes Mellitus in urban and rural areas of cuttack district. *J. Assoc Physicians India;* 19: 681-92.

Zargar, A.H., Khan, A.K., Masood, S.R., Laway, B.A., Wani, A.I., Bashir, M.I. and Dar, F.A., 2000. Prevalence of Type-2 D.M and impaired glucose tolerance in Kashmir Valley of India subcontinent. *Diab. Res. Clini Pract,* 47: 135-46.

Clinical Evaluation of Coded Unani Drugs in Daul-Feel (Filariasis)

[1]M. Ishtiyaque Alam,
[1]Bilal Ahmad,
[1]Mahboob-us-Salam,
[1]L.A. Khan*,
[1]S.S. Ali Khan
and
[2]M.K. Siddiqui

[1]Regional Research Institute of Unani Medicine (CCRUM), East Boring Canal Road, Patna-800001

[2]Central Council for Research in Unani Medicine, 61-65, Institutional Area, Janakpuri, New Delhi-110058

Abstract

A clinical trial of two coded Unani drug combinations with and without Munzij and Mushil therapy was conducted on the patients of Daul-Feel at Regional Research Institute of Unani Medicine, Patna. Out of all the cases registered, only thirty six patients completed the study. According to the clinical and pathological findings both the combinations were observed to possess significant efficacy except in the case of filarial oedema which reduced minimally, showing that these combinations are less effective in the chronic cases.

Key Words: Unani Medicine, Filariasis

Introduction

Filariasis is a major health problem in India, especially in the state of Bihar. It is a disease which has serious economic and social implications as it affects many young working adults of both sexes (WHO, 1992). It leads to irreversible chronic manifestations which are responsible for social stigma. Besides causing considerable economic loss and severe physical disability, frequent acute attacks of filariasis traumatize the patients. Slum dwellers with inadequate housing and no basic sanitation are at the highest risk of infection with *W. bancrofti* transmitted by *C. quinquefasciates*, and it is the rural poor who are affected by filariasis transmitted by other species (WHO, 2002).

According to Unani System of Medicine, the disease is caused by abnormal accumulation of Black Bile (Sauda) or Phlegm of thick consistency (Balgham-e-Ghaleez) or Pure Blood (Khan, 1885).

The drug of choice Diethyl carbamazine (DEC) in filariasis did not show uniform efficacy in all the patients. Furthermore, the acceptance of DEC is limited due to severe side effects. On the other hand, Unani classical books are full of references of drugs, which have been found efficacious during trials conducted by eminent scholars of yesteryears, but no clinical data is available to support such claims. The present study was carried out in the O.P.D. and I.P.D. section of RRIUM, Patna. Thirty six patients completed the study.

Material and Methods

Patients of either sex within age group of 11-60 years were selected for the study after careful clinical examination. After diagnosis patients were subjected to laboratory investigations. The filarial oedema was measured with the help of measuring tape.

Present address: Regional Research Institute of Unani Medicine (CCRUM), Post Box 70, Aligarh-202002 (U.P.)

The patients were randomly subdivided into following 4 groups:

Group A : UNIM–268 2 tables of 500 mg twice daily

UNIM–270 5 grams powder

UNIM–272 20 grams oil

UNIM–270 and UNIM–272 are mixed together and used for application on the affected part

UNIM–271 20 grams crude (used as Nutool i.e. irrigation of the affected part with the luke warm decoction of the crude drug)

Group B : UNIM–269 2 tables of 500 mg twice daily

UNIM–270 5 grams powder

UNIM–270 and UNIM–272 are mixed together and used for application on the affected part

UNIM–271 20 grams crude (used as Nutool/Irrigation)

Group C : Munzij (DF-9) Decoction of crude drugs in the dose of 125 ml for a period of 21 to 30 days depending upon Nuzj

Mushil (DF-18) 07 Mushils (Decoction of crude drugs in the dose of 125 ml) at bed time alternated by 07 Tabreeds (DF-10)

After MM Therapy the patients were given the same treatment as mentioned in group A

Group D : Munzij (DF-9) Decoction of crude drugs in the dose of 125 ml for a period of 21 to 30 days depending upon Nuzj

Mushil (DF-18) 07 Mushils (Decoction of crude drugs in the dose of 125 ml) at bedtime alternated by 07 Tabreeds (DF-10)

After MM Therapy the patients were given the same treatment as mentioned in group B

Elastocrape bandage was used in all four groups.

The duration of treatment was 120 days in group A and group B, while in group C and group D this duration was MM Therapy + 120 days.

Investigations included urine analysis for chyluria, haemogram, night and day peripheral blood smears for identification of microfilaria. Results were compared on the basis of outcome of laboratory findings at base line and after treatment.

Results and Discussion

Clinical responses are presented in tables 05 to 08. The clinical parameters such as fever, lymphangitis and lymphadenitis showed marked regression after treatment in all the 4 groups (Table 5).

Table-1. Age-wise distribution of the patients

Age Group (Years)	Number of Cases	Percentage
11–20	02	5.55
20–30	06	16.66
30–40	15	41.66
40–50	06	16.66
50–60	07	19.44
Total	36	100

Table-2. Sex-wise distribution of the patients

Sex	Number of Cases	Percentage
Male	20	55.55
Female	16	44.44
Total	36	100

Table-3. Socio–economic status of the patients

Socio-economic status	Number of Cases	Percentage
Poor	21	58.33
Average	15	41.66
Good	Nil	Nil
Total	36	100

Table-4. Chronicity status of disease

Chronicity in years	Groups			
	A	B	C	D
Up to 1 year	03	02	01	01
1 Year – 5 Years	05	05	02	02
5 Years –10 Years	04	03	02	03
Above 10 Years	01	01	Nil	01

Table-5. Clinical Parameters Before and After Treatment

S. No.	Group	No. of Patients	Lymphadenitis		Lymphangitis		Fever	
			Base Line	After Treat-ment	Base Line	After Treat-ment	Base Line	After Treat-ment
1	A	13	12	00	13	02	03	00
2	B	11	10	00	10	02	03	00
3	C	05	05	00	05	01	03	00
4	D	07	07	00	07	03	02	00

Table-6. Filarial Oedema in Centimeters, Before and After Treatment

Day of Measurement	Statistics	Treatment Group			
		Group A	Group B	Group C	Group D
Base Line	Mean	110.86 cm	97.11 cm	138.2 cm	85.07 cm
After Treatment	Mean	103.81 cm	91.55 cm	129.3 cm	80.07 cm
	% of reduction	6.36%	5.73%	6.44%	5.88%

Table-7. Total Eosinophil percentage, Before and After Treatment

Day of Estimation	Statistics	Treatment Group			
		Group A	Group B	Group C	Group D
Base Line	Mean (%)	6.46	4.54	5.6	8
	N=	13	11	5	7
After Treatment	Mean (%)	4.46	3.63	2.4	4.28
	N=	13	11	5	7
Percentage reduction in eosinophil count after treatment		31%	20%	57.15%	46.5%

N = Number of subjects/observations

Table-8. Absolute Eosinophil count Before and After Treatment

Day of Estimation	Statistics	Treatment Group			
		Group A	Group B	Group C	Group D
Base Line	Mean	460	412.72	400	487.14
	N =	13	11	05	07
After Treatment	Mean	276.15	312.72	273.2	241.42
	N =	13	11	05	07
Percentage reduction in AEC after treatment		40%	24.23%	31.7%	50.5%

N = Number of subjects/observations

6.36%, 5.73%, 6.44%, 5.88% reductions in filarial oedema was observed in group A, B, C, and D respectively (Table-6).

The decrease in eosinophil percentage was found to be 31% and 20% in group A and B respectively, while 57.15% and 46.5% decrease was observed in group C and D, respectively (Table-7). The percent decrease in absolute eosinophil count after treatment was found to be 40, 24.23, 31.7 and 50.5 in group A, B, C and D, respectively (Table-8).

The results suggest that all the treatment groups showed good response on all the parameters but reduction in filarial oedema was negligible.

Out of 36 patients, only 7 patients showed chronicity of one year or less while the remaining twenty nine patients were suffering from filariasis for a period of more than two years. Therefore, it may be that the drugs used during this trial which showed marked efficacy on all parameters except filarial oedema may help to reduce the oedema also if given in less chronic cases of filariasis.

Acknowledgement

Financial support by the Central Council for Research in Unani Medicine, New Delhi for the present study is thankfully acknowledged.

References

Khan, M.A., 1885. Ikseer-e-Azam, Vol. IV, Matba Nizami, Kanpur, p.11.

WHO, 1992. Lymphatic Filariasis – The disease and its control. 5th Report of WHO Expert Committee on Filariasis. WHO Technical Report Series No. 821, Geneva.
WHO, 2002. Weekly Epidemiological Record, Geneva, 16 (77): 1125-132.

Effect of Essential Oil of Rose on Aggressive Behaviour of Siamese Fighting Fish

[1]Munawwar Husain,
[2]Shariq Zafar
and
[1]Masroor A. Qureshi

[1]Regional Research Institute of Unani Medicine (CCRUM), Sir J.J. Hospital Compound, Byculla, Mumbai-400008

[2]Dr M.I.J. Unani Medical College 60 Yari Road, Versova, Andheri (W), Mumbai-400061

Abstract

The behavioral effects of the essential oil of rose (ER) were seen on the aggressive behavior of the male Siamese fighting fish. A total of twelve fully mature male Siamese fighting fish (*Betta splendens*) were taken for the behavioral study. They were kept separately in peaceful place in the jars and were fed the standard feed ad libitum. They were divided randomly into two groups, control and test.

The study shows that the test drug increases the aggressive behaviour as compared to central fishes despite being reported and used as an anti-anxiety agent. It could be analogous to the aggression increasing effect of depressants like morphine and alcohol.

Key Words: Anti-anxiety, Anti-stress

Introduction

The incidence of anxiety, depression and other psychiatric disorders is increasing day by day in the fast mechanical and materialistic age. As a result, the use of Allopathic psychotropic agents is also increasing, which have further deleterious effects on the brain, nervous system and other organs. The drugs like diazepam, alprazolam may also cause dependence. Their adverse effects include aggression, excitement, confusion, headache, giddiness, alimentary tract upset, skin rashes, reduced libido etc.

There are many herbs like Sumbul-ut-teeb, Asgandh, Brahmi, Sankhaholi etc., which are in use for the treatment of psychiatric disorders, especially anxiety and stress in Unani Medicine. However, a lot of work has already been carried out and published on these herbs. There are different reports available on the psychopharmacological activities of different plant origin drugs, like anxiolytic activity of the extracts of *Sphaeranthus indicus* flowers in mice (Ambavade et al., 2006); antidepressant-like activity of glycyrrhizin in mice that was supposed to be medicated through an interaction with adrenergic and dopaminergic systems; and the efficacy of glycyrrhizin was also comparable to that of imipramine and fluoxetine (Dhingra and Sharma, 2005); facilitatory effect of the extract of *Bacopa monnieri* on the learning responses and augmentation of both cognitive function and mental retention capacity (Singh and Dhawan, 1997); attenuation of stress induced elevation of norepinephrine of brain and hypothalamus and also suppression of stress-induced elevation of plasma corticosterone by *Panax ginseng* (Bhattacharya and Sur, 1999); CNS depressant and anticonvulsant activity of Bramhi Ghrita, a formulation containing *Bacopa monnieri*, *Evolvulus alsinoides, Acorus calamus, Saussurea lappa* and cow's ghee (Achliya et al., 2005); nootropic activity and modification of 5-HT and noradrenaline mediated behavior by the pet. ether extract of *Lawsonia inermis* (Iyer

et al., 1998); *Myristica fragrans* showed anxiogenic, sedative and analgesic activities in mice (Sonavane et al., 2001).

The Gul-e-Surkh (*Rosa damascena,* Mill.) is one of such drugs, which has high esteem amongst the psychoactive and mood enhancing Unani remedies. Its virtues were described by Pliny (23-70 C.E.) and Dioscorides (1st centaury C.E.). The rose oil has been highly valuable and the Roman emperors were very fond of it, as they poured rose water into the canals running in their gardens. The famous Unani scholar Ibn-e-Sina has written an entire book on rose.

The essential oil of rose has been used by Unani scholars in their Aromatherapy, and they have described that the aroma of the fresh rose works as *mufarreh* and claimed that it acts as a brain and heart tonic and is useful for depression and anxiety related disorders. There were some limited reports available regarding the sympathetic activity of rose oil fragrance in normal adults (Haze et al., 2002); anti-conflict activity of rose oil in mice using Geller and Vogel conflict tests (Umezu, 1999); anti-anxiety-like effect the pharmacologically active constituents of rose oil in mice using Geller and Vogel conflict tests (Umezu et al., 2002); anxiolytic-like properties of rose oil in rats using elevated plus-maze (EPM) test (de Almeida et al., 2004); antidepressant activities of rose oil in mice using forced swim test (Farzin et al., 2004).

However, there was a need to further validate its psychopharmacological actions like anti-anxiety, anti-stress or antidepressant etc. Therefore, the essential oil of Gul-e-Surkh was selected for the present study.

Methodology

The present study was carried out in the Ilmul Advia (Pharmacology) lab, MIJ Tibia College Mumbai during 2005-2007. The fighting fish were purchased from the authorized supplier of fisheries University Mumbai and was identified by department of Aquarium fish, fisheries University Mumbai.

As the abolition of the fighting behavior in Siamese fighting fish is indicative of the possible tranquillizing/anti-aggressive/anti-anxiety property of the Test drug, therefore this test was carried out to evaluate the effect of ER.

The behavioral effects of the essential oil of rose (ER) were seen on the aggressive behavior of the male Siamese fitting fish. Total twelve fully mature male Siamese Fitting fish (*Betta splendens*) were taken for the behavioral study. They were kept separately in an isolated place in the jars and were fed the standard feed ad libitum. They were divided randomly into two groups.

The 2% (wt/wt) solution of ER was made in the propylene glycol (PG). Then this solution was further diluted with distilled water to make a final solution for the test group, containing 0.01% of ER and 0.49% of PG. Another solution of 0.49% of plain PG was made in the distilled water, which was used for the fishes of control group.

The fishes of both the groups were put individually into their respective solutions for a period of 45 minutes each; and then they were transferred into their jars containing normal water. This procedure was repeated for 5 days.

The aggressive behavior was observed on the 6th day by introducing one fish from control group and another fish of the test group into a water tank measuring 30 cm. x 17.5 cm. x 23 cm. (length x width x height) containing plain water filled up to the height of 15 cm. *(Editor's note: 'It would have been better to test the aggressive behaviour of control and test animals by challenging with untreated animals, rather than with each other').* The tank was kept under sufficient light, and the parameters of aggressive behaviour were observed by the naked eye and simultaneously the video clips were also recorded to see it further into slow motion, and re-confirming and matching the scores of parameters of aggression already recorded by naked eye observation.

The intra species aggressive behavior of the Siamese fitting fish (Miczek and Barry, 1976) were observed on the basis of two major components viz. 'Threat' and 'Attack', which were assessed on the basis of following sub-parameters.

1. ***Threat components***

    a) Orientation towards opponent

    b) Execution of undulating movements

    c) Erection of gill covers

    d) Erection of median fins (fin display)

    e) Extension of branchiostegal membranes

    f) Darkening of the body and fin colors

    g) Chasing

2. ***Attack***

    a) Bites

    b) Nips

    c) Vigorous fin and tail movement

    d) Tail whips

    e) Lock-jaw with the opponent

The behavioral components (sub-parameters) of 'Threat' were assigned the score of one (1) on each occurrence; while the components (sub-parameters) of 'Attack' were assigned the score of two (2) on each occurrence. The mean scores of the control and test groups were compared statistically by the Student's't' Test.

Table-1. Individual scores of aggressive behaviors in Siamese fighting fish

S. No.	Threat behaviors										Attack behaviors										Total Aggressive Behavior scores	
	Orientation towards opponent		Execution of undulating movements		Erection of gill covers		Fin display		Extension of branchio-stegal membranes		Chasing		Bites		Nips		Vigorous fin and tail movement		Tail whips			
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
1.	10	15	26	26	17	25	12	19	16	24	7	40	6	16	18	34	40	44	32	64	174	307
2.	12	16	25	21	16	24	13	20	17	25	6	41	7	15	18	33	41	42	31	60	186	297
3.	11	18	24	23	18	22	11	17	15	26	9	39	5	19	17	31	45	46	30	65	185	306
4.	13	19	22	28	15	28	16	21	18	21	8	42	9	16	20	30	43	44	34	64	198	313
5.	10	14	23	25	14	26	12	19	19	20	6	41	6	14	19	35	42	43	32	65	183	302
6.	12	17	28	30	17	25	14	18	14	26	9	45	7	16	17	38	45	46	30	68	193	329
7.	14	20	21	31	17	21	15	16	15	24	7	40	8	15	21	34	40	44	35	62	193	307
8.	12	21	26	29	16	20	11	17	16	26	8	44	6	18	19	32	43	45	32	60	199	312

Table-2. Analysis of aggressive behavior of Siamese fighting fish

Parameters of aggressive behavior	Mean		S.E.		p Value
	C	T	C	T	
Threat components:					
h) Orientation towards opponent	11.75	17.50	0.591	0.866	<0.0001*
i) Execution of undulating movements	24.375	26.625	0.8224	1.238	0.1825***
j) Erection of gill covers	16.25	23.875	0.4532	0.9531	0.0005*
k) Erection of median fins (fin display)	13.00	18.375	0.6547	0.5957	0.0002*
l) Extension of branchiostegal membranes	16.25	24.00	0.5901	0.8238	0.0007*
m) Chasing	7.50	41.50	0.4226	0.7319	<0.0001*
Attack components:					
f) Bites	6.75	16.125	0.4532	0.5806	<0.0001*
g) Nips	18.625	33.375	0.4978	0.8851	<0.0001*
h) Vigorous fin and tail movement	42.375	44.25	0.7055	0.4910	0.0058**
i) Tail whips	32.00	63.50	0.6268	0.9636	<0.0001*
Total aggressive behavior	188.88	309.13	2.985	3.367	<0.0001*

C: control; T: test; S.E.: Standard error; *extremely significant; **very significant; ***not significant

Discussion

The analysis of aggressive behavior of Siamese fighting fish (Table 1 Table 2 revealed that the score of the orientation towards opponent is 11.75 ± 0.591 (mean ± S.E.) in the control group; while it is 17.50 ± 0.866 (mean ± S.E.) in the test group. The p value is <0.0001 (extremely significant). The scores of execution of undulating movements are 24.375 ± 0.8224 (mean ± S.E.) in the control group; while the score is 26.625 ± 1.238 (mean ± S.E.) in the test group. The p value is 0.1825 (not significant). The score of the erection of gill covers is 16.25 ± 0.4532 (mean ± S.E.) in the control fishes; while the score is 23.875 ± 0.9531 (mean ± S.E.) in the fishes of test group. The p value is 0.0005 (extremely significant). The score of erection of median fins (fin display) is 13.00 ± 0.6547 (mean ± S.E.) in the control group; while the score is 18.375 ± 0.5957 (mean ± S.E.) in the test group. The p value is 0.0002 (extremely significant). The scores of the extension of branchiostegal membranes is 16.25 ± 0.5901 (mean ± S.E.) in the control group; while the score is 24.00 ± 0.8238 (mean ± S.E.) in the test group. The p value is 0.0007 (extremely significant). The scores of chasing are 7.50 ± 0.4226 (mean ± S.E.) in the test group; while the scores are 41.50 ± 0.7319 (mean ± S.E.) in the test group. The p value is <0.0001 (extremely significant). The scores of bites are 6.75 ± 0.4532 (mean ± S.E.) in the control group; while the scores in the test group are 16.125 ± 0.5806 (Mean ± S.E.). The p value is <0.0001 (extremely significant). The scores of nips are 18.625 ± 0.4978 (mean ± S.E.) in the control group; while the scores are 33.375 ± 0.8851 (mean ± S.E.) in the test group. The p value is <0.0001 (extremely significant). The score of vigorous fin and tail movement are 42.375 ± 0.7055 (mean ± S.E.) in the control group; while the scores are 44.25 ± 0.491 (mean ± S.E.) in the test group. The p value is 0.0058 (very significant). The scores of tail whips are 32.00 ± 0.6268 (mean ± S.E.) in the test group; while in the test group the scores are 63.50 ± 0.9636 (Mean ± S.E.). The p value is <0.0001 (extremely significant). The scores of Total Aggressive Behavior are 188.88 ± 2.985 (mean ± S.E.) in the control group; while the scores are 309.13 ± 3.367 (mean ± S.E.) in the test group. The p value is <0.0001 (extremely significant).

Discussion In the test of aggressive behavior of Siamese fighting fish, among the threat component the aggregate scores of the orientation towards opponent, erection of gills covers, erection of median fins (fins display), extension of branchiostegal membranes and chasing in the test group, were much higher than the control group, and extremely significant difference was found. Only in one threat component, i.e. execution of undulating movements, the difference was not significant. Among attack component, the aggregate score of the bite, nips and tail-whips in the test group were nearly double the scores of the same parameters in the control group; and the differences were extremely significant. One attack component, i.e. vigorous fin and tail movement in the test group was slightly higher than in the control group;

and the difference of the aggregate score was very significant. As the total aggressive behavior in the test and control group was also calculated, the score of the test group were markedly higher than the control group and statistically the difference was extremely significant. This experiment shows that the test drug increases the individual components as well as total aggressive behavior of the Siamese fighting fish as compared to the control fishes. It is very strange in this experiment, that the test drug which is showing anti-anxiety, anti-stress activity and also suppression of sympathetic activity, is increasing aggression in the Siamese fighting fish. There is a possibility that a drug could show different pharmacological activity in different animals and the mode of administration of drug could also alter the mechanism of action of the test drug. However if we analyze the previous experiments, the Siamese fighting fish (*Betta splendens*) showed different responses to different drugs. As in two experiments, the morphine in low concentrations up to 40µg/ml. of tank water was found to facilitate attack and threat response in the Siamese fighting fish (Walaszek and Abood, 1956; Braud and Weibel, 1969). In other experiments, low doses of alcohol were found to facilitate whereas high doses of alcohol were found to suppress certain intraspecies aggressive behavior in Siamese fighting fish. (Raynes *et al.,* 1968; Raynes and Ryback, 1970).

In the present experiment of Siamese fighting fish, it is revealed that the mechanism of action of rose oil on the Siamese fighting fish would be similar as that of low doses of morphine and alcohol. In experiments with L.S.D., the doses ranging from 1-50 µg/ml. of tank water were found to increase aggressive behaviors (bites, chasing, fin display and tail-whips), in the Siamese fighting fishes (Abramson and Evans, 1954; Evans et al., 1958). Although the effect of L.S.D. of that experiment were having similarity with the effects of rose oil appeared in the present experiment on the Siamese fighting fish; but the open field behavior experiment in rats done in the present study excludes the similarity of action of the rose oil with the L.S.D.; as no sign of hallucinogenic or L.S.D.-like effect of the rose oil was found on the parameter of open field behavior of rats.

In a previous study, the cannabis extract had also shown effects, just opposite to the action of rose oil appeared in the present study. As, Gonzalez et al. (1971) reported suppression of aggressive displays by pairs of Siamese fighting fish after 2hr in tanks containing cannabis extracts (1mg/litre) or THC (0.5 mg/litre). The swimming movements were slower but normal coloration was maintained. Development of tolerance was indicated by a lessened suppression of aggressive displays by the ninth day of the drug treatment.

Acknowledgments

Authors are grateful to Dr Mohammad Khalid Siddiqui, Director Central Council for Research in Unani Medicine, New Delhi for his support, encouragements and coordination in the present study.

References

Achliya, G.S., Wadodkar, S.G. and Dorle, A.K., 2005. Evaluation of CNS activity of Bramhi Ghrita, *Indian J. Pharmacology*, 37(1): 33-36.

Ambavade, S.D., Mhetre, N.A., Tate, V.D. and Bodhankar, S.L., 2006. Pharmacological evaluation of the extracts of *Sphaeranthus indicus* flowers on anxiolytic activity in mice, *Indian J. Pharmacology*, 38(4): 254-259

Bhattacharya, S.K., 1992. Evaluation of adaptogenic activity of Trasina, an Ayurvedic formulation, In: Proceedings of International Seminar on Traditional Medicine (7-9 November, Calcutta), Oxford and IBH Publishing, Calcutta, India, pp. 322-326.

de Almeida, R.N. et al, 2004**.** Anxiolytic-like effects of rose oil inhalation on the elevated plus-maze test in rats, *Pharmacol Biochem Behav.* Feb; 77(2):361-4.

Dhingra, Dinesh and Sharma, Amandeep, 2005. Evaluation of antidepressant-like activity of glycyrrhizin in mice, *Indian J. Pharmacology*, 37(6): 390-394.

Farzin, D., Zarghami, M. and Khalaj, L., 2004. Evaluation of antidepressant activities of rose oil and geranium oil in the forced swim test in mouse, Abstracts of Posters/Medicinal Plants and the Nervous System, *Iranian J. Pharmaceutical Research,* Supplement 1: 70

Haze, Shinichiro, Sakai, Keiko and Gozu, Yoko, 2002. Effects of fragrance inhalation on sympathetic activity in normal adults, *Jpn. J. Pharmacol.*, 90: 247-253.

Haze, Shinichiro, Sakai, Keiko and Gozu, Yoko, 2002**.** Effects of fragrance inhalation on sympathetic activity in normal adults, *Jpn. J. Pharmacol.*, 90: 247-253.

Iyer, M.R., Pal, S.C., Kasture, V.S. and Kasture, S.B., 1998. Effect of *Lawsonia inermis* on memory and behavior mediated via monoamine neurotransmitters, *Indian J. Pharmacology*, 30: 181-185.

Miczek, K.A. and Barry Herbert, 1976. Pharmacology of sex and aggression, In Behavioral Pharmacology (eds. Stanley D. Glick and Joseph Goldfarb), The Mosby Company, Saint Louis pp. 198, 201, 204, 206, 208-211, 213, 215, 219, 222, 228.

Singh, H.K. and Dhawan, B.N., 1997. Neuropsychopharmacological effects of the Ayurvedic nootropic *Bacopa monnieri*, Linn. (Brahmi), *Indian J. Pharmacology*, 29: S359-S365.

Sonavane, Ganeshchandra; Sarveiya, Vikram; Kasture, Veena and Kasture, S.B., 2001. Behavioral actions of *Myristica fragrans* seeds, *Indian J. Pharmacology* 33: 417-424.

Umezu, T., 2000. Behavioral effects of plant-derived essential oils in the geller type conflict test in mice, *Jpn. J. Pharmacol.,* 83(2): 150-53.

Umezu, T., 1999. Anticonflict effects of plant-derived essential oils, *Pharmacol. Biochem. Behav.* 64(1): 35-40.

Pharmacological Study of Earthworm *(Perionyx excavatus* Perrier) for Anti-ulcer and Anti-oxidant Activity

Sachi Bharti and Shweta

Vermiculture Research Station, Department of Zoology, D.S. College, Aligarh-202001

Abstract

The drugs from the Natural Kingdom, chiefly those used by traditional medicines, are being increasingly explored to obtain more effective and safer drugs. The herbal drugs have been quite extensively studied but animal-origin drugs are still unexplored. So, earthworm (*P. excavatus*), used in Unani Medicine, was studied as adjuvant, alongwith modern drug 'Ranitidine', for Anti-ulcer activity against gastric ulcer induced by pyloric ligation and immobilization stress in the rat; and for anti-oxidant activity by observing its effect on TBARS, SOD, catalase and glutathione. The study revealed that the test drug significantly reduces gastric ulceration and gastric juice volume. It also significantly decreases TBARS and increases SOD, catalase and reduced glutathione levels. So, the study shows earthworm (*P. excavatus*) to possess anti-ulcerogenic effect, atleast partly, due to a strong anti-oxidant effect.

Key Words: *Perionyx excavatus* Perrier, Anti-ulceral, Anti-oxidants, Superoxide dismutase, Thiobarbituric acid reactive substances

Introduction

The treatment of peptic ulcer, the most common gastro-intestinal disorder, by modern medicines is known to cause many side effects such as arhythmias, impotence, gynaecomastia, haematopoietic changes etc. Drugs used in the treatment of peptic ulcer act by blocking H_2 histaminic receptor, inhibiting proton pump, affecting the mucosal barrier and drugs that act on central nervous system (Jaup, 1981). A review of literature reveals that numerous herbo-mineral derivatives/formulations and even many single plants are known to possess anti-ulcer activity such as Shankha bhasma, cauvery-100, Normacid, *Solanum nigrum, Brassica oleracea*, *Ocimum sanctum* and *Trigonella foenum-graecum* (Pandit *et al.,* 2000; Bafna *et al.,* 2000; Shah *et al.,* 2002).

Many tribes and people in remote villages of India were known to use earthworms for treating various kinds of ailments (Bhatnagar and Palta, 2002). The Unani system of medicine also makes use of earthworm for treating ulceration (Vohora and Khan, 1978). Recently the coelomic fluid of earthworm was found to exhibit cytolytic, agglutinating, proteolytic, haemolytic, mitogenic, anti-pyretic, tumorstatic and antibacterial activity (Lange *et al*., 1997; Liu *et al*., 2004).

Besides these, Vohora and Khan (1978) have also reported the role of earthworms in the healing of wounds, chronic folds, piles and sore throat. The anti-inflammatory activity of total 'earthworm paste' and its extracts in different solvents is reported by Yegnanaraya (1987). But there has been very few attempts to study anti-ulcer and anti-oxidant effect in comparison of a known standard modern drug such as 'ranitidine'. The investigations aim at evaluating the earthworm (*Perionyx excavatus*

Perrier), an indigenous species, for anti-ulcer and anti-oxidant activity with a view to discover new drugs of natural origin to combat ulceration.

Materials and Methods

Preparation of "earthworm paste"

Earthworms (*Perionyx excavatus* Perrier) were washed (500 sexually mature clitellated worms) with running tap water and then fed with wet blotting paper for 18-20 hours for gut clearance. The gut cleared worms were again washed with distilled water. The worms were kept in a plastic trough covered tightly with polythene cover and exposed to sun light for three days to kill the earthworms. Mucus and coelomic fluid that oozed out digested the dead worms forming a brown coloured paste called "earthworm paste".

Animals

Healthy and pure strain male albino rats ranging 150-200 g body weight were used. The animals were housed in polypropylene cages at 24°±2°C and fed with standard diet and water ad libitum throughout the study. The study got clearance from Institutional Animal Ethical Committee (IAEC).

Drugs

Aspirin, earthworm paste and the standard drug ranitidine were suspended in 1% carboxy methyl cellulose and given orally for 10 days during the experiment.

Aspirin plus pyloric ligation induced ulcer model

The methods of Goel *et al*. (1986), Shay *et al*. (1945) and Parmar *et al*. (1984) were followed for the evaluation of anti-ulceral activity. The animals were divided into 7 groups of 6 animals each.

Group I Normal control (water)
Group II Ranitidine (50 mg/kg)
Group III Ranitidine (50 mg/kg) + Earthworm paste (20 mg/kg)
Group IV Ranitidine (50 mg/kg) + Earthworm paste (40 mg/kg)
Group V Ranitidine (50 mg/kg) + Earthworm paste (80 mg/kg)
Group VI Ranitidine (50 mg/kg) + Earthworm paste (160 mg/kg)
Group VII Ranitidine (50 mg/kg) + Earthworm paste (320 mg/kg)

All the doses were administered orally once daily for 10 days. Group II to VII received aspirin 200 mg/kg orally, 1 hour after the oral administration of test and standard drugs. On the 11th day Pyloric ligation was carried out on the 18 hours

fasted rats. After 4 hours of pyloric ligation, the animals were sacrificed by decapitation. The stomach was cut open along the greater curvature and the gastric juice was collected and centrifuged at 3000 rpm for 10 minutes. The supernatant was measured and used for the estimation of total and free acidity. The stomach was washed with normal saline and lesions were observed using a binocular magnifier. The gastric lesions were measured by the following score and ulcer index was determined.

1 point - Loss of normal morphology
1 point - Discolouration of mucosa
2 point - Ulcer upto 1 mm (dia)
3 point - Ulcer upto 2 mm (dia)

Estimation of free and total acidity

From the supernatant collected, 1 ml was pipetted out and titrated against 0.01 N sodium hydroxide using Topfer's reagent and phenolphthalein as indicator.

Anti-oxidant studies

The glandular part of the stomach was removed and used for the assay of thiobarbituric acid reactive substances (TBARS) and anti-oxidants: superoxide dismutase, catalase, glutathione peroxidase and non-enzymatic reduced glutathione.

Assay of thiobarbituric acid reactive substances

The tissue homogenate was prepared in Tris-HCl buffer (0.025 M, pH 7.8). To 10 ml of the tissue homogenate, 20 ml of TCA-TBA-HCl reagent was added and mixed thoroughly. The mixture was kept in a boiling water bath for 15 minutes. After cooling the tubes were centrifuged at 1000 rpm for 10 minutes and the pink colour developed in the supernatant was measured in a UV spectrophotometer at 535 nm against a reagent blank. A series of standard solution in the concentration of 2.5-10 moles were treated in a similar manner and the values were expressed as m mol/ mg protein in tissue.

Assay of superoxide dismutase

Tissue was homogenized in sodium pyrophosphate buffer (0.025 M, pH 8.3) 0.5 ml tissue homogenate was diluted to 1.0 ml with distilled water followed by addition of chilled 2.5 ml ethanol and 1.5 ml chloroform.

Assay of catalase

Tissue homogenate was prepared by using phosphate buffer (0.01 M, pH 7.8). To 0.9 ml phosphate buffer, 0.1 ml tissue homogenate and 0.4 ml hydrogen peroxide

were added the reaction was arrested after 15, 30, 45 and 60 seconds by adding 2.0 ml of dichromatic acetic acid mixture. The tubes were kept in a boiling water bath for 10 minutes, cooled and the colour developed was read at 620 nm in UV Spectronic spectrometer. Standards in the concentration range of 2-100 m moles were taken and processed as for the test. The specific activity of the enzymes was expressed as m mol H_2O_2 consumed/min/mg/protein.

Assay of glutathione peroxidase

To 0.2 ml Tris buffer, 0.2 ml EDTA, 0.1 ml sodium azide and 0.2 ml enzyme preparation (tissue homogenate) were added and mixed well. To this 0.2 ml of GSH followed by 0.1 ml of H_2O_2 were added. The contents were mixed and incubated at 37ºC for 10 minutes. The reaction was arrested by the addition of 0.5 ml 10% TCA. The tubes were centrifuged and the remaining was determined as in the GSH procedure and the activities were expressed as mg of GSH consumed/min/mg/ protein.

This mixture was shaken for 90 minutes at 4ºC and then centrifuged. The enzyme activity in the supernatant was determined as follows. The assay mixture contained 1.2 ml sodium pyrophosphate buffer, 0.1 ml phenazine methosulphate and 0.3 ml nitroblue tetrazolium and appropriately diluted enzyme preparation in a total volume of 3 ml. The reaction was started by the addition of 0.2 ml NADH. After incubation at 30ºC for 90 seconds, the reaction was stopped by the addition of 1 ml glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 ml n-butanol. The mixture was allowed to stand for 10 minutes, centrifuged and butanol layer was separated. The coloured intensity of the chromogen was measured in UV spectronic spectrometer at 560 nm. A system devoid of enzyme served as control. The enzyme concentration required to produce 50% inhibition of chromogen formation in one minute under standard conditions was taken as one unit. The specific activity of the enzyme was expressed as units/mg protein.

Assay of reduced glutathione

A known weight of tissue was homogenized in phosphate buffer. 0.5 ml of the homogenized mixture was treated with 20 ml 5% TCA, mixed and centrifuged 2.0 ml of the supernatant was treated with 1.0 ml Elman's reagent and 4.0 ml 0.3M disodium hydrogen phosphate. The absorbance of the yellow colour developed was read in UV specronic spectrophotoemter at 412 nm. A series of standards (20-100 m g) were treated in a similar manner along with a blank containing 1.0 ml buffer. The amount of glutathione was expressed as m moles/mg of protein.

Statistical analysis

The statistical significance of difference was tested at 0.05 level using one-way analysis of variance (ANOVA).

Results and Discussion

Study of anti-ulcer activity

Aspirin, a known ulcerogenic drug and pyloric ligation had significantly increased the gastric juice volume (64%) free acidity (32%) and total acidity (28%) except pH compound to normal control. These symptoms of ulcer were brought to near normal condition when the standard drug 'ranitidine' was administered. Earthworm paste 160 mg/kg had significantly decreased the gastric juice volume, free acidity and total acidity. These values were better than treatment with ranitidine.

Study of antioxidant activity

Aspirin plus pyloric ligation had significantly increased the peroxidation level indicator TBARS and decreased the anti-oxidants SOD, CAT, GPx and GSH. Similar results were observed in the ranitidine treated animal. 160 mg/kg earthworm paste shows much better results.

Ulcer, a common human disease occurs due to the imbalance between two opposing factors (a) increase in attacking factors including *Helicobacter pylori*, bile salts, acid, pepsin (b) decrease in defensive factor including mucus and gastric mucosal barrier particularly cyclo-oxygenase which is responsible for mucus production improved blood flow and bicarbonate production in the duodenum. Attacking factors predominate in duodenal ulcer and failed defensive mechanism in gastric ulcer. Consequently, the treatment of peptic ulcer is directed against either reduction of the aggressive factors or enhancement of defensive mechanism. Ulcerogens, like ACTH, carticosone, aspirin and phenylbatazone reduce the rate of secretion of mucus and reduce the concentration of protein bound carbohydrates in these secretions. Aspirin, a non-steroid, anti-inflammatory drug induces gastric ulcer by causing back diffusion of H^+ ions into the mucosal cells reduced mucosed defense through inhibition of cyclo-oxygenase (Cox) and prostaglandin synthesis and also known to increase gasric juice secretion, total acidity, free acidity and reduce the pH.

In the present study the earthworm paste particularly 160 mg/kg was found to bring down the gastric juice volume, free acidity and total acidity indicating the efficacy in reducing these factors, which tend to ulcer. These results seems even better than results of the standard drug-ranitidine used for treatment of ulcer (Table 1). It was also reported, aspirin plus pyloric ligation to increase the lipid peroxidation index (TBARS) and decrease SOD. CAT, GPx and GSH, thus leading to oxidative span. But all these parameters were reversed by the treatment with the standard drug ranitidine and earthworm paste. Administration of earthworm paste particularly 160 mg/kg had resulted in the levels of antioxidant enzymes and the lowering of peroxidation index (TBARS) indicating the scavenging of free radicals and reducing lipid peroxidation.

Table-1. Anti-oxidant activity of earthworm paste on stomach of *Rattus norvegicus*

Treatments	Thiobarbituric acid reactive substances (μ mol/mg protein)	Superoxide dismutase μ mol H_2O_2 consumed/min/mg	Catalase Unit s/mg protein	Glutathione peroxidase μg/mg/min	Reduced glutathione μg/mg protein
Normal control	0.2115 ± 0.01	4.5397 ±0.02	5.0324 ± 0.02	5.9109 ± 0.01	3.3034 ± 0.03
Aspirin induced ulcer control	0.6132 ±0.06	1.1005 ±0.02	1.5310 ±0.03	0.2421 ±0.15	0.7395 ±0.01
Standard drug (Ranitidine 85 mg/kg)	0.5835 ±0.02	1.4848 ±0.02	2.4244 ±0.02	2.9239 ±0.02	1.1014 ±0.03
Earthworm paste (mg/kg)					
20	0.5190 ±0.01	1.4228 ±0.03	1.8218 ±0.02	1.6127 ±0.01	0.9278 ±0.02
40	0.5589 ±0.07	1.4863 ±0.02	1.8218 ±0.02	2.8147 ±0.01	1.8360 ±0.01
80	0.4808 ±0.02	2.0672 ±0.01	2.2094 ±0.01	2.8147 ±0.01	1.8360 ±0.01
160	0.3906 ±0.05	3.1546 ±0.09	3.6463 ±0.01	4.7303 ±0.02	2.3512 ±0.01
320	0.5426 ±0.01	2.0520 ±0.02	2.4777 ±0.01	3.4235 ±0.01	1.3517 ±0.04

Table-2. Anti-ulceral activity of earthworm paste on stomach of *Rattus norvegicus*

Treatments	Gastric juice volume	Gastric juice pH	Free acidity ml/100g	Total acidity mEq/l
Normal control	3.5 ±0.07	5.1 ± 0.09	08.2 ±0.14	15.29 ±0.27
Aspirin induced ulcer control	5.4 ±0.06	2.9 ±0.05	25.2 ±0.16	53.15 ± 0.17
Standard drug (Ranitidine 85 mg/kg)	4.7 ±0.05	4.0 ±0.05	11.5 ± 0.25	24.68 ±0.16
Earthworm paste (mg/kg)				
20	4.5 ±0.05	3.7 ±0.05	12.9 ±0.15	33.17 ±0.10
40	4.0 ± 0.04	3.8 ±0.05	12.2 ±0.10	30.60 ± 0.54
80	4.2 ±0.15	4.2 ±0.05	11.1 ± 0.05	22.38 ±0.21
160	3.9 ±0.05	4.7 ±0.05	10.5 ±0.29	20.08 ± 0.41
320	4.3 ±0.10	4.1 ±0.05	11.9 ±0.30	29.30 ±0.28

The study suggests that the earthworm paste could be a drug of choice for treatment and cure of ulcer, particularly when world is looking for natural remedies in view of alarming side effects of synthetic drugs. However, more studies shall be needed to further substantiate the results and bring a therapeutic agent for commercial use.

Acknowledgements

We wish to thank Dr. M.D. Owais, Department of Biotechnology, A.M.U. Aligarh, for help in the present investigation. Financial support of Department of Biotechnology, Government of India, New Delhi, for this programme is also acknowledged.

References

Bafna, P.A. and Balaraman, K., 2004. Anti-ulcer and anti-oxidant activity of Normacid, a herbomineral formulation. *Indian J. Exp. Biol.* 42: 674-680.

Bhatnagar, R.K. and Palta, R.K., 2002. Vermiculture and vermicomposting, Kalyani Publisehrs, New Delhi, India.

Gilbert, D.A., Surawicz, C.M., Silverstein, F.E., Wernberg, C.R., Saunders, D.R. and Feld, A.P. 1984. Prevention of acute aspirin induced gastric mucosal injury by methyl prostaglandin EZ: A microscopic study. *Gastroenterol*. 86: 339-345.

Goelm, R.K., Gupta, S., Shankar, R. and Sanyal, A.K., 1986. Antiulcerogenic effects of banana powder (*Musa sapientum* var. *paradisiaca*) and its effect on mucosal resistance. *J. Ethnopharmacol*. 18: 33-44.

Jaup, B., The mode of action of Pirenzopine in man with special reference to its anticholinergic muscarinic properties. *Scand. J. Gastroenterol*. 78: 11-26

Pandit, S., Sur, T.K., Jana, U., Bhattacharya, D., Debnath and P.K., 2000. Anti-ulcer effect of Shanka bhasma in rats: A Preliminary study. *Indian J. Pharmacol*. 32: 378-380.

Parmar, N.S., Hennings, G. and Gulati, O.P. 1984. The gastric antisecretary activity of 3-methoxy 5,7,3,4-tetra hydroxyl flavan (ME). A specific histidine decarobyxlase inhibitor in rats. *Agents Actions*, 15: 143-145.

Shah, Z.A. and Vohora, S.B., 2002. Anti-oxidant/restorative effects of calcined gold preparations used in Indian systems of medicine against flobal and focal models of Ischaemia. *Pharmacol. Toxicol*. 254-259.

Shay, H., Komarav, S.A., Fels, S.S., Meranze, D., Gruenstein, M. and Siplet, H., 1945. A simple method for the uniform production of gastric ulceration in rat. *Gastroenterology*, 5: 43-61.

Vohora, S.B. and Khan, M.S.Y., 1978. Animal origin drugs used in Unani Medicine, Institute of History of Medicine and Medical Research, Tughlaqabad, New Delhi, p. 137, 1978.

Yegnanarayan, R., Sethi, P.P., Rajhans, P.K., Dulandiran, K. and Ismail, S.A. 1987. Anti-inflammatory activity of total earthworm extracts in rats. *Indian J. Pharamco*., 19: 221-224.

Study of Beekh Papeeta (Male Papaya Root) for its Antifertility Effect in Experimental Models

[1]Jalis Ahmad,
[2]S.H. Afaq,
[2]N.A. Khan
and
[2]Kunwar Mohammad Yusuf Amin

[1]Dept. of Ilmul Advia, ZVM Unani Medical College and Hospital, New Modi Khana Road, Azam Campus, Camp, Pune (Maharashtra)

[2]Department of Ilmul Advia, Ajmal Khan Tibbiya College, Aligarh Muslim University, Aligarh-202002 (U.P.)

Abstract

Aqueous extract of male papaya (*Carica papaya* Linn) root was studied for its anti ovulatory and anti implantation effects in rats and rabbits to determine its potential to be a contraceptive or antifertility agent. In the test for anti ovulatory effect in rats, the animals were treated with 200 mg/kg of extract for 10 days and the vaginal smear was examined daily for oestrus/diestrus phase. Presence of diestrus phase persistently was considered as positive. While in rabbits the treatment was given only for three days (120 mg /kg) and the rabbits were thereafter treated with Cupric Acetate to induce ovulation. Forty eight hrs later the animals were laparotomised and the bleeding points in uterus were observed as the indicator of ovulation. Test for anti implantation activity was carried out in pregnant rats after 10 days of treatment; they were laparotomised and the two horns of the uterus were examined for implantation sites. The test drug was found to possess 100% anti ovulatory effect in both the rat and the rabbit models, while it produced weak anti implantation effect, as only in 20% of animals, absence of implantation sites was recorded. It can be concluded therefore that male papaya root (Beekh Papeeta, as known in Unani Medicine) possesses good anti ovulatory and weak anti implantation activity.

Key Words: Anti ovulatory, Anti implantation, Contraceptive, Antifertility, *Carica papaya*, Unani Medicine

Introduction

Population explosion is one of the foremost problems of the modern age. Therefore, the development of efficient birth control methods particularly reversible chemical contraception is a high priority area of research and development. Despite extensive efforts, fool-proof chemical contraceptives that are safe and cheap have still not been developed. Tibbe-Unani a proficient and highly sophisticated system of medicine is not wanting in this important group of drugs and possesses a large number of oral contraceptive drugs. Unani physicians have discussed this issue as early as in the middle ages and mentioned in their writings that contraception is worthy of discussion and a legitimate part of the medical practice and that a woman should not have children except of her own free will. They also suggested coitus interruptus and vaginal douching as means of preventing conception and also suggested a number of drugs for this purpose (Adil, 1969; Himes, 1963). However, Unani antifertility agents have not received adequate scientific attention.

The root of male papaya (*Carica papaya*) is an important drug claimed to possess anti fertility activity in Unani Medicine. Although, various parts of papaya are used in Unani as well as other traditional medicines (Ghari, 1921; Akah *et al.* 1997) but different pharmacological effects and the therapeutic value ascribed to these parts have still not been scientifically evaluated. Male papaya root is one such part that

is used as a contraceptive agent but has not yet been evaluated scientifically for this important effect. We also obtained the interesting verbal report about the folk use of male papaya root in Malaysia and several parts of India particularly the north east as a female contraceptive. The purgative effects of papaya root have been reported[4] but probably studies for its anti fertility potential has not been carried out. The Unani, ethnobotanical and modern reports about the antifertility action of papaya pertain mainly to unripe fruit, its milky juice (Latex) and seed (Satyavati et al., 1976; Amonymous, 1992; Saha and Sareen, 1961; Garg and Garg, 1971). It has been reported that unripe fruit may induce miscarriage in susceptible pregnant women (Adebiyi *et al.,* 2002). Its crude latex derived from unripe papaya fruits stimulates contraction even in non pregnant rat uterus (Schmidt, 1995). The papain found in its latex when administered to pregnant rats during the initial days of gestation produced teratogenic and embryo toxic effect (Adebiyi *et al.,* 2002). The abortifacient activity in the extract of papaya root has been mentioned without the specification of the sex of the plants (Saha and Sareen, 1961). Therefore, in the present study the aqueous extract of male papaya root was subjected to experimental testing for contraceptive activity. Since, the main aim of contraception is to prevent fertilization or implantation, therefore, both anti ovulatory as well as anti implantation effects were studied.

Material and Methods

Preparation of extract

The root of male papaya was obtained from Aligarh. Prof. S.H. Afaq, pharmacognosist at the department of Ilmul Advia, A.K. Tibbiya College, A.M.U., Aligarh confirmed the identity of the test drug. The bark of the root was separated and the root was crushed in an iron mortar. The crushed root was then extracted for 6 hours in soxhlet apparatus in distilled water. The extract was filtered and the solvent was evaporated on water bath. The yield percentage of the dried extract was 10% of the crushed drug. The dried extract was reconstituted in distilled water for the administration to the animals. The dose for albino rats was calculated by multiplying the human therapeutic dose by the conversion factor of seven (Freirich, 1966).

Test for anti ovulatory activity in Rats

The test for anti-ovulatory activity in rats was carried out by the method of Kamboj (Kamboj, 1982). Regularly cyclic adult female rats weighing 100-150 gm were taken and divided into 2 groups of 6 animals each such that the total weight of animals in various groups was approximately the same.

The animals in Group I served as plain control and treated with normal saline. The animals in Group II were fed with the extract of the root of male papaya orally at

a dose of 200 mg/kg once a day, for 10 days and the vaginal smear of the animals was examined daily for oestrus/diestrus phase. The animals persistently showing diestrus phase were recorded as positive and the percentage of such animals in each group was calculated.

Study for anti ovulatory activity in Rabbits

The test drug was studied for anti-ovulatory activity by the method of Chaudhry et al (Chaudhry *et al.,* 1970). Adult female rabbits weighing 1.3–1.5 kg were kept in isolation for 21 days to ensure that they were not pregnant and to prevent the induction of ovulation by mating. After isolation these animals were divided into 3 groups of 3 animals each.

The animals in Group I served as plain control and were treated with normal saline, while the animal in Group II served as standard control and administered norethisterone (German Remedies) at a dose of 0.25 mg/kg, once a day for 3 days by oral route with intragastric soft rubber catheter. The animals in Group III were treated with the extract of male papaya root at the dose of 120 mg/kg orally, once a day for 3 days. Thirty minutes after the administration of the last dose in each group, a freshly prepared 0.4% solution of Cupric Acetate was administered to each animal intravenously through the marginal ear vein at the dose of 4 mg/kg to induce ovulation.

To observe the ovulation laparotomy of the animal was carried out 48 hrs after the Cupric Acetate injection under light ether anaesthesia and the number of bleeding points on each ovary was noted as the indicator of ovulation. The percentage reduction of ovulation in the animals of standard and test groups was determined.

Study for anti implantation activity

The method of Khanna and Chaudhury was used to study the anti implantation activity (Khanna and Chaudhry, 1968).

Adult female albino rats of known fertility weighing 125-150 gm were used. The vaginal smear of the animal was studied for selecting the animals in pro estrus phase. They were allowed to mate with the male rats of proven fertility. Next morning the vaginal smear was examined for evidence of copulation as shown by the presence of thick clumps of spermatozoa. This day was designated as 1st day of pregnancy.

The pregnant animals were divided into 3 Groups of 5 animals each, such that the total weight of animals in each group was approximately the same.

The animals in group I served as the plain control and were administered normal saline. The animals in group II were treated with the extract of the test drug at the dose of 200 mg/kg once a day for 11 days. The rats were laparotomized on 12th

day of pregnancy under light ether anesthesia and the two horns of the uterus were examined for implantation site.

Observations and Results

Anti ovulatory effect in rats

The animals in control group showed complete absence of diestrus phase. While in test group all the animals showed persistently diestrus phase (Table-1).

Anti ovulatory effect in rabbits

Laparotomy of animals was carried out 48 hours after Cupric Acetate administration and the bleeding points on each ovary were noted as the indicator of ovulation.

All the animals in control group showed bleeding points in the ovary whereas the animals in test group and the standard group did not show bleeding points in the ovary, thus showing 100% inhibition of ovulation (Table-2).

Anti implantation Activity in rats

None of the control animals showed absence of implantation site, indicating that 100% animals retained the pregnancy. The papaya root treated animals showed that in 20% of animals the implantation site was absent while 80% retained the pregnancy (Table-3).

Discussion

The aqueous extract of the test drug produced 100% anti ovulatory effect, while exhibited only 20% anti implantation effect indicating strong anti ovulatory and weak

Table-1. Effect of Test Drug on ovulation in rats.

Group	No. of Rats used	Rats persistently showing diestrus phase	Rats showing regular cycle	% inhibition of ovulation
Control	10	0	10	0%
Male Papaya Root Extract 200 mg/kg	10	10	0	100%

Table-2. Effect of Test Drug on ovulation in rabbits

Group	No. of Rabbits used	Rabbits showing ovulation point	Rabbits showing no ovulation point	% inhibition of Ovulation
Control	3	3	0	0%
Root of male papaya root extract 120 mg/kg	3	0	3	100%
Northisterone 0.25 mg/Kg	3	0	3	100%

Table-3. Effects of Test Drug on implantation in rats

	No. of rats used	No. of rats showing implantation sites on 12 days	No. of rats showing no implantation sites 12^{th} day	Mean ±SE of implantation sites	Percentages of rats having no implantation sites on 12^{th} day
Control	5	5	0	5.4+0.357	0
Male papaya root extract 200 mg/kg	5	4	1	1.4 + 0.455*	20

* = $p < 0.01$

anti implantation activity in male papaya root.Since,the anti ovulatory effect, is more important than the anti implantation effect in contraception, the test drug is suggested to be a contraceptive agent and can be used to prevent or delay pregnancy.

In the test for anti ovulatory effect it was shown that inhibition of ovulation was 0% in control group while in the animal treated with the test drug inhibition was found to be 100% showing absolute anti ovulatory effect. The absolute inhibitory effect is considered a pre requisite when a drug is to be used as a contraceptive agent because failure of such a drug even in a single case will defeat the very purpose of such an important preventive measure. In the light of the importance of the anti ovulatory effect it was studied in two different animal groups. The test drug produced same degree of effect in both the groups despite the inter species difference of

animals. This further confirmed the anti ovulatory effect possessed by Papaya root and also provided an indication of its mechanism of action. Thus the root can be categorized as an important source of antifertility agent that possesses probably more striking effect as compared to the other parts or constituents of papaya.

The second test carried out for anti implantation activity demonstrated that 20 % of the animals showed absence of implantation sites while the mean implantation site in the group was found to be 1.4 + 0.455. The implantation sites were significantly lesser ($P< 0.01$) than the control group. The study, therefore, shows that the extract of root of male papaya possesses weak anti implantation activity .

The findings of the test drug as a whole are in consonance with the earlier reports. The root of papaya (sex of plant unspecified) is reported to possess abortifacient activity (Saha and Sareen, 1961). but there are no reports about its anti implantation effect. However, the petroleum ether extract of the pulp of unripe papaya fruit is reported to possess 60% anti-implantation activity whereas the alcoholic and aqueous extract are reported not to possess such an activity (Adebiyi, 2002). The latex of the green fruit is reported to possess oxytocic activity[8]. The seeds of the papaya[8] and oil of papaya plant (Garg, 1974) are reported to possess anti fertility activity without the specification of the mechanism of antifertility effect. The hexane extract of the seed has been shown to exert post coital contraceptive effect in high dose indicating anti implantation or early abortifacient effect (Keshri and Singh, 1993). Adebiyi and co-workers (Adebiyi, 2002). claimed that unripe fruit may induce miscarriage in pregnant women, while the ripe fruit or its marker compound papain in purified form do not possess such an effect at least in rats (Adebiyi, 2002; Schmidt, 1995). Thus the anti implantation activity has been shown only in Pet ether extract of the unripe fruit and even the alcoholic and aqueous extract of the unripe fruit lacks this property. The demonstration of weak anti implantation activity is therefore not absolutely in conflict with the earlier reports. Recently a complete loss of fertility has been demonstrated in male rabbits, rats and monkeys fed an extract of papaya seed (Lohiya *et al.,* 1999; Pathak et al., 2000; Lohiya, 2002). It appears that two component viz. latex and seed of papaya have adverse effect on the fertility; the former probably on female and the later on male species. As mentioned earlier that root of papaya has not been extensively studied for pharmacological activities; only few reports for its pharmacological actions such as purgative effect are available and that too without sex specification. Our study thus probably provides one of the earliest reports suggesting contraceptive effect in the root of male papaya. It has wide therapeutic potential and the delivery of a cheap female contraceptive is possible if the test drug is further subjected to experimental and clinical studies.

It can be concluded therefore that the present study scientifically validated the usage of male papaya root as antifertility and contraceptive agent in Unani Medicine and it also suggested the mechanism of the antifertility effect by demonstrating the anti ovulatory effect.

References

Adebiyi, A., Adaikan, P.G. and Prasad, R.N.V., 2002. Papaya comsumption is unsafe in pregnancy: Fact or Fable? Scientific evaluation of a common belief in some part of Asia using a rat model. British *Journal of Nutrition* 88, 199-203.

Adil, E., 1969. Proceeding of the Pakistan International F.P. Conference, Dacca, p. 36-47.

Akah, P.A., Oli, A.N., Enwerem, N.M., Gamaniel, K., 1997. Preliminary studies on purgative effect of *Carica papaya* root extract, *Fitoterapia,* 68, 327-331.

Anonymous, The wealth of India (Raw Material) 1992. Vol 1A, 1B, Publication and Information Directorate, C.S.I.R., New Delhi, 2786-293.

Chaudhry, R.R., Saksena, S.K. and Garg, S.K., 1970. Preliminary observations in rabbits on the anti ovulatory activity present in *Taxus baccata* Linn. Leaves, *J. Reprod. Ferti.* 22, 151-153.

Freirich, E.J., 1966. Quantitative comparision of toxicity of anti cancer agents in mouse, rat, dog, monkey and man, Cancer Chemotherapy Report, 50(4), 219-244.

Garg, S.K., 1974. Antifertility effect of oil from few indigenous plants on female albino rats, *Planta Medica,* 26(4), p. 391-393.

Garg, S.K. and Garg, G.P., 1971. Antifertility effect of *Areca catechu* and *Carica papaya* Linn in female albino rats *Ind. J. Pharmcol.,* 3(1), 23.

Ghani, N., 1921. Khazanatul Advia, Vol. II, Mataba Nawal Kishore, Lucknow, 34-35.

Himes, N.E., 1963. Medical History of Contraception. Gamut Press, New York p. 167-183.

Kamboj, V.P., 1982. Testing for the antifertility agents, lectures UNESCO to C.D.R.I. workshop on the use of Pharmacological techniques for Evaluation of Natural Products. C.D.R.I., Lucknow, p. 110-114.

Keshri, G. and Singh, M.M., 1993. Post coital antifertility activity of the seeds of *Carica papaya* Linn. in female albino rats, *Ind. Drugs* 30 (9), 453-457.

Khanna, U. and Chaudhury, R.R., 1968. Antifertility screening of Plants I: Investigation of *Butea monosperma* (Linn) Kuntze, *Indian J. Med. Res.,* 56 p. 1574-1580.

Lohiya, N.K., Mishra, P.K., Pathak, N., Manivannan, B., Jain, S.C., 1999. Reversible azoospermia by oral administration of the benzene chromatographic fraction of the chloroform extract of the seeds of C*arica papaya* in rabbits, *Advances in Contraception* 15, 141 161.

Lohiya, N.K., Mishra, P.K., Pathak, N., Manivannan, B., Sriram, S., Bhande, S.S., Panneerdos, S., 2002. Chloroform extract of *Carica papaya* seeds induces long term reversible azoospermia in langur monkey. *Asian Journal of Andrology* 4, 17-26.

Pathak, N., Mishra, P.K., Manivannam, B., Lohiya, N.K., 2000. Sterlity due to inhibition of sperm motility by administration of benzene chromatographic fraction of chloroform extract of the seed of *Carica papaya* in rats, *Phytomedicine* 7, 325-327.

Saha, J.C. and Sareen, A.D., 1961. Ecbolic properties of Indian medicinal plants part I. Ind. J. Med. Res., 49, 144.

Satyavati, G.V., Gupta, K.A. and Tandon, N., 1976. Medicinal plants of India, Cambridge printing works, Kashmiri Gate, Delhi, Vol. I, p. 185-187 and p. 292-293.

Schmidt, H., 1995. Effect of papain on different phases of parenteral ontogenesis in rats, *Reproductive Toxicology* 9 (1978), 49-55.

Singh, S. and Devi, S., 1978. Changes in placenta of rat fetuses induced by maternal administration of papaya, *Ind. J. Exp. Biol,.* 16(12), 1256-1260.

The Possible Role of Glutathione Reductase in the Glutathione-mediated Antioxidant Activity of Zahr Mohra (Serpentine) – An Experimental Study

[1]Sauduz Zafar Ali,
[2]Shamim Jahan Rizvi,
[3]S.M. Kashif R Zaidi,
[3]Naheed Banu,
[1]N.A. Khan
and
[1]Kunwar Mohammad Yusuf Amin

[1]Department of Ilmul Advia
Ajmal Khan Tibbiya College

[2]Interdisciplinary Brain Research Centre
Department of Forensic Medicine
Jawahar Lal Nehru Medical College

[3]Department of Biochemistry
Faculty of Life Sciences

Aligarh Muslim University
Aligarh 202002 (U.P.), India

Abstract

Zahr Mohra (Serpentine) a well-known, exclusively Unani drug has been shown by us to possess striking Antioxidant activity by increasing Reduced Glutathione in rats subjected to oxidative stress by immobilization (Ali *et al.,* 2008). One likely mechanism of this effect could be by increasing the activity of the enzyme Glutathione reductase (GR), responsible for the reduction of oxidized Glutathione. Therefore, in the present study it was tested for its effect on the level of this enzyme in serum, in rats subjected to oxidative stress by immobilization, with α-tocopherol (Vitamin-E) as the standard antioxidant agent for comparison. The study revealed that Zahr Mohra produces a significant increase in GR activity, in comparison of the untreated, stressed animals, which was not significantly different from the GR level in α-tocopherol-treated animals as well as in the nonstressed animals. Therefore, the study indicates that Zahr Mohra exerts Antioxidant activity, at least partially, by increasing Glutathione reductase activity. It also supports the demonstration of Antioxidant activity in Zahr Mohra in an earlier study by us.

Key Words: Zahr Mohra, Serpentine, Antioxidant, Glutathione, Glutathione reductase

Introduction

During the last two decades, there has been growing interest in studies that concern with the prevention of uncontrolled oxidative stress leading to various diseases in living system. Several studies have shown the role of oxidative stress in the causation and progression of different disease including atherosclerosis, carcinogenesis, neurodegenerative diseases, inflammatory conditions and early ageing (Stadman & Berlet, 1999) as well as prophylactic and therapeutic role of Antioxidant Drugs in such pathological condition such as Vit.E, Vit.C etc (Rice-Evans & Arif, 1999). Most of the effective and safe antioxidants are of Natural origin, so Traditional Medicines, mainly employing Natural drugs are being explored for better antioxidants (Farnsworth & Soejarto, 1985).

Restraint stress is a well known method for induction of physical and psychological stress (Kevtnansky, 1970) that results in restricted mobility and aggression (Singh, 1993) and produces a considerable pituitary-adrenocortical activation (Culman, 1980). It was demonstrated that it causes increased free radical generation (Liu J, 1994), brings about changes in activities of various free radical scavenging enzyme in plasma of rat (Hoidal, 1988), increases the lipid peroxidation in plasma and brain (Manolli, 2000) and decreases the concentration of glutathione and Vit C which play an important role in protection of tissues from oxidative.

Zahr Mohra or Serpentine is used exclusively in Tibb-e-Unani. Although, the most important use of Zahr Mohra in Unani Medicine is as a Tiryaq (Antidote) but other

Unani reports about it e.g. being protective to health, vital faculties, vital organs and nerve also suggest Anti-oxidant activity which is further supported by its reported Unani use in pathologies that may arise due to oxidative stress e.g. neuroasthenia, amnesia cardiac asthenia and several other inflammatory diseases (Ghani, 1920). In an experimental study, a compound Unani formulation having Zahr Mohra as the chief ingredient, namely, Jawahar Mohra, was found to possess adaptogenic activity (Ahmad *et al,* 1998). Similarly, in a clinical study, Kharmeera-e-Marwareed, which contains Zahar Mohra as an important ingredient, was reported to be effective in palpitation during pregnancy (Suboohi *et al,* 2001). Therefore, it can be hypothesized that Zahr Mohra may also act as a potent Anti-oxidant in oxidative stress. Thus, we subjected Zahr Mohra to a study for its effect on Reduced Glutathione, an important Anti-Oxidant parameter, in Serum of rats subjected to oxidative stress by immobilization. The drug has shown to produce a significant increase in GTH level (Ali *et al.,* 2008). Since, one of the mechanisms of GTH increase could be augmentation of the activity of the enzyme Glutathione Reductase (GR), responsible for the reduction, hence, reactivation of Glutathione. Therefore, in the present study Zahr Mohra was studied for its effect on GR in serum of rats subjected to oxidative stress by immobilization, with α-Tocopherol acetate (Vitamin-E) as the standard Anti-Oxidative agent for comparison.

Materials and Methods

Test Drug

The test drug, Zahr Mohra (Serpentine*)*, was obtained from Dawakhana Tibbiya College, Aligarh Muslim University, Aligarh, India. The identity was confirmed in the light of its Unani morphological description. A voucher specimen (No. B-21) was deposited in the Museum, Department of Ilmul Advia. A micro fine powder of Zahar Mohra (Serpentine) was prepared in china clay mortar and pestle and homogenized in Teflon Homogeniser at 2000 rpm and suspended in distilled water without any suspending agent. Vit.E (α-tocopherol acetate) used as the standard drug was obtained from Loba Chemicals. It was suspended in 5% gum acacia, for administration. The dose was determined by multiplying the Unani clinical dose with appropriate conversion factor (Dhawan, 1982) and found to be 15 mg/Kg for μ-tocopherol acetate and 35 mg/Kg for Zahr Mohra.

Chemicals

All the chemicals and reagents were of analytical grade and were obtained from various sources. Tris, Succinic acid, Potassium dihydrogen phosphate, Disodium hydrogen phosphate, KCL, Methanol, TCA, EDTA (S.D. Fine. India). α-tocopherol acetate, Glycyl glycine (Loba, India), CDNB, DTNB, GSH, GSSG, NADPH (SRL, India).

Animals and Treatment

Twenty eight male albino rats (Wistar strain), weighing 120-130 gm, were divided into 4 groups of 7 animals each. The animals were provided with standard diet (Purina) and tap water *ad libitum* and maintained at 20-25°C with 12 hour light and dark cycle. The animals were deprived of food for 12 hours before the administration of treatment, water was provided throughout the study. The animals in all the groups were administered with the treatment by oral route once a day for 7 days. The animals in all the groups except Gp I (Plain Control), were subjected to stress on the 7th day, as described later. The animals in Gp I & II that served as Plain Control and Stressed Gp, respectively, were administered with only the vehicle i.e. distilled water, while animals in Gp III serving as the Standard Gp were administered with µ-tocopherol acetate (15 mg/Kg), the animals in Gp IV, serving as test group, were administered with microfine powder of Zahr Mohra (35 mg/Kg BW). On the 7th day, immediately after giving the treatment, all the animals were subjected to immobilization stress in individual cages of their size for 6 hours (Hasan *et al.,* 1980, modified by Zaidi *et al.,* 2003). The animals were then removed from the cages and post-stress treatment was given as above. Forty-five minutes after the post-stress treatment, the animals were sacrificed by cervical dislocation.

Collection and Preparation of Biological Samples

After sacrificing the animals, the blood was collected and centrifuged at 2500 rpm for 10 minutes and separated serum was collected carefully and used for estimating the Glutathione reductase (GR).

Biochemical Investigations

Estimation of Glutathione Reductase (GR) activity: GR activity in serum was estimated by the method of Hazelton et al. (1985). Assay was based on the ability of GR to induce reduction of oxidized glutathione by NADPH to reduced glutathione observed by decrease in absorbance at 340 nm spectrophotometrically. Specific activity of enzyme was expressed as hM of NADPH oxidized/min/mg protein.

Protein estimation: Protein estimation was made since the concentration of all the test parameters, namely, GSH, GR, and GST, was expressed per mg of Protein. The estimation was carried out by the method of Lowry *et al.* (1951).

Statistical Analysis

The findings were statistically compared for determining significance of difference by one-way ANOVA Test followed by pair-wise comparison of various groups by LSD. The analysis was carried out by using the software of the website, www.analyseit.com.

Observations and Results

The Glutathione Reductase (GR) activity was found to be 0.0248 ± 0.0004 (n mole of NADPH oxidized/min/mg protein) in the Plain Control (Non-stressed) Gp, while restraint stress, applied on 7th day for 6 hours, induced marked decrease in the in GR in the Control (Stressed) Gp to 0.011 ± 0.0002 (n mole of NADPH oxidized/ min/mg protein), which was significantly lower as compared to the Plain Control (Non-stressed) Gp (P<0.001). In the Standard Gp, administered with a tocopherol acetate, the GR activity was increased in a highly significant manner to 0.034 ± 0.0014 (n mole of NADPH oxidized/ min/ mg protein), in comparison to both the Control (Stressed) Gp and the Plain Control (Non-stressed) Gp (P<0.001). In the animals treated with the test drug, namely, Zahr Mohra, GR was significantly increased to 0.0149 ± 0.0005 (n mole of NADPH oxidized/min/mg protein), in comparison of the Control (Stressed) Gp. (P< 0.05). However, it was not increased in comparison of the Standard Group or the Plain Control (Non-stressed) Gp. (Table-1).

Table-1. Effect of Zahr Mohra (Serpentine) on Glutathione reductase in serum of rats subjected to restraint stress

Group	GR (Mean ± SE)
Plain Control Gp.	0.0248 ± 0.0004
Control (Stressed) Gp.	0.011 ± 0.0002^{b2}
Standard Gp.	0.034 ± 0.0014^{a2b2}
Test Gp.	0.0149 ± 0.0005a^{1}

a = Control (stressed animals)
b = Against plain control (Non-stressed animals)
1 = P < 0.05; 2 = < 0.001
n = 7

Discussion

Reduced glutathione (GSH) is one of the most important non-enzymatic physiological antioxidant, which mainly scavenges and blocks the hydroxyl radical and singlet oxygen (Meister,1985) . Highly reactive hydroxyl radicals, against whom there is no enzymatic defense, have greater affinity for lipid molecules and are known to be one of the most important factors for initiation of chain reaction of lipid peroxidation, hence, cellular damage. So, OH blockade by increase in the concentration of GSH (Ali *et al.,* 2008), strongly indicates Antioxidant Activity in the Test Drug. Secondly, Glutathione also opposes the ROS by acting as a co-factor with Glutathione Peroxidase enzyme to catalyze the Catalase-like conversion of H_2O_2 to water and

molecular oxygen that additionally catalyzes the conversion of Lipid hydroperoxide to lipid alcohols (Larson, 1997). Thus, by diminishing H_2O_2, GSH blocks the generation of reactive oxygen. Thirdly, by re-reducing the oxidized enzyme, it causes their reactivation[45].

As mentioned, it was considered interesting to explore the possible mechanism of the increase in Reduced Glutathione by Zahr Mohra. One of the very important means of increasing Reduced Glutathione is an increase in the activity of Glutathione Reductase enzyme, that catalyzes the reduction of oxidized Glutathione with NADPH as the co-factor (Halliwell and Gutteridge,1985). Therefore, a striking increase in GR activity indicates that the test drug may be increasing Glutathione concentration at least partly by increasing the GR activity.

The present study revealed that Zahr Mohra produces a striking increase in the concentration of GR in serum of rats subjected to immobilization induced oxidative stress. Thus, the study indicates that the Reduced Glutathione-increasing, hence, Antioxidant Activity of Zahr Mohra is, at least partially, due to its ability to increase the activity of Glutathione Reductase enzyme. The study also provides support to the finding of our earlier study that Zahr Mohra increases Reduced Glutathione during oxidative stress. By showing that there is no significant difference in GR activity in animals treated with the test drug and the animals treated with the standard Antioxidant agent α-tocopherol, as well as, the unstressed animals, the study indicates that Zahr Mohra has a strong Antioxidant activity.

References

Ahmad, G., Amin, K.M.Y., Khan, N.A., Tajuddin, 1998. The anti stress activity of a gem containing Unani formulation against stressors. *J. of Ethnopharmacology.* January; Vol. 59. pp. 187-193.

Ali, S., Rizvi, S.J., Khan, N.A., Kashif, S.M., Zaidi, R., Banu, N. & Amin, K.M.Y., "The Study of Zahr Mohra (Serpentine) for Glutathione-mediated Antioxidant Activity in Rats Subjected to Oxidative Stress." (Accepted for publication in *Unimed*)

Culman, J., Kvetnansky, A., Kiss, E., Mczey, K., Murgas, K., 1980. Interaction of serotonin and catecholamines in individual brain nuclei in adrenocortical activity regeneration during stress. In: Usdin E, Kventnansky R, Kopin I (Eds), Catecholamine and Stress. Elsevier/ North Holland. Biochemical Press, The Netherlands, p.113.

Dhawan, B.N., 1982. Organization of biological screening of medicinal plants with special reference to cdri programme. Appendix-1, lectures UNESCO-CDRI Workshop on the Use of Pharmaco Techniques for evaluaion of Natural Product, CDRI, Lucknow p. 61.

Farnsworth, N.R., Soejarto, D.D., 1985. Potential consequence of plant extinction in the United States on the current and future availability of prescription drugs. *Econ. Bot.* 39: 231-246.

Ghani, M.N., Zahar Mohra, 1920. In: Khazain al Advia, Lucknow: Matbaa Munshi Nawal Kishore. Vol. 2. p. 690-691.

Halliwell, B., Gutteridge, M.C., 1985. Free Radical in Biology and Medicine. London Oxford Press, pp. 104, 110, 114, 135, 246-7, 268, 274-275, 287, 305, 312.

Hasan, M., Ali, S.F., 1980. Organophosphate pesticide dichrovos induced increase in the rate of Lipid Peroxidation in the different region of rat brain, supporting ultra structural findings. *Neurotoxicity;* 2: 43-52.

Hoidal, J.R., Van Asbek, B.S., Mann, J., Jacob, H.S., Kenedy, T.P., 1988. Therapy with red blood cell decreases hyperoxic pulmonary injury. *Exp. Lung Res.*; 14 (Suppl): 977-85.

Kvetnansky, R., Mikulaj, L., 1970. Adrenal and urinary catecholamines in rats during adaptation to repeated immobilization stress. *Endocrinol.;* 87: 738-43.

Larson, R.A., 1997. Naturally occurring Antioxidants. New York: Lewis Publishers. p. 67-79, 169-172.

Liu, J., Wang, X., Mori, A., 1994. Immobilization stress induced antioxidant defense changes in rat plasma: effect of treatement with reduced glutathione. *International J. Biochemistry.* 26: 511-517.

Manolli, L., Gamaro, G.D., Silveira, P.P., Dalmaz, C., 2000. Effect of chronic variate stress on thiobarbituric acid reactive species and on total radical trapping potential in distinct regims of brain. *Neurochemical Research,* 25: 915-921.

Meister, A., 1985. Methods in Enzymology; Meister A., (Eds) New York. Academic Press. Vol. 113, p 499

Meister, A., 1985. Methods for selective modification of glutathione metabolism and study of glutathione transport. In: Methods in Enzymology; Meister A., (Eds) New York. Academic Press; Vol. 113, p. 571-585.

Rice-Evans, Arif, S., 1999. Dietary Antioxidant and Nutrition, In: Gilbert, L.D., and Colton, C.A., (Eds.), Reactive Oxygen Species in Biological System: An Interdisciplinary Approach. New York: Kluwer Academic/ Plenum Publishers; p. 367 –399.

Singh, L.K., Ray, X., Alexacos, N., Netaumen, R., 1993. Theoharides. Acute immobilization stress triggers skin mast cell degranulation via corticotropin releasing harmone neurotension and substance link to neurogenic skin disorders. *Brain Behav. Immunol.* 3: 225-239.

Stadman, E.R., Berlett, B.S., 1999. Reactive Oxygen-Mediated Protein oxidation in aging and Disease. In: Gilbert, L.D. and Colton, C.A. (Eds.), Reactive Oxygen Species in Biological System: An Interdisciplinary Approach. New York. Kluwer Academic/ Plenum Publishers; p. 657-671.

Suboohi Mustafa, 2001. The effect of Khameera Marwareed in palpitation during pregnancy. *Hamdard Medicus,* Vol XLIV No (2):123-126

Zaidi, S.M.K.R., Al-Qirim, T.M., Hoda, N., Bano, N., 2003. Modulation of restraint stress induced oxidative changes in rats by antioxidant vitamins. *The J. Nutritional Biochemistry.* 4: 633-636.

A Clinical Study of the Unani Formulation UNIM-210 for Anti-Diabetic Effect

[1]Radhey Shyam Verma,
[1]Shabana Parveen,
[1]Imranullah Khan,
and
[2]M.K. Siddique

[1]Regional Research Institute of Unani Medicine (CCRUM), Post Box No. 70, Aligarh-202002, U.P. (India)

[2]Central Council for Research in Unani Medicine, 61-65, Institutional Area, Janakpuri, New Delhi-110058

Abstract

The effect of Unani coded drug UNIM-210 was evaluated in twenty patients with type-2 diabetes. The patients included in trial group were given UNIM-210 two tablets of 500 mg each twice daily, orally with water for a period of 150 days along with normal diet. UNIM-210 significantly lowered the biochemical parameters such as fasting (FF) glucose level (28%), post prandial (PP) glucose level (18%), blood urea (23%), serum creatinine (10%), serum glutamate pyruvate transaminase (SGPT) (44%) and serum glutamate oxaloacetate transaminase (SGOT) (33%), whereas no significant changes were observed in total protein level. A significant decrease was observed in the level of serum albumin (13%), when compared with before treatment to the after treatment of drug UNIM-210. In follow-ups (I to IVth) studies a gradual reduction in fasting glucose but not gradual decrease was observed in post prandial glucose level. Pathological studies had shown that a significant reduction were observed in erythrocyte sedimentation rate (ESR) (59%), total leucocyte counts (TLC) (12%), polymorph (15%) and eosinophil counts (EOS) (13%) and statistically non significant except ESR and polymorphs ($P< 0.0001$), when compared with before treatment to the after treatment of the patients to this drug. No significant changes had been observed in hemoglobin level and red blood corpuscles (RBC) counts.

Key Words: Type-2 diabetes, Unani Medicine.

Introduction

Diabetes is a heterogeneous metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism (Das *et. al*, 1996). The management of diabetes is considered as a global problem. The modern drugs including insulin and oral hypoglycemic agents control the blood sugar level as long as they are regularly administered, and also produce a number of undesirable side effects (Upadhaya *et.al*., 1996; Reynolds, J.E.F., 1997). Unfortunately, none of the oral synthetic hypoglycemic agents has been successful in maintaining euglycaemia and controlling long term microvascular and macrovascular complications (Larner, J., 1985; Momin, A., 1987; Stenman *et. al*., 1990).

The use of herbal medicines for the treatment of diabetes mellitus has gained importance throughout the world. Although, there are numerous traditional medicinal plants reported to have hypoglycemic properties (Aiman; 1970), many of them proved to be not very effective in lowering glucose levels in severe diabetes (Nagarajan *et. al*., 1978). Therefore, there is a need to search for effective safe drug for the treatment of diabetes mellitus (DM). Keeping in view the above facts,

Present address: Regional Research Centre, Unani Medical College, Himmatganj, Allahabad-211016 (U.P.)

the efficacy of Unani coded drug UNIM-210 was evaluated in the management of diabetes mellitus.

Materials and Methods

UNIM-210 was obtained from Central Council for Research in Unani Medicine, New Delhi. The study was carried out at Regional Research Institute of Unani Medicine (RRIUM), Aligarh. Sixty patients attending in the out patients department (OPD), RRIUM of either sexes, age (25-65 yrs) were screened to assess the various biochemical and pathological parameters. Out of sixty patients, twenty patients were selected for clinical trail. Criteria for selection of patients were based on the measurement of sugar level in fasting state (FF) (12 hours) and post prandial (PP) blood sugar after 1.30 hours after meals.

Collection of blood and serum

Blood samples were collected by puncturing the vein at each investigation. One ml. of blood with ethylene diamine tetra acetic acid (EDTA) was used for various pathological parameters and other 2-3 ml of blood samples were allowed to clot for 30 min and serum was separated by centrifugation, which was used for various biochemical parameters. Biochemical and pathological investigations were carried out as follows:

Biochemical analysis

Biochemical parameters, carried out are the followings. Blood sugar was estimated by O-toluidine method (1959). Serum cholesterol was measured by Wybenga and Pileggi method (1970). Triglyceride was measured by GPO/PAP method (1969). Blood urea was done by di acetyl mono-oxime (DAM) method (1951). Serum creatinine was measured by alkaline picrate method (1945). Total protein, albumin and globulin were measured by modified Biuret and Dumas method (1971). Serum glutamic pyruvic transaminase (SGPT, E.C.2.6.1.2) and Serum glutamic oxaloacetic transaminase (SGOT, E.C. 2.6.1.1.) were estimated by Reitman and Frankel method (1957).

Pathological analysis

It includes, Hemoglobin (Hb), Erythrocyte Sedimentation Rate (ESR), Red Blood Corpuscles (RBC), Total Leukocyte Count (TLC), Differential Leukocyte Count (DLC): (Polymorphs, Lymphocytes, Eosinophils, Monocytes, and Basophils). The percent hemoglobin was done by Sahli's Acid Haematin Method, Newcomer (1919). ESR was measured by westergreen method, Mukherjee (1990). TLC and RBC counts were done by haemocytometry method, Plum (1936). DLC was done by Leishman stain method, Mukherjee (1990).

Drug, Dose and mode of administration

Compound Unani formulation coded as UNIM-210 was administered as two tablets of 500mg each, twice daily, orally with water after meals.

Duration of treatment and follow-up

Duration of treatment was 150 days. After registration of patients; base line observations were made before starting the treatment was carried out by investigating all the biochemical and pathological parameters. Post-treatment observations were made at intervals of 30 days, 60 days, 90 days, 120 days and 150 days in which all biochemical and pathological parameters were carried out.

Statistical Analysis

Values are expressed as mean ± standard error of mean (n=20). Data were analysed statistically by one-way analysis of variance (ANOVA) followed by Dennett's't' test. The values were considered significant when the P-value was less than 0.05.

Results and Discussion

Biochemical Study

In normal physiology glucose homeostasis is maintained by two kinds of hormones, including growth hormone, cortisol and catecholamine (Cryer and Polonsky, 1998; Pilkis and EI-Maghrabi, 1988; Gerich, 1988). Theoretically, a herbal drug with hypoglycemic activity may act via the following fundamental mechanisms, at the intestinal level, by delaying or inhibiting glucose absorption (Anderson and Akanji, 1991; Adamson and Okafor, 1990). At the pancreatic level, by stimulating the secretion of insulin (Noor *et. al*., 1989).At the peripheral level, by facilitating the entry of glucose into cells (Miura and Kato, 1995; Kato *et. al*., 1995) and at peripheral level by decreasing the glucose utilization and at hepatic level by decreasing the hepatic glucose production (Vats *et. al*., 2004; Hanson and Reshef, 1997).

The present study has shown that UNIM-210 causes a significant decrease in fasting blood glucose (28%) and post prandial glucose (18%) level, blood urea (23%), Serum creatinine (10%), serum albumin (14%), serum globulin (20%), S.G.P.T. (44%) and S.G.O.T. when compared with pre-treatment values. These values were statistically significant ($P<0.01$) (Table 1 & 2). There was no significant change in serum cholesterol and albumin. A significant reduction were observed in both the fasting as well as Post prandial glucose levels in Ist, II, III and IV^{th} post-treatment testing, when compared with pre-treatment levels (Table 3). (Sharma *et. al*., 1983) had reported that the possible mechanism may be either increase in the utilization

Table-1. Effect of Unani coded drug UNIM-210 on the levels of blood glucose, serum cholesterol, serum triglycerides, blood urea and serum creatinine.

	Sugar level (mg/dl)		Serum Choles-terol (mg/dl)	Serum Trigly-cerides (mg/dl)	Blood Urea (mg/dl)	Serum Creatinine (mg/dl)
	Fasting Sugar (FF)	Post Prandial Sugar (PP)				
Before treatment	190.77 ± 7.47	253.31 ± 1.41	179.77 ± 10.1	165.05 ± 9.73	35.61 ± 1.26	1.29 ± 0.02
After treatment	136.56 ± 8.48**	206.82 ± 1.73**	175.04 ± 9.64NS	133.57 ± 2.91NS	27.55 ± 1.46***	1.16 ± 0.04**

*p<0.05, **p<0.01, ***p<0.001, NS p<0.1 (not significant).

Table-2. Effect of Unani coded drug UNIM-210 on the levels of SGPT, SGOT, serum protein, serum albumin, serum globulin and A/G ratio.

	S.G.P.T. (Unit/ml)	S.G.O.T. (Unit/ml)	Serum Total Protein (gm/dl)	Serum Albumin (gm/dl)	Serum Globulin gm/dl	A/G Ratio
Before treatment	25.07 ± 0.83	21.80 ± 1.66	6.93 ± 0.19	4.82 ± 0.13	4.24 ± 0.15	1.84 ± 0.12
After treatment	14.00 ± 1.05***	14.60 ± 0.96***	7.67 ± 0.16**	4.18 ± 0.15**	2.99 ± 0.20***	1.6 ± 0.10NS

*p<0.05, **p<0.01, ***p<0.001, NS p<0.1 (not significant).

in the periphery or decrease in the endogenous glucose production in the liver and also there is a possibility that the extracts may inhibit the proximal tubular reabsorption mechanism for glucose in the kidney which can also contribute towards blood glucose lowering effect.

Pathological Studies

The present study has shown that there was a significant decrease in ESR (59%), (P<0.0001), TLC (12%), polymorphs (15%), (P<0.001) and eosinophils count (13%)

Table-3. Effect of UNIM-210 on the blood glucose level of different follow-up in diabetic patients.

Group → Parameter ↓	Before treatment	First follow-up	Second follow-up	Third follow-up	Forth follow-up
Blood Glucose Fasting (FF) (mg/dl)	190.77 ± 12.92	144.23 ± 7.55**	155.54 ± 6.93**	127.42 ± 3.84***	128.94 ± 5.34***
Blood glucose Post Prandial (PP) (mg/dl)	253.31 ± 9.23	206.59 ± 8.68***	237.86 ± 10.85NS	207.57 ± 6.77***	227.24 ± 11.68*

*p<0.05, **p<0.01, ***p<0.001, NS P<0.1 (not significant).

Table-4. Effect of Unani coded drug UNIM-210 in the levels of haemoglobin, E.S.R., R.B.C. counts, total leucocytes count (T.L.C.) and differential leukocytes count (D.L.C.).

	Hemo-globin (Hb) (gm %)	E.S.R. (mm/hr)	R.B.C. (10^{-6}/ m^3)	T.L.C. (10^3/ mm^3)	Differential Leucocyte Count (DLC)		
					Poly-morphs (%)	Lympho-cytes (%)	Eosino-phils (%)
Before Treatment	12.98 ± 0.17	15.31 ± 1.62	4.70 ± 0.16	8.15 ± 0.36	71.81 ± 1.81	35.75 ± 1.53	2.94 ± 0.67
After Treatment	13.29 ± 0.24***	6.25 ± 1.46***	5.03 ± 0.14NS	7.17 ± 0.54NS	61.19 ± 2.23***	36.19 ± 2.33NS	2.56 ± 0.35NS

*p<0.05, **p<0.01, ***p<0.001, NS p<0.1 (not significant).

but these values were within the normal range, when compared with pre- treatment values. The increase in pre-treatment values may be due to severe infections such as emphysematous pyelonephritis, papillary necrosis, perinephric abscess, Candida pyelonephritis, otitis, rhinocerebral mucormycosis, foot infection, pulmonary infection, urinary tract infection in type-2 diabetic patients (Wheat, 1980; Earhart and Baugh, 2005; Ljubic *et. al.*, 2005; Stamm and Hooton, 1993). Pickup (2004) had also reported that activated innate immune system is closely involved in pathogenesis

of type-2 diabetes and associated complications such as dyslipidemia and atherosclerosis.

The findings of the present study show thatUNIM-210 increased hemoglobin and decreased the ESR and polymorphs, significantly. Similar results had also reported by Shim *et. al.*; 2006, they observed that WBC, neutrophil, lymphocyte, monocyte and eosinophil counts were higher in the patients with metabolic syndrome (MS) features than those without MS features.

Further investigations are required to find out the mechanism. In conclusions, the present study indicates that Unani coded drug UNIM-210 exhibited hypoglycemic activity in diabetic patients.

Acknowledgement

The work is supported by the Central Council for Research in Unani Medicine, New Delhi. We are thankful to all the staff of Biochemistry and Pathology Laboratory of RRIUM, Aligarh and Mr. Shamsul Huda, typist, for their help.

References

Adamson, I., Okafor, C., 1990. A supplement of dikanut (*Irvingia gabonensis*) Improves treatment of type II diabetes. *African J. Medicine* 9, 108-115.

Aiman, R., (1970). Recent research in indigenous anti-diabetic medicinal plants, an overall assessment. *Indian J. Physiol. Pharmacol.* 14, 95-105.

Cryer, P.E., Polonsky, K.S., 1998. In: Wilson, J.D., Foster, D.N., Kronenberg, H.M., Larsen, P.R. (Eds.), Williams Textbook of Endocrinology, 9th ed. W.B. Harcovert Publishers Limited pp. 939-971.

Das, A.V., Padayutti, P.S., Paulose, C.S., 1996. Effect of leaf extract of *Aegle marmelos L* on the histological and ultrastructural changes in tissues of streptozotocin induced diabetic rats. *Indian J Exp Biol* 14, 340.

Earhart, K.C., Baugh, W.P., 2005. Rhinocerebral mucormycosis. Available at: http: //www.emedicine.com/med/topic 2026.htm.

Gerich, J.E., 1988. Glucose counterregulation and its impact on diabetes mellitus. *Diabetes*, 37, 1608-1617.

Hanson, R.W., Reshef, L., 1997. Regulation of phosphoenolpyruvate carboxkinase GTP) gene expression. *Annu. Rev. Biochem. 66, 581-611.*

Hultman, E., (1959). Rapid specific method of determination of aldosaccharides in body fluids. *Nature*, London 183, 108-111.

Kato, M., Miura, T., Usami, M., Kato, A., Kadowaki, S., 1995. Hypoglycemic effect of the rhizomes of Ophiopogonis tuber in normal and diabetic mice. *Biological and Pharmaceutical Bulletin* 18, 875-877.

Larner, J., 1985. Insulin and oral hypoglycemic drugs, glucagons. In the Pharmacological Basis of Therapeutics (Eds.), Gilman, A.G., Goodman, L.S., Rall, T.W., Murad, F., Macmillan, New York, PP: 1490-1516.

Ljubic, S., Balachandran, A., Pavliae-Renar, I., 2005. Pulmonary infections in diabetes mellitus. *Diabetologia Croatica* 4, 115-124.

Miura, T., and Kato, A., 1995. The difference in hypoglycemic action between *Polygonati rhizome* and *Polygonati officinalis* rhizome. *Biological and Pharmaceutical Bulletin* 18, 1605-1606.

Momin, A., 1987. Role of indigenous medicine in primary health care. In proceedings of First International Seminar on Unani Medicine, New Delhi, India, pp. 54.

Mukherjee, K.L., 1990. Medical Laboratory Technology, 3rd Edition pp 228-307, Tata Mc Graw-Hill Publishing Company Limited, New Delhi.

Newcomer, H.S., 1919. Absorption spectra of acid hematin, oxyhemoglobin and carbon monoxide, hemoglobin: A new hemoglobinometer. *J. Biol. Chem.* 37, 465.

Noor, H., Hammond, P., Sutton, R., Ashcroft, S.J.H., 1989. The hypoglycemic and insulinotropic activity of *Tinospora crispa*: Studies with human and rat islets and HIT-TIS B cells. *Diabetologia* 32, 354-359.

Pickup, J.C., 2004. Inflammation and activated innate immunity in the pathogenesis of type-2 diabetes. *Diabetes Care* 27, 813-823.

Pilkis, S.J., EI-Maghrabi 1988. Hormonal regulation of hepatic gluconeogensis and glycolysis. *Annual Review of Biochemistry* 57, 755-785.

Plum, P., 1936. Accuracy of haematological counting method. *Acta Med. Scandinav* 90, 342.

Steinman, S.P.H., Groop, K., Laakkonen, E., Wahlin-Boll, E., Melander, A., 1990. Relationship between sulphonyl urea dose and metabolic effect. *Diabetes* 39, 108 A.

Reitman, S., Frankel, S., 1957. A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* 28, 56-63.

Reynolds, J.E.F., Martindale, 1997. The Extra Pharmacopoeia, 30th Ed, (Pharmaceutical Press, London), 276.

Sharma, M.K., Khare, A.K., Feroz, H., 1983. Effect of neem oil on blood glucose levels of normal, hyperglycemic and diabetic animals. *Indian Medical Gazette* 117, 380-383.

Shim, W.S., Kim, H.J., Kang, E.S., Ahn, C.W., Lim, S.K., Lee, H.C., Cha, B.S., 2006.The association of total and differential white blood cell count with metabolic syndrome in type-2 diabetic patients. *Diabetes Research & Clinical Practice* 73, 284- 291.

Stamm, W.E., Hooton, T.M., 1993. Management of urinary tract infections in adults. *N Engl. J. Med.* 329, 1328-1334.

Vats, V., Yadav, S.P., Grover, J.K., 2004. Ethanolic extract of *Ocimum sanctum* leaves partially attenuate streptozotocin-induced alterations in glycogen content and carbohydrate metabolism in rats. *J. Ethnopharmacology,* 90: 155-160.

Upadhayay, O.P., Singh, R.M., Dutta, K., 1996. Studies on the antidiabetic medicinal plants used in Indian folklore. *Aryavaidyan* 9, 159.

Wheat, L.J., 1980. Infection and diabetes mellitus. *Diabetes Care 3, 187-197.*

Standardization of an Antioxidant Unani Herbal Drug "Zafran"

[1]Sonali Sajwan,
[2]Kunal Sajwan,
[3]V.K. Singh
and
[3]M.K. Siddiqui

[1]Drug Standardization Research Institute (CCRUM), PLIM Building, Kamla Nehru Nagar, Ghaziabad, U.P.

[2]Drug Standardization Research Unit (CCRUM), Jamia Hamdard, New Delhi

[3]Central Council for Research in Unani Medicine, 61-65, Institutional Area, Janakpuri, New Delhi-110058

Abstract

Zafran, a Unani drug of repute is known as Saffron in English and Kesar in Hindi, consists of dry stigmas and top of style of *Crocus sativus* L. (Fig. 1), belongs to family Iridaceae. The drug is used for the treatment of amenorrhea, colic, leucorrhoea and headache, besides exhibiting antioxidant and anti-cancerous properties. It is produced mainly in Spain (70% of world supply), and other producers are Iran and India, mainly Pampur (Kashmir). It contains Crocin as a main constituent, which is responsible for its colouring property and offers the distinctive aroma, taste and aromatic oil.

Present studies deal with the standardization of Zafran, and include macroscopy, microscopy and powder analysis, TLC with marker compound, extractive values and ash values. All these standards may be fruitful for correct identification and quality assurance of the drug and help manufacturing quality medicines of Indian Systems of Medicine.

Key Words: *Crocus sativus,* Crocin, Standardization, Zafran, Stigma, TLC.

Introduction

Zafran is used frequently in Unani system & other Indian Systems of Medicine. It consists of stigmas and style of *Crocus sativus* L., and belongs to the family Iridaceae (Evans, 2002; Iyenger, 1997; Sarin, 1995; Anonymous, 2004). Plant is native of South Europe and cultivated in Spain, Itly, India and China. In India its cultivation is mostly confined to Pampur (in Kashmir) and Kishtwar (in Jammu) (Anonymous, 1950).

True Zafran should not be confused with American saffron (safflower), i.e. *Carthamus tinctorious*, which is produced from tubular florets and is lighter red then true Zafran. It is used in Chinese medicine and in the West it is employed to a limited extent as a colouring and flavouring agent (Youngken, 2004).

Fig. 1. Flowers of *Crocus sativus* L.

The drug is used for the treatment of amenorrhoea, colic, leucorrhoea and headache, besides exhibiting antioxidant and anti-cancerous properties (Takashi, 2004; Evans, 2002). It is produced mainly in Spain (70% of world supply), and other producers are Iran and India, mainly Pampur (Kashmir) (Youngken, 2004; Anonymous, 2004; Evans, 2002). It contains Crocin as a main constituent, which is responsible for its colouring property and offers the distinctive aroma, taste and aromatic oil.

Material & Methods

The material has been collected from Pampur (Srinagar, J & K), India. For microscopic study, material was first soaked in water for 24 hours and then stained with safranin or other staining materials and studied microscopically. Microphotographs were taken with the help of Microscope with camera attachment. The physico- chemical studies of powdered drug were carried out according to the methods given in The Ayurvedic Pharmacopoeia (2004). For TLC fingerprint profile, the extract was prepared with Ethanol and water. 1 mg of crocin was dissolved in 1 ml of Ethanol separately. These extracts and the crocin were applied with a Desaga applicator on a precoated silica gel TLC plates (E. Merck) of uniform thickness of 0.2 mm. The fingerprint profiles were obtained with a Desaga Video Documentation Unit.

Observations

Macro-morphology

Saffron occurs in loose masses consisting of reddish cylindrical funnel shaped reddish brown three (3) stigmas with dentate or fimbricate margin, odour strongly aromatic, taste bitter (Fig. A).

Micro-morphology

T.S. shows thin walled elongated paranchymatous cells containing colouring matter covered with thick cuticle (fig. 1). At the upper end numerous cylindrical papillae (fig.3) up to 145µ in length. Pollen grains few, spherical, nearly smooth measuring 50-106µ in diameter (fig. 2).

Fig. A. Crocus sativus L Style & Stigma

Microphotographs of Zafran

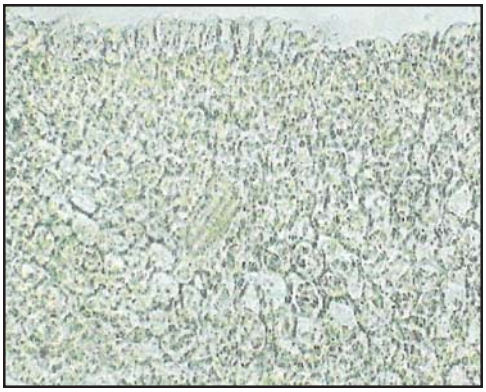


Fig. 1. T.S. Stigma of C. sativus x 40

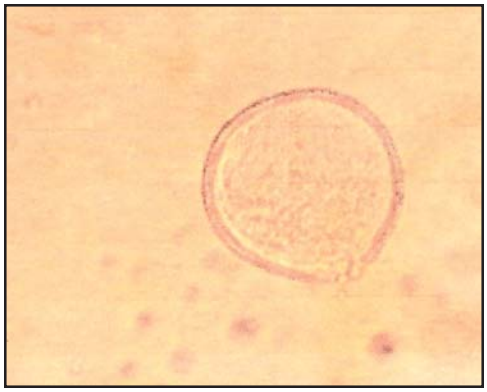


Fig. 2. Pollen grain of C. sativus x 40

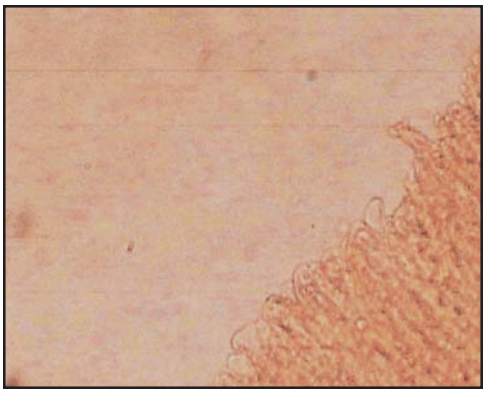


Fig. 3. Pappilae in stigma of C. sativus x 40

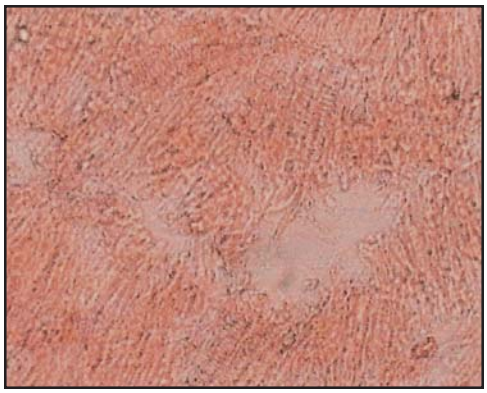


Fig. 4. Annular and spiral vessels of C. sativus

Powder Analysis

Powder studies reveals following diagnostic features: pieces of papillae (fig. 3), parenchyma cells (fig. 2), and smooth spherical pollen grains 50-106µ in diameter (fig. 2). Some vessels with annular and spiral thickenings (fig. 4) were present.

Adulterants & substitutes

1. Zafran has been adultrated with ligulate flowers of *Calendulla officinalis*.
2. Tubular florets of *Carthamus tinctorious*.
3. Basal portion of the styles and dried floral parts, such as stamens and petals of *Crocus* have also been employed as adulterants of crude drug.
4. Dried style of *Zea mays* (corn silk).

Test for Zafran

When Zafran stigmas placed in Sulphuric acid, it takes blue colour immediately and then changing to violet and finally to deep wine colour.

Physico chemical studies

The determination of ash values, alcohol and water extratives were made from air-dried material. The procedures recommended by Anonymous (2004) were followed for calculating total ash, acid insoluble ash, alcohol and water extractive percentages. Total ash 4.5-5.0%, acid insoluble ash 0.45-0.48%, Hexane extractive 14.5-16%, Ethanol soluble extractive 18-21% and water soluble extractive 55-58%.

HPTLC with marker compound

Crocin is the main constituent of Zafran, 1 mg of Crocin is dissolved in to 1 ml of Methanol for preparing standard solution water and alcoholic extracts of the Zafran was taken for this purpose and the TLC plate was developed in n-butanol: Acetic Acid: Water in 50 : 10 : 20 ratio (Wagner 1996) and sprayed with Anisaldehyde Sulphuric acid reagent and the finger print profiles are documented under visible light, UV 254nm, 366nm & after derivatization (fig. 5a, 5b, 6a, 6b & 6c).

ZAFRAN: HPTLC PROFILE

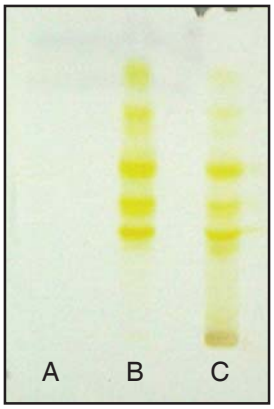


Fig. 5(a) Visible

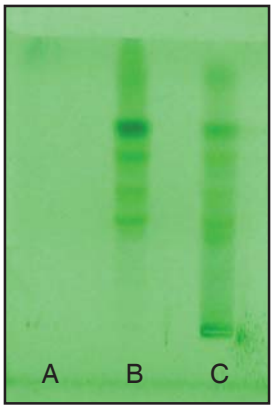


Fig. 5(b) UV 254 nm

A: Pet. Ether Ext. B: Alcohol Ext. C: Aqueous Ext

Solvent System: n-Butanol: Acetic Acid: Water (50:10:20)

Results and Discussion

Zafran, one of the highly efficacious herbal drugs in Unani system of medicine has been used since long but often found adulterated or substituted, because of lack of knowledge and standardization skills.

Thus, the study is likely to help in the quality assurance of drug used in traditional system of medicine, particularly in development of standard parameters.

ZAFRAN: HPTLC PROFILE WITH MARKER

Fig. 6(a) Visible

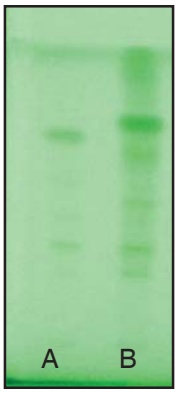


Fig. 6(b) UV 254 nm

Fig. 6(c) Derivatization

A: Crocin (5µl) B: Alcohol Ext.

Spray Reagent: Anisaldehyde-Sulphuric Acid

Solvent System: n-Butanol: Acetic Acid: Water (50:10:20)

Acknowledgements

The authors are thankful to Dr. Shamshad Ahmad Khan, Asstt. Director Chemistry, CCRUM Hqs, New Delhi and Dr. Rajeev Kumar Sharma, Senior Scientific Officer, PLIM, Ghaziabad, for encouragement and Dr. Seema Akbar, Research Officer Chemistry, RRIUM, Srinagar J & K, for providing the authentic sample of drug investigated from Pampur, Srinagar (J & K).

References

Anonymous, 1950. Wealth of India, Vol II, CSIR, pp. 370-371.

Anonymous, 2004. The Ayurvedic Pharmacopoeia of India, Ministry of Health & Family Walfare, New Delhi, Part I Vol. IV, pp. 52-54.

Evans, W.C., 2002. Pharmacognosy, W.B. Saunders Publications, XV Edition, 437-438.

Iyenger, M.A., 1997. Pharmacognosy of Powdered Crude Drugs, V Edition, Iyenger Series, Manipal, India, p. 77.

Sarin, Y.K., 1995. Illustrated Manual of Herbal Drugs used in Ayurveda CSIR & ICMR, New Delhi, p. 185.

Takashi Ochiaia, Shigekazu Ohnoa, Shinji Soedaa, Hiroyuki Tanakab, Yukihiro Shoyamab and Hiroshi Shimeno, 2004. *Neuroscience Letters*, 362(1): 61-64.

Wagner, H. and Bladt, S., Plant Drug Analysis, 1996. Springer Verlag, Berlin Hiedelberg, pp. 288-289.

Youngken, H.W., 2004. Natural Drugs, Biotech Books, Delhi, pp. 123-126.

Ingredient Identification in "Itrifal-e-Kishneezi" – A Polyherbal Formulation in Unani System of Medicine

[1]Kiran Negi,
[2]V.K. Singh
and
[2]M.K. Siddiqui

[1]Drug Standardisation Research Unit
(Central Council for Research
in Unani Medicine)
Hamdard University,
New Delhi-110062

[2]Central Council for Research
in Unani Medicine
61-65, Institutional Area,
Janakpuri, New Delhi-110058

Abstract

Quality control and standardization of the drug is a dire need today. The raw materials brought into the market in various forms are frequently adulterated with cheap or less potent plant materials. Some greedy manufacturers in order to achieve maximum profit utilize these plants and their products to prepare spurious and unbalanced therapeutic formulations which prove to be ineffective to cure any ailment. Hence proper identification and authentication of all the plant ingredient present in a compound formulation is a must. Present paper deals with ingredient identification in 'Itrifal-e-Kishneezi', a polyherbal formulation in Unani System of Medicine which is frequently used by unani physicians to cure gastric headache, conjunctivitis, otalgia, flatulence, bleeding piles and chronic catarrah since time immemorial. All the ingredients which are required in the preparation of Itrifal-e-Kishneezi are examined separately (Both macroscopically and microscopically) followed by the microscopic examination of this formulation as a whole. This will provide a key of diagnostic histological characters which serves as an important tool for quality control purpose.

Key Words: Itrifal-e-kishneezi, Polyherbal formulation, Standardization.

Introduction

In Unani System of Medicine, "Itrifal" is a semisolid medicinal preparation where one or more single drugs of plant, animal or mineral origin are mixed along with Triphala (three myrobalan fruits) in powder or liquid form in the base (Qiwam) made of purified honey, sugar, candy or jaggery. "Itrifal-e-Kishneezi" is a classical preparation of the Unani System of Medicine which is considered as carminative, stomachic, laxative and aperiant in action. It is frequently used by the unani physicians to cure gastric headache, conjectivitis, otalgia, flatulence, bleeding piles and chronic catarrah since time immemorial (Anonymous, 2006).

To test the efficacy of this important formulation identification and authentication of all the ingredient present in it is a must.

Methodology

Standard formulation of Itrifal-e-Kishneezi was prepared using following ingredients as per National Formulary of Unani Medicine (2006):

Macroscopic and microscopic characters of all the ingredients of Itrifal-e-Kishneezi are examined individually. Approx. 10 g. of Itrifal-e-Kishneezi is dissolved in 250 ml. of distilled water and filtered. The powdered drug materials present on the filter paper are washed thoroughly and dried. Mounts are made in different reagents and cells/tissues/cell contents etc. are examined under a

S. No.	Plant Name	Botanical Name	Family	Part used	Quantity
1.	Post-e-Halela Zard	*Terminalia chebula* Retz.	Combretaceae	Fruit pulp	100g.
2.	Post-e-Halela Kabli	*Terminalia chebula* Retz.	Combretaceae	Fruit pulp	100g.
3.	Halela Siyah	*Terminalia chebula* Retz.	Combretaceae	Fruit pulp	100g.
4.	Aamla	*Emblica officinalis* Gaertn.	Euphorbiaceae	Fruit pulp	100g.
5.	Post-e-Balela	*Terminalia bellerica* Roxb.	Combretaceae	Fruit pulp	100g.
6.	Kishneez Khushk	*Coriandrum sativum* Linn.	Apiaceae	Fruit	100g.
7.	Asal or Qand Safaid	Sugar	–		1.8 kg.
8.	Raughan-e-Badam or Raughan-e-Zard	Almond oil	–		Q.S.

microscope according to the method laid down by Johnsen (1940) and Trease and Evans (1983).

Observation

Ingredients

All the ingredients of Itrifal-e-Kishneezi belong to same morphological group (fruit) but has characteristic histological features which are diagnostic for their identification in the formulation.

Post-e-Halela Zard (*Terminalia chebula* Retz. C.B. Baker)

Part used: Pericarp of mature fruits

Macroscopy: Broken pieces of yellowish brown fruit of various size, drupaceous, glabrous, wrinkled and ribbed longitudinally, fracture brittle with agreeable odour and astringent taste.

Microscopy: T.S. of pericarp shows epicarp consisting of single layered epidermis covered by thick cuticle. Epidermal cells radially elongated showing thickness

on inner tangential walls; mesocarp consists of 2-3 layers of collenchyma followed by a broad zone of parenchyma in which fibres, sclereids in groups and vascular bundles scattered. Mesocarpic parenchyma cells round to oval in shape, slightly thick walled with little intercellular space and filled with numerous starch grains and rosette crystals of calcium oxalate. Fibres tangentially elongated, with or without simple pits and having width 9 μ - 27 μ. Starch grains numerous, simple, round to oval in shape measuring 2.25 μ - 4.50 μ - 6.75 μ in diameter; calcium oxalate crystals rosette shaped having diameter of 4.50 μ - 18 μ - 22.50 μ. Endocarp consists of thick walled sclereids of various shapes, lignified having simple pits.

All the cells gives positive test for tannin.

Post-e-Halela Kabli (*Terminalia chebula* Retz. C.B. Baker)

Part used: Pericarp of fruits

Macroscopy: Broken pieces of yellowish brown fruit of various size, drupaceous, glabrous, wrinkled and ribbed longitudinally, fracture brittle with agreeable odour and astringent taste.

Microscopy: T.S. of pericarp shows epicarp consisting of single layered, radially elongated epidermal cells covered by cuticle. Mesocarp consisting of two to three layers of collenchyma followed by a broad zone of parenchyma. Mesocarpic parenchyma cells round to oval in shape, slightly thick walled and filled with abundant starch grains which are simple, round to oval in shape, measuring 2.25 μ - 6.75 μ in diameter. Tangentially elongated fibers and sclereids are present at the outer mesocarpic region.

Halela Siyah (*Terminalia chebula* Retz. C.B. Baker)

Part used: Fruit pulp

Macroscopy: Fruit deep brown to black, elongated, ovoid both sides tapering, drupaceous, glabrous, surface hard with longitudinal ribs and wrinkles, fracture brittle, yellowish pulp enclosing a large rough seed. Odourless, taste astringent.

Microscopy: T.S. of fruit shows:

Epicarp: single layered, rectangular shaped epidermal cells covered by cuticle; mesocarp: several layered, slightly thick walled, isodiametric parenchyma cells. After four – five layers of parenchyma two – three layers of tangentially elongated cells present. Numerous vascular bundles are scattered at the inner mesocarpic region. Abundant rosette shaped calcium oxalate crystals present at the inner mesocarpic region, near and within the vascular region.

Amla (*Emblica officinalils* Gaertn.)

Part used: Fruit pulp

Macroscopy: Gray to black curled pieces of dried fruit, highly shriveled and wrinkled, texture rough, cartilaginous, tough; taste sour and astringent.

Microscopy: T.S. of fruit shows:-

Epicarp: Single layered tabular epidermal cells covered by cuticle. Hypodermis 2-4 layered, tangentially elongated, thick walled parenchyma cells.

Mesocarp: Several layered, thin walled parenchyma cells with intercellular spaces; stone cells present either isolated or in small groups.

Post-e-Balela (*Terminalia bellerica* Roxb.)

Part used: Fruit pulp

Macroscopy: Broken pieces of whitish brown fruits of various sizes; velvety surface, wrinkled and ribbed longitudinally, fracture hard with characteristic odour and slightly bitter taste.

Microscoy: T.S. of fruit shows:-

Epicarp: Single layered epidermis hairs.

Mesocarp: Several layered; thin walled parenchyma cells with intercellular spaces. Outer 2-5 layers contain tannin and rosette shaped crystals of calcium oxalate. Peripheral portion of mesocarp shows patches of lignified sclerotic cells. Collateral vascular bundles scattered throughout.

Kishneez Khushk (*Coriandrum sativum* Linn.)

Part used: Fruit

Macroscopy: Fruit is cremocarp; subspherical, approx. 3-5 mm. in diameter; brownish-yellow made up of 2 mericorps; each mericarp has five wavy inconspicuous primary ridges and four straight, prominent secondary ridges. Seed coelospermous; odour aromatic with spicy taste.

Microscopy: T.S. of fruit shows two vittae on the commissural surface of each mericarp. Epidermis of pericarp composed of polygonal tabular cells with single prism like crystals of calcium oxalate; occasional stomata present. Mesocarp composed of an outer and an inner layer of parenchyma with a sclerenchymous layer in between. Sclerenchymatous cells fusiform and lignified. Outer few layers of sclerenchyma run longitudinally whereas inner one or two layers runs tangentially. Inner epidermis of the pericarp composed of parquetry cells.

T.S. of seed shows that testa consists of dark brown flattened cells; endosperm curved, parenchymatous cell containing numerous oil globules, aleurone grains and rosette shape crystals of calcium oxalate.

Test Sample (Formulation):

Microscopic examination of Itrifal-e-Kishneezi shows following components of diagnostic characteristics:-

1. *Sclereids:* Abundant sclereids, either single or in groups; show great variation in size and shape; sclereids square-rectangular-oval-isodiametric-triangular in shape; lumen either broad or very narrow; showing variable striations and pitted walls. Elongated sclereids having thick walls; narrow lumen and pitted walls.
2. *Fibers:* Abundant elongated fibers, occur mostly in groups; thick walled, lignified with simple pits; single fiber occur in pieces of various size, narrow, thick walled with tapering ends.
3. *Sclerenchymatous layer:* Masses of thick walled, sinuous, fusiform cells with narrow lumen and indistinct pits; occur in several layers and at times crossing with each other or with thin walled lignified cells of the mesocarp.
4. *Trichomes:* Few trichomes which are simple, unicellular, elongated and uniserriate.
5. *Vittae:* Vary occasional brown fragments of the vittae present.
6. *Epicarp:* In surface view, rectangular-polygonal in outline with straight thick walls.
7. *Endocarp:* Fragments of thin walled, lignified cells; arranged in groups adherent to the polygonal cells of the mesocarp.
8. *Xylem vessel:* Occur in pieces of various sizes, either single or in groups having spiral, scalariform or reticulate thickenings with pitted walls.
9. *Parenchyma cells:* Mesocarpic parenchyma cells shape vary from oval-polygonal, rectangular-irregular; moderately thick walled, some possess simple pits.
10. *Calcium oxalate crystals:* Occur in rosette shape of variable sizes or frequently as broken pieces; found either scattered or within the parenchyma cells.
11. *Starch grains:* Abundant starch grains; found scattered; majority of the grains are simple, oval to round, spherical-polygonal; some are compound with three or more components.

Results and Conclusion

The histological characters that are microscopically examined in Itrifal-e-Kishneezi (test sample) reveals that:-

1. Itrifal-e-Kishneezi shows abundant sclereids either single or in groups, oval-isodiametric, narrow lumen, showing variable striations and pits; elongated sclereids with thick walls, narrow lumen and pitted walls; abundant elongated fibers occur mostly in groups, thick walled, lignified with distinct simple pits; mesocarpic parenchyma moderately thick walled, oval-polygonal in shape having rosette shape crystals of calcium oxalate; abundant starch grains which are simple, oval to round in shape. All these characters confirms the presence of *Terminalia chebula* Retz. in it. (Fig.1-10)

2. Presence of trichomes which are simple, unicellular, elongated, uniseriate; fiber trachied having simple pits in their lateral walls; xylem fibers which are simple, llignified, narrow, thick walled with tapering ends; thick walled xylem parenchyma, rectangular to irregular in shape possessing simple pits; sclereids which are elongated with pointed or flattened ends, striated walls, pitted and highly lignified confirms the presence of *Terminalia bellerica* Roxb. In Itrifal-e-Kishneezi. (Fig. 11-14)

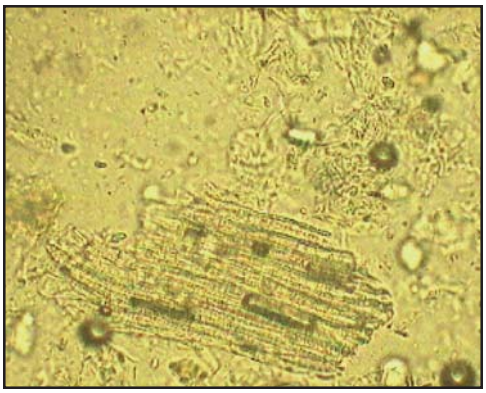


Fig. 1. Sclerenchyma fiber of *Terminalia chebula* Retz. x 40

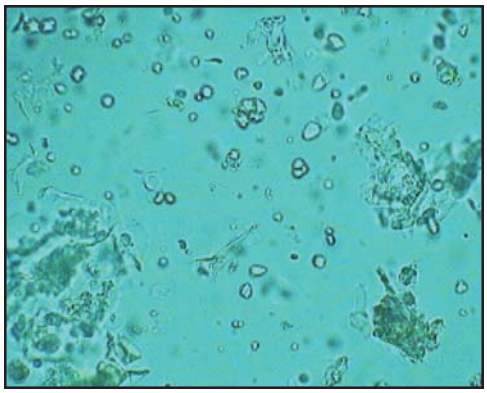


Fig. 2. Starch grains of *Terminalia chebula* Retz. x 40

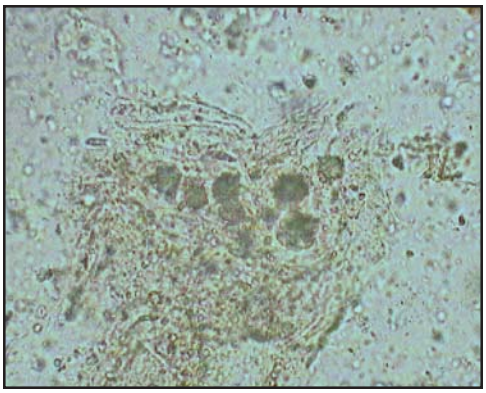


Fig. 3. Crystals of *Terminalia chebula* Retz. x 40

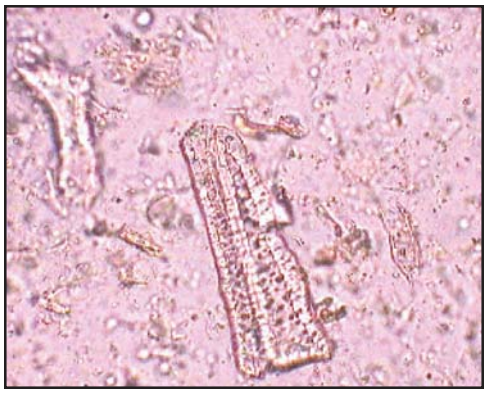


Fig. 4. Pitted sclereid of *Terminalia chebula* Retz. x 40

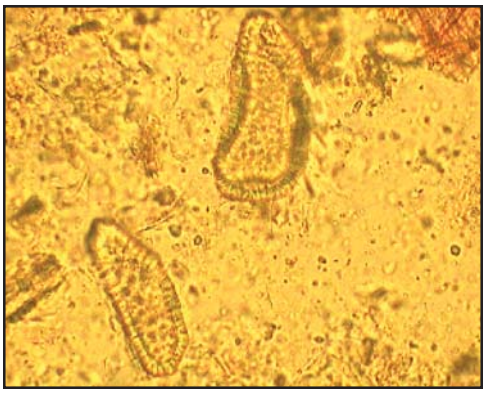


Fig. 5. Pitted sclereid of *Terminalia chebula* Retz. x 40

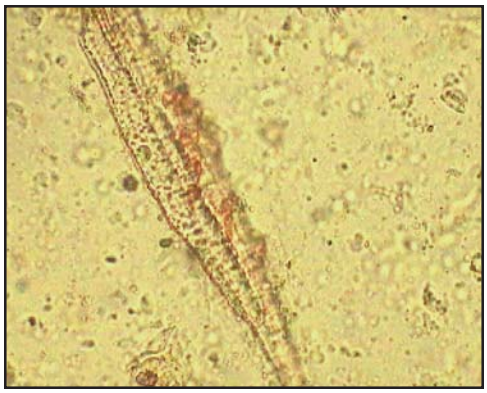


Fig. 6. Elongated pitted sclereid of *Terminalia chebula* Retz. x 40

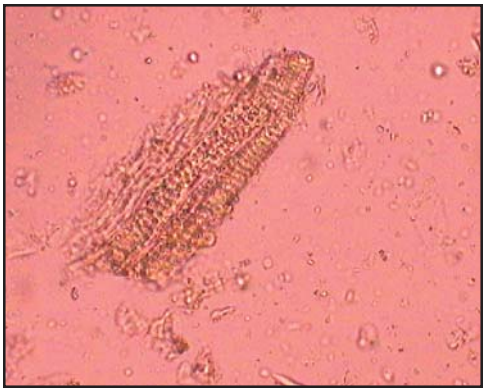


Fig. 7. Spiral vessel of *Terminalia chebula* Retz. x 40

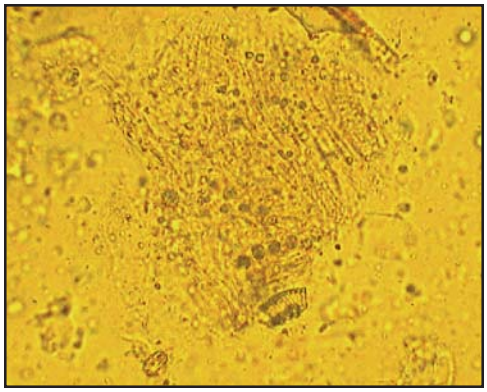


Fig. 8. Calcium oxalate crystals of *Terminalia chebula* Retz. x 40

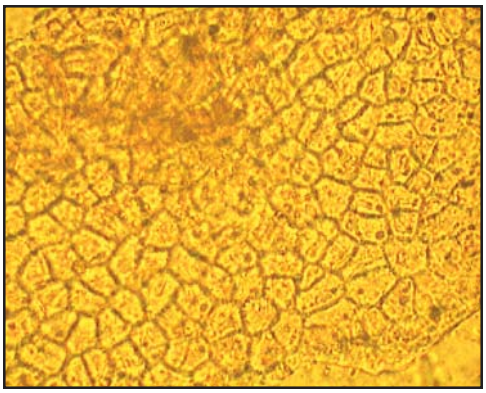


Fig. 9. Epicarp of *Terminalia chebula* Retz. x 40

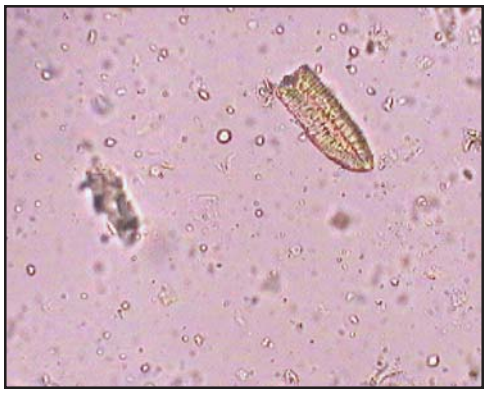


Fig. 10. Sclereid of *Terminalia chebula* Retz. x 40

3. Presence of vittae; sclerenchyma fibers which occur in groups as masses of thick walled, sinuous, fusiform cells with narrow lumen and indistinct pits crossing with each other or with thin walled lignified cells of mesocarp; fragments of endocarp having thin walled, lignified cells with polygonal cells of mesocarp; fragments of endosperm with aleurone grains and oil globules confirms the presence of *Coriandrum sativum* Linn in Itrifal-e-Kishneezi. (Fig. 15-19)

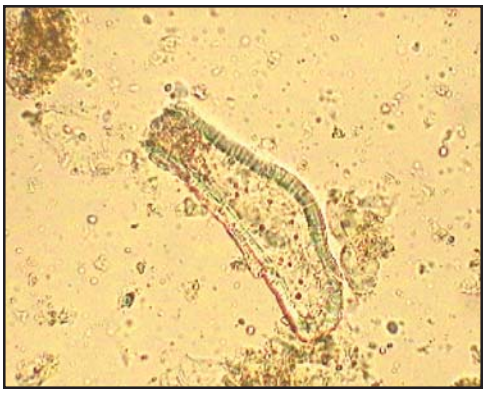


Fig. 11. Pitted sclereid of *Terminalia bellerica* Roxb. x 40

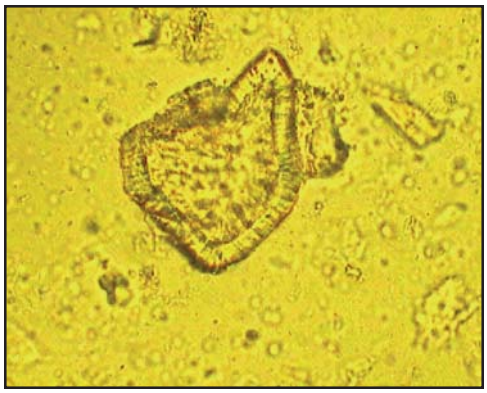


Fig. 12. Pitted sclereid of *Terminalia bellerica* Roxb. x 40

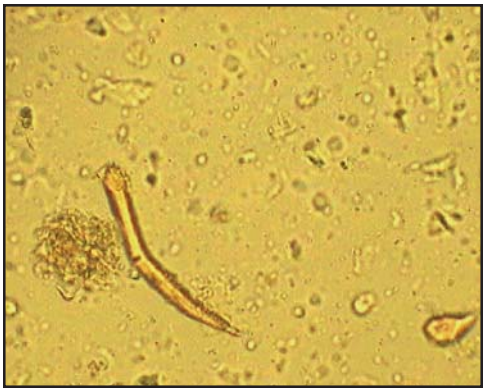


Fig. 13. Trichome of *Terminalia bellerica* Roxb. x 40

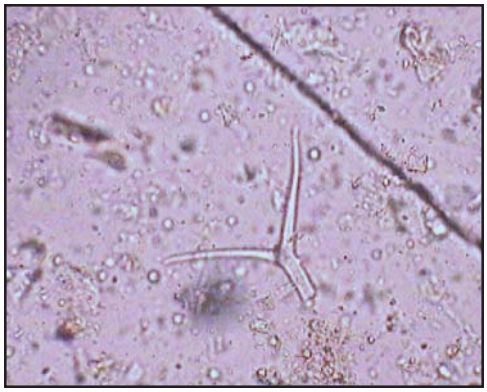


Fig. 14. Trichome of *Terminalia bellerica* Roxb. x 40

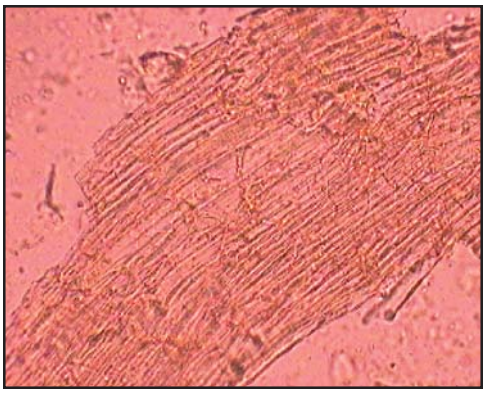


Fig. 15. Fibre from pericarp of *Coriandrum sativum* Linn. x 40

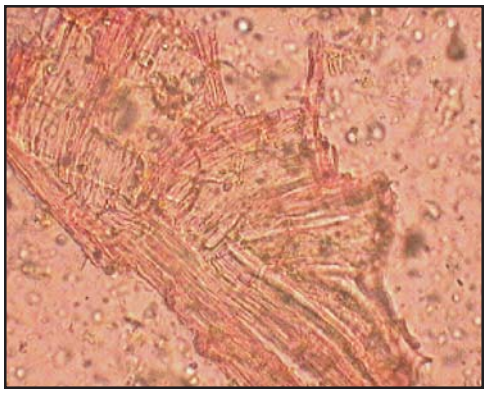


Fig. 16. Endocarp of *Coriandrum sativum* Linn. x 40

4. Sclereids which are square-rectangular-triangular-isodiametric in shape with very broad lumen and pitted walls and mesocarpic parenchyma cells confirms the presence of *Emblica officinalis* Gaertn. in Itrifal-e-Kishneezi. (Fig. 20)

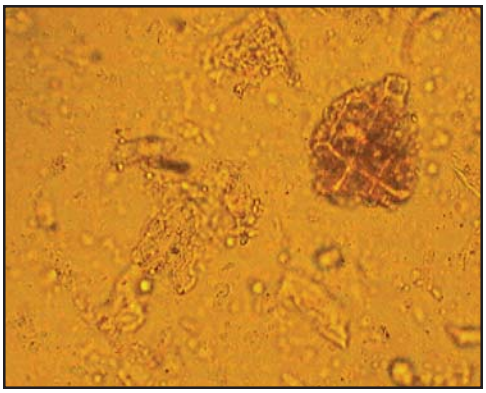


Fig. 17. Vittae of *Coriandrum sativum* Linn. x 40

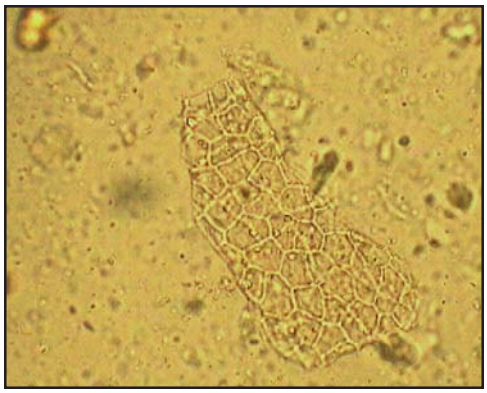


Fig. 18. Endosperm of *Coriandrum sativum* Linn. x 40

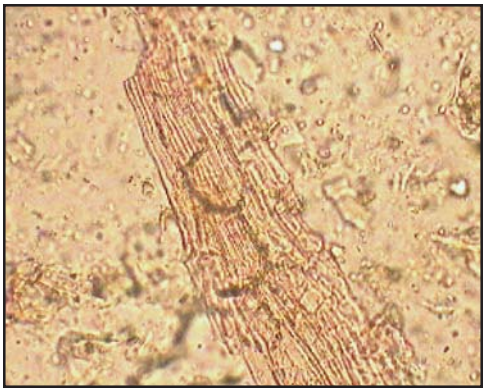


Fig. 19. Endocarp of *Coriandrum sativum* Linn. x 40

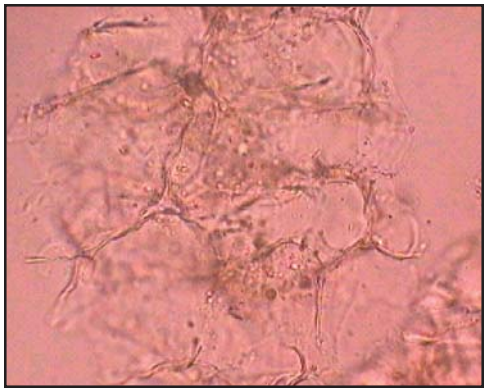


Fig. 20. Mesocarp parenchyma of *Emblica officinalis* Gaertn. x 40

Acknowledgements

Financial support by the Central Council for Research in Unani Medicine for the present study in thankfully acknowledged.

References

Anonymous, 1989. The Ayurvedic Pharmacopoeia of India, Part I, Vol. 1, Govt. of India, Ministry of Health and Family Welfare, Dept. of AYUSH, New Delhi.

Anonymous, 2006. National Formulary of Unani Medicine Part I. Ministry of Health and Family Welfare, Dept. of Ayush, New Delhi (reprinted).

Anonymous, 1976. The Wealth of India (Raw Materials), Vol XI, PID, (CSIR), New Delhi.

Anonymous, 1986. Physico-chemical standards of Unani formulations, Part I, CCRUM, Ministry of Health and Family Welfare, Dept. of AYUSH, Govt. of India, New Delhi.

Johansen, D.A., 1940. Plant Microtechniques, Mc. Grew Hill Book Company, New York.

Kirtikar, K.R. and Basu, B.D., 1988. Indian Medicinal Plants , Vol. I. Bishan Singh, Mahendrapal Singh Book Depot, Dehradun (Reprint).

Kokoski, J., Kokoski, R. and Siama, F.J., 1958. Fluorescence of Powdered Vegetable Drugs under U.V. Radiation, *J. Amer. Pharm. Assoc.* 47 (10): 715.

Trease and Evans, W.C., 1983. Pharmacognosy. 12th Ed. Bailliere Tindall, London.

Pharmacognostical Studies on the Tubers of *Cyperus rotundus* Linn.

Rajeev Kr. Sharma

Pharmacopoeial Laboratory
for Indian Medicine,
Ghaziabad-201002, India

Abstract

Pharmacognosy of the tubers of *Cyperus rotundus* Linn. has been carried out to lay down standards for the genuine drug. Other parameters studied include physico-chemical constants, fluorescence behavior, U.V. Spectrophotometry, Chromatography etc.

Key Words: *Cyperus rotundus* Linn, Pharmacognosy, Drug Standardisation, 'Nagarmotha', 'Sad kufi'.

Introduction

Cyperus rotundus Linn. (Family- Cyperaceae) is an annual weed of the pasture lands, road sides and other moist places and grows throughout Indian sub-continent. The dried tubers of plants are officially regarded as 'Musta' in Ayurveda and 'sad kufi' in Unani system of Medicine. It is used as anthelmintic, antipoisonous, astringent, attenuant, Carminative, emmenagogue, expectorant, febrifuge, galactogogue, lithontriptic, nervine tonic, sedative (intestinal), stomachie and tonic. It is medicinally utilized in appearing of thirst, disorders of stomach, irritations of bowls, febrile and dyspeptic affections, heals wounds and ulcers, pain in abdomen and in sorpion stings (Kirtikar and Basu, 1933; Nadkarni, 1954; Chopra et al., 1958). Besides its medicinal potentialities, it is also used in certain dye preparations to impart perfume to the fabrics. In Bengal, dried and pounded tubers are largely used as perfume in the weddings of natives. The generic title of the plant '*Cyperus'* is supposed to be derived from '*Cypris'* – a name of lord venus, as the underground parts of some of the species of *Cyperus* being aphrodisiacal. It is reported that Romans used it as emmenagogue in uterine complaints. The drug is mentioned in Ashtang Hridya, Bhav Prakash Nighantu, Charak Samhita, Dhanvantari Nighantu, Sushruta Samhita etc. and also mentioned as 'Nagarmotha' in Unani system of medicine , The drug is often adultrated with allied species and other generic members of family Cyperaceae. (Herman, 1868, Watt, 1889-93; Anonymous, 1950, 1981; Nadkarni, 1954; Chunekar, 1972).

Methodology

Drug samples were collected from different places with a view to find out any significant difference present within the same species. Hand sections were stained and mounted in Canada balsam for anatomical studies. Lignification on smoothed cross-surfaces was studied with phloroglucinol-HCl. For studying powder, Jackson and Snowdon (1968) was followed. To determine physico-chemical constants, Indian Pharmacopoeia (Anonymous, 1966) was consulted and for fluorescence study schedules mentioned by Trease and Evans (1972) were followed. Colours were named by consulting Rayner (1970). Standard prescribed procedures for

Histochemical studies (Johanson, 1940; Youngken, 1951; Cromwell, 1955, Trease and Evans, 1978), Organic group detection (Robinson, 1963), Elemental quantitation (Khan, *et.al.*, 1985), U.V. Spectrophotometry (Willard, *et.al.*, 1965) and Chromatography (Shellard, 1968, Stahl, 1969, Smith and Feinberg, 1972) were adopted.

Systematics

Family: Cyperaceae Juss. Endl. Gen. 109, Lindl. Veg. Kingd. 117, Gen. Pl. III: 1037.

The family is spreaded over about 90 genera and 4,000 species, which have global distribution. In India, this family comprise 22 genera with over 405 species distributed in temperate and alpine Himalaya and chiefly in plains.

Genus: Cyperus Linn. Gen. n. 66, Gen. Pl. III: 1043, F.B.I. 6:697.

This genus comprises 1,468 species distributed in tropical and warm teperate regions of the world. In India, the genera consists of about 100 species. The genus is distributed chiefly in the plains, abundant in Eastern India.

C. rotundus Linn. Sp. Pl. 45. 1753, F.B.I. 6 : 614, FUGP 3:322, Kiik. Pfreich: 107. 1935, FD 356.

Synonyms: C.curvatus Llanos, *C. hexastachyus* Rottb., *C. leptostachyus* Griff., *C. odoratus* Osbeck., *C. radicans* Fl. Grace., *C. tenuiflorus* Royle.

An erect perennial, glabrous tufted herb with a subterranean, woody, stoloniferous rhizome thickened at intervals into black, woody tubers, stem nodose at base, 3 gonosis. Leaves basal usually shorter or as long as the stem, some times longer, linear. Inflorescence a simple or compound umbel often overtopped by the longest of 3. Bracts foliar, generally 3, unequal, spikelets brown with reddish tings. Glumes ovate, 20-50 obtuse, imbricate, decurrent below as hyaline wings, stamens 3, obovoid, glabrous. Nut blackish brown, one third of length of the glume, narrowly ovoid (Plate I).

Flowering and Fruiting: July-October.

Distribution: Throughout India ascending upto 1800 m a.m.s.l. Common weed in waste and unused dry, gravelly places and in crevices amongst stones (Chopra et al., 1958).

Observations

I. *Organoleptic Characteristics*

A. The drug comprises of dried tubers of varying sizes. The tubers are oval to spindle shape, somewhat compressed and tapered at both the ends spreading

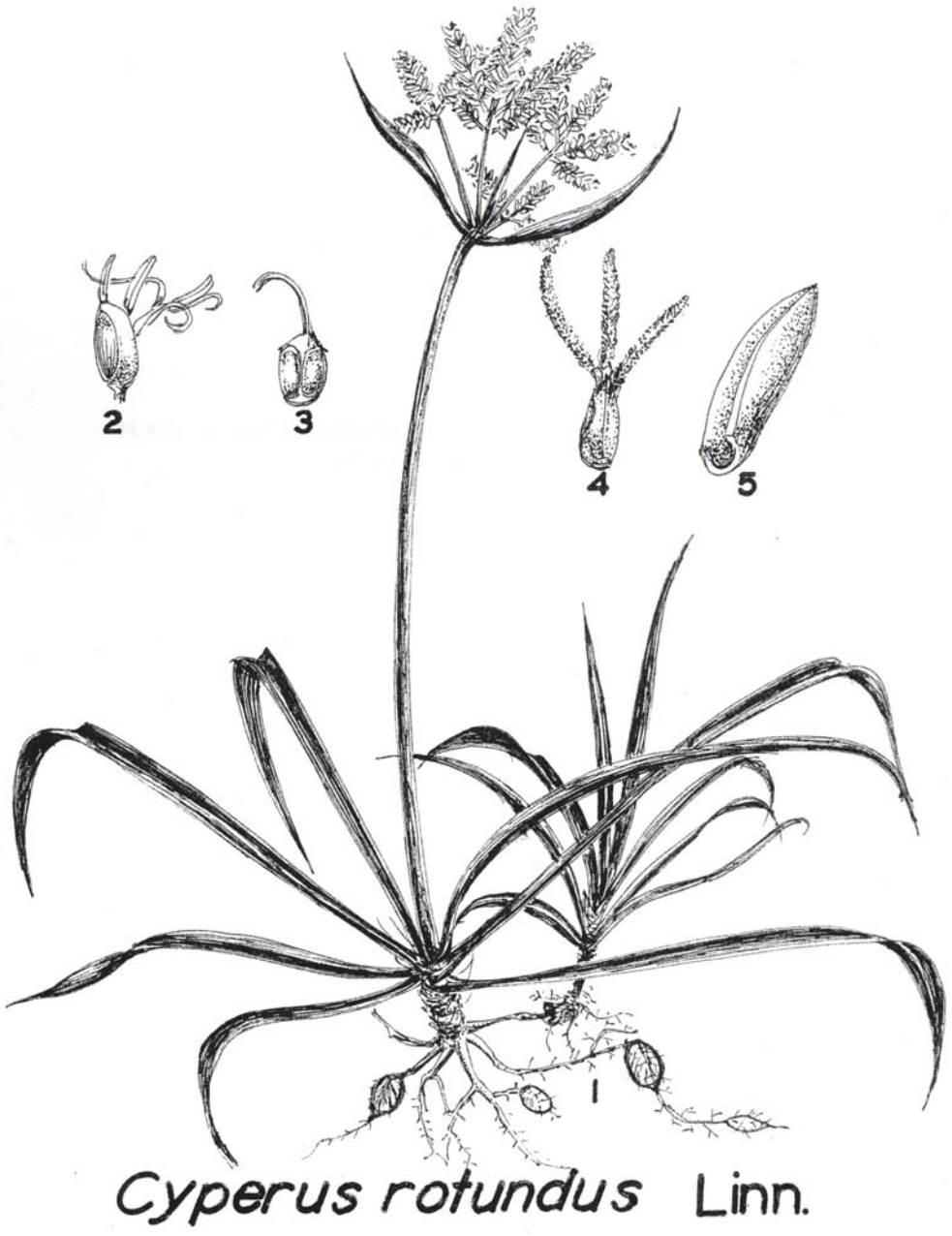


Plate-I. Habit and Taxonomic Details of Drug Plant (*Cyperus rotundus* Linn.)
1. Habit Sketch, 2. Floret, 3. Nut, 4. Carpel and 5. Glume

the root system. The tubers generally range from 1.5-3.5 cm in length and 0.5-2.5 cm in diameter. The tubers are unbranched and sometimes flattened or uniformly cylindrical with comparatively longer center portion. These are slightly semi-succulent when fresh, but turn hard in nature after drying. These are dark brown to black in colour and are covered with numerous rootlets. Some of the tubers have scares or remains of rootlets (Plate II). Tubers are not easily breakable due to smaller size and hardened nature. The fracture is short exposing

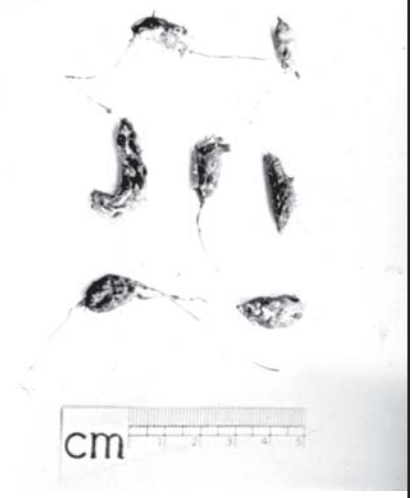


Plate-II. Macrosopical Feature of Drug 'Musta' (Dried tubers *Cyperus rotundus* Linn.)

white interior with light brown dots. The tubers have an aromatic fragrance and a slightly agreeable taste.

B. *Powdered Drug:* The powdered drug is brown in colour with aromatic odour and agreeable aromatic taste.

II. *Micro-morphological Charateristics*

A. Transverse section of the tuber is circular to oval in outline (Plate III). It shows a single layered epidermis consisting of radially elongated cells. Epidermis is followed by two to four layers of hypodermis composed of thick walled, polygonal lignified cells. Epidermis and hypodermis are filled with dark brown content (Plate IV A). Ground tissues of the cortex consist of thin walled compact parenchymatous cells which are circular to polyhedral in shape (Plate IV C, B). The cells of cortex are filled with starch grains which are simple and oval in shape with indistinct hilum. A number of amphivasal vascular bundles are distributed throughout the cortical region comprising of xylem and phloem elements (Plate IV B). Innermost layer of the cortex is followed by highly thickened endodermis encircling the stele. The distinct layer of endodermis is composed of thick walled barrel shaped cells, which have no casparian bands on the radial walls. These cells are also devoid of starch grains. The pericycle layer is not distinct. A few patches of sclerenchymatous cells are present at certain places adjacent to endodermis (Plate IV D). Ground tissues of the steler region are composed of thin walled parenchymatous cells containing starch grains similar to those of cortical cells (Plate IV E). A large number of vascular bundles are found scattered asymmetrically throughout the steler region. The vascular

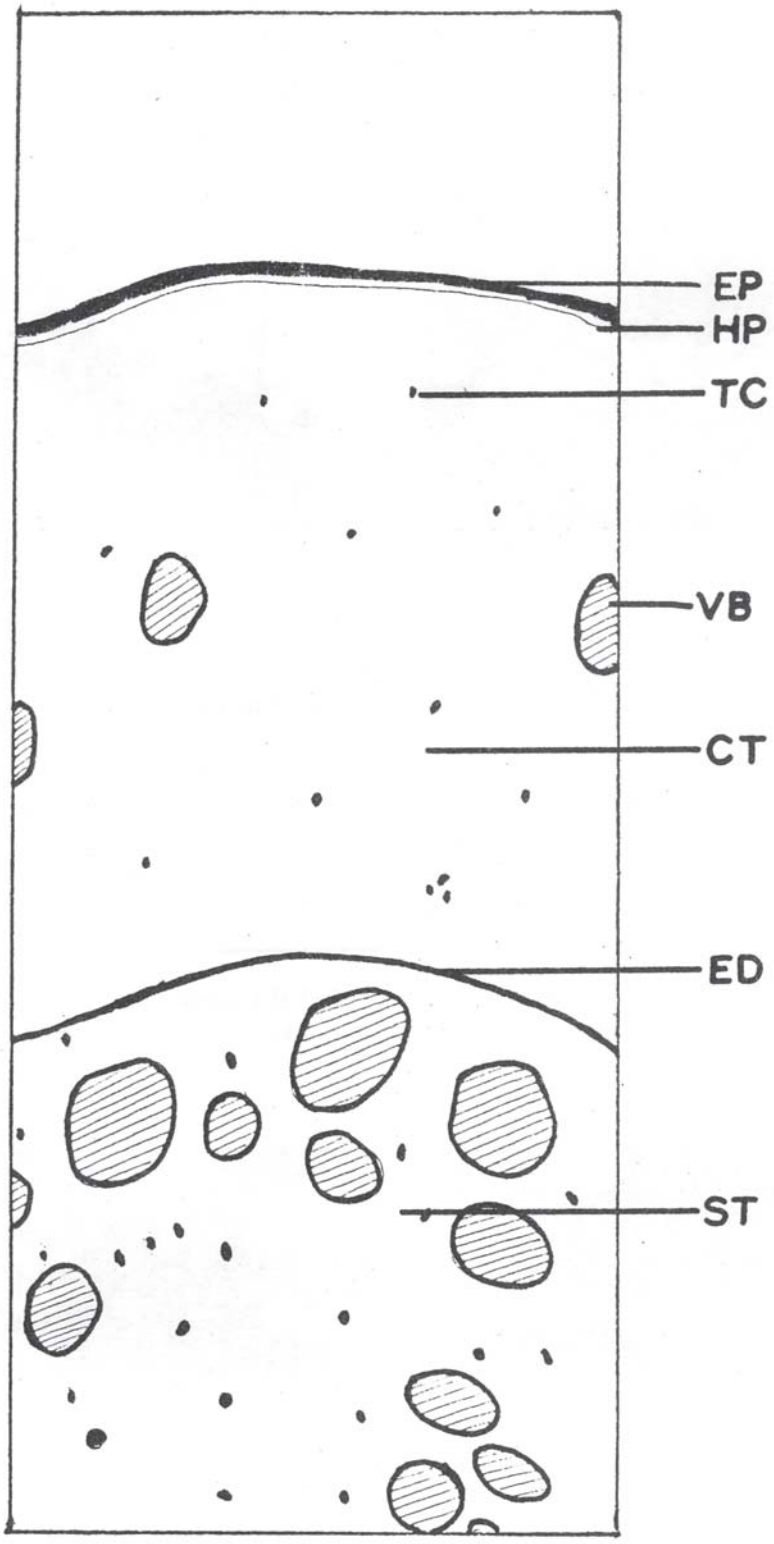


Plate-III. Diagramatic Representation of Transection of Drug (Dried tubers *Cyperus rotundus* Linn.), 25 X)

Abbreviations: CT-Cortical region, ED-Endodermis, EP- Epidermis, HP-Hypodermis, ST-Steler region, TC-Tannin containing cell and VB- Vascular bundle.

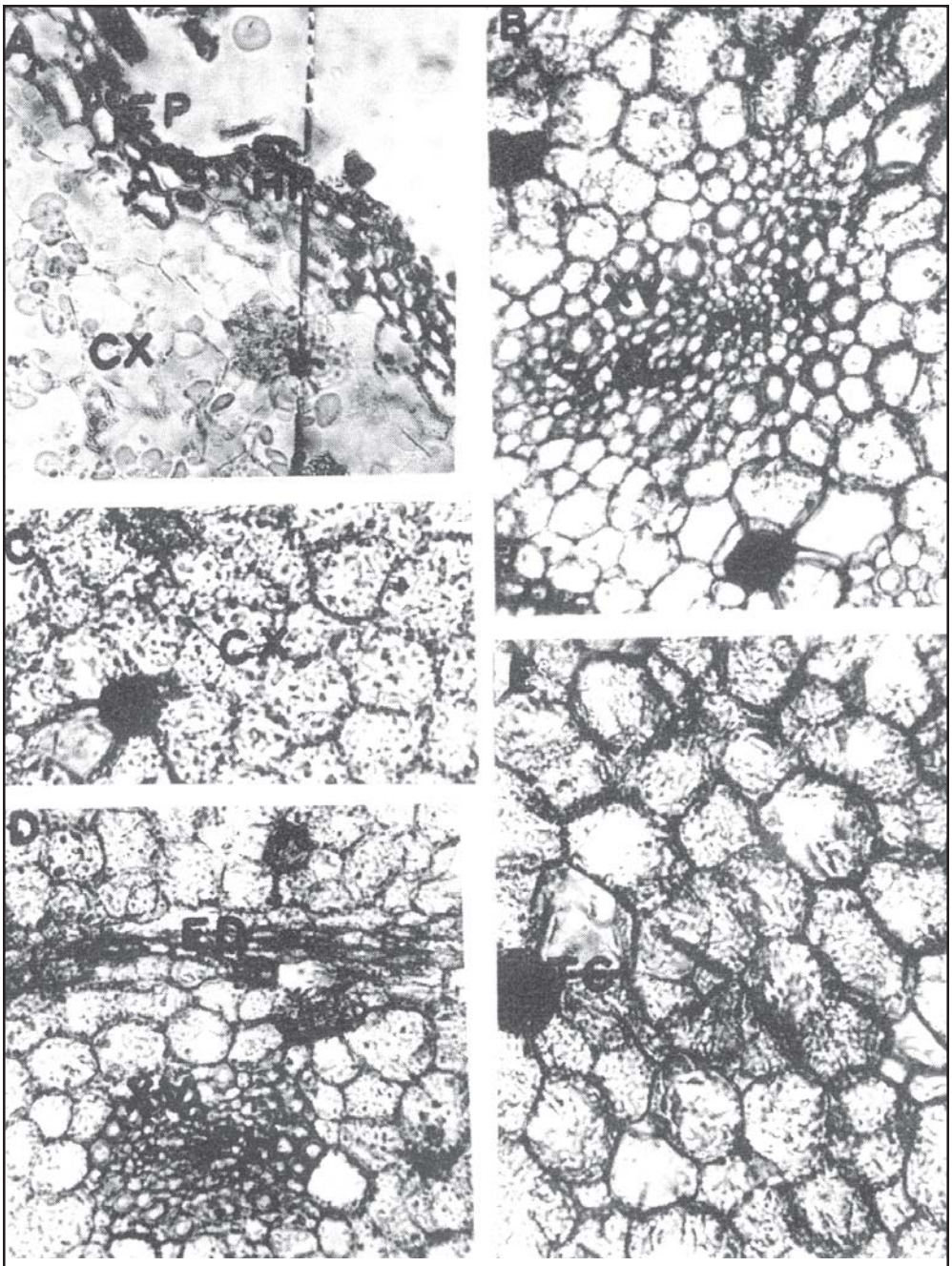


Plate-IV. Photomicrogrpahs of Transection of Drug (Dried tubers of *Cyperus rotundus* Linn.)

Transection of drug showing a portion of –

A. Epidermis, hypodermis and cortex, 1200 X.
B. Vascular bundles distributed in cortical region, 1200 X.
C. Ground tissue of cortical region, 1200 X.
D. Cortical region, endodermis and vascular bundle distributed in steler region, 1200 X.
E. Ground tissue of steler region, 1200 X.

Abbreviations: CX- Cortex, ED-Endodermis, EP- Epidermis, HP- Hypodermis, PH-Phloem,
TC- Tannin containing cells and XV-Xylem vessel.

bundles are of two types- amphivasal and collateral. The majority of vascular bundles are collateral, closed and are surrounded by lignified fibre sheath. Vascular bundles towards the periphery are smaller in size and from almost a complete ring neighbouring the endodermis (Plate IV D). The xylem vessels are lignified and have simple pits and reticulate thickening on the walls. Lignified fibres surround the phloem on both sides and are of various shapes and sizes with tapering or blunt ends. These are originated by the conversion of the ground parenchymatous cells and surround each of the vascular bundle forming sclerenchymatous sheath. Phloem is composed of mainly phloem parenchyma, sieve tubes and companion cells. The phloem parenchyma consists of polyhedral cells. A number of pigment cells containing tanniferous contents are found scattered in cortical and steler regions.The microscopical measurements of individual cell of different tissues and cell contents in microns given below (Table-1).

Abbreviation: 'D' – Diameter

C. *Powdered Drug:* The powdered drug occasional fragments of epidermis, a few of them adhering to the cells of hypodermis, abundant thin walled compact, parenchymatous cells of cortical and steler region filled with starch grains; rarely, cells of endodermis associated with parenchymatous or sclerenchymatous cells and occasional moderately thick walled fibres with tapering or blunt ends. The vessels often fragmented occur singly or usually in groups and have reticulate thickening. Starch grains are simple and abundant in occurrence. Parenchymatous cells containing brown tannin content are also fairly common in powdered drug.

Table 1. Dimensional data of Cellular elements in transactions and cell contents.

Sl.No.	Cellular Elements/Cell Contents	Measurements in microns
1.	Epidermis Cells	10.0-24.4x10.2-20.5
2.	Hypodermis Cells	16.1-40.0x12.0-24.0
3.	Cortex Cells	32.2-96.0X 16.0-84.4
4.	Endodermis Cells	10.0-32.5x12.0-20.6
5.	Xylem vessels	5.0-20.2x10.5-29.5
6.	Phloem parenchyma Cells	3.4-5.5x2.3-3.6
7.	Cells of ground tissue (Steler region)	28.0-88.6x20.1-52.2
8.	Starch grains	2.0-7.2 (D)
9.	Tannin containing Cells	25.0-60.1x20.0-50.7

III. Histochemistry

A. *Micro-Chemical Tests and Behaviour of specific reagents towards Plant/Drug Tissues:* Observations and results pertaining to micro-chemical tests and behaviour of specific reagent towards plant tissues are presented in Table-2.

B. *Organic Groups of Chemical Constituents:* The extracts of the drug were tested for presence of different organic groups and results are presented in Table-3.

IV. Identity, Purity & Strength

A. *Physico-Chemical Constants:* The analytical values in respect of physico-chemical constant of drug were established and results are reported in Table-4.

B. *Medicinal Inorganic Elements:* The quantitative data in respect of medicinal inorganic elements detected in drug are presented in Table-5.

V. Fluorescence & Spectroscopy

A. *Fluorescence Characteristic of Powdered drug under Ultra-Violet Light:* Powdered drug was screened for fluorescence characteristic with or without chemical treatment. The observations pertaining to their colour in daylight and under ultra-violet light were noticed and are presented in Table-6.

B. *Ultra-Violet Spectroscopy:* The data related to Ultra-Violet Spectrophotometric characteristics as computed in Table-7.

VI. Chromatography

A. *Paper Chromatography:* The amino acids and free sugars were resoluted and detected by paper chromatographic techniques. The comparison of Rf. Values of reference standards of different amino acids and free sugars confirms the presence of –

(i) Amino Acids – DL-2-Amino-n-butyric acid, L–Arginine monohydrochloride and L-cysteine.

(ii) Free Sugars – D-Fructose and sucrose.

B. *Thin-Layer Chromatography:* Best separation for TLC fingerprinting were obtained by using different layers and solvent systems. Inferences are shown in Table-8.

Discussion

Datta and Mukerjee (1950) carried out the work on the pharmacognosy of *Cyperus rotundus* Linn. But they investigated the rhizome. The tubers of the plant are

Table-2. Micro-chemical Tests and behaviour of specific reagents towards plant tissues and cells contents.

Sl. No.	Reagent	Test for	Infer-ence	Histological zone/ cell contents responded
1.	Dragendorff's reagent	Alkaloids	+	A few cells of peripheral portion xylem and phloem
2.	Marme's reagent	Alkaloids	+	Same as above.
3.	Wagner's reagent	Alkaloids	+	Same as above.
4.	Potassium hydroxide solution (5% w/v)	Anthocynin	–	Not Responded
5.	Sulphuric acid (66% v/v)	Anthocynin	–	Not Responded
6.	Acetic acid	Calcium oxalate	–	Not Responded
7.	Potassium hydroxide solution (5% v/v) + Hydrochloric acid	Calcium oxalate	+	Not Responded
8.	Sulphuric acid	Calcium oxalate	+	Not Responded
9.	Kedde reagent	Cardiac glycoside	–	Not Responded
10.	Iodine Solution followed by Sulphuric acid	Cellulose	+	Parenchymatous cells of cortex and stele.
11.	Sudan III	Fixed oil and fats	–	Not Responded
12.	Chlor-zinc-Iodine Solution	Latex	–	Not Responded
13.	Aniline sulphate Solution followed by Sulphuric acid	Lignin	+	Cells of hypodermis, sclerenchyma, phloem xylem vessels and fibres
14.	Phloroglucinol HCl	Lignin	+	Same as above
15.	Lugol's solution	Protein	+	Most of parenchy-matous cells of cortex, phloem and xylem.
16.	Millon's reagent	Protein	+	Same as above
17.	Picric acid	Protein	+	Same as above
18.	Heating with KOH (5% w/v) + H_zSO_4	Suberin	+	A few cells of epidermis and endodermis
19.	Sudan III	Suberin	+	Same as above
20.	Weak Iodine solution	Starch	+	Starch grains
21.	Potassium hydroxide solution (5% w/v)	Starch	+	Same as above
22.	Sulphuric acid	Starch	+	Same as above

Table-3. Major Group of Organic Chemical Constituents of Drug.

Sl.No.	Organic Groups of Chemical Constituents	Reagents/Tests	Inference
1.	Alkaloid	Dragendorff's and Mayer's reagents	+
2.	Anthraquinone	Borntrager reaction	+
3.	Coumarin	Alcoholic potassium hydroxide	-
4.	Flavonoid	Shinoda reaction	+
5.	Glycoside	Mollisch's test	+
6.	Protein	Xanthoprotein test	+
7.	Resin	Ferric chloride regent	-
8.	Saponin	Libermann-Burchard reaction	+
9.	Steroid	Salkowski reaction	+
10.	Tannin	Gelation test	+

subjected in the present studies. The diagnostic characters by which the drug can be macroscopically identified are dried tuber, dark brown to black, oval to spindle somewhat compressed and tapered, length 1.5-3.5 cm, diameter 0.5-2.5 cm; unbranched, some times flattened, semi-succulent when fresh but dried ones hard; surface have scars or remains of rootlets. Fracture-short, odour-aromatic and taste slightly aggreable. Microscopically (transverse section of drug) exhibit epidermis single layered; hypodermis comprise 2-4 layers of thick walled, polygonal, lignified cells; cortex; endodermis single layered; few patches of sclerenchymatous cells

Table-4. Analytical Values of Physico-chemical Constants

Sl.No.	Physico-Chemical Constants	Analytical values
1.	Moisture content, % w/w	8.0
2.	pH	5.8
3.	Crude fibre, % w/w	20.5
4.	Total Ash, % w/w	7.2
5.	Acid insoluble ash, % w/w	1.3
6.	Alcohol soluble extractive % w/w	5.1
7.	Water soluble extractive % w/w	9.2

Table-5. Quantitative estimation of Medicinal Inorganic Elements

Sl.No.	Physico-Chemical Constants	Analytical values Mg/g of ash
1.	Cadmium	0.0011
2.	Calcium	0.3950
3.	Copper	0.1950
4.	Iron	2.1700
5.	Magnesium	0.4330
6.	Manganese	0.0170
7.	Nickle	0.1190
8.	Potassium	9.2100
9.	Sodium	24.2400
10.	Zinc	0.0006

Table-6. Fluorescence Characteristic of Powdered Drug under Ultra-Violet Light.

Sl. No.	Treatments	Colour in day light	Nature of colour in fluorescence
1.	Powder as such	Brown	whitish
2.	Powder with		
	a. Carbon tetra chloride	Colourless	Buff
	b. Ethyl acetate	Colourless	Colourless
	c. Hydrochloric acid	Golden yellow	Yellow
	d. Nitric acid + water	Golden yellow	Yellow
	e. Sodium hydroxide + methanol	Yellowish tinge	Buff
	f. Sodium hydroxide + water	Orange	Yellowish green
	g. Sulphuric acid + water	Light brown	Buff
	h. Buffer- pH 5	Yellow tinge	Greenish yellow
	i. Buffer- pH 7	Colourless	Colourless
	j. Buffer- pH 9	Colourless	Yellow tinge

Table-7. Ultra-Violet Spectrophotometer characteristic of drugs.

Sl.No.	Specifications	Data
1.	Tincture dilution ml/ml	0.012
2.	Maximum absorption peak	0.460
3.	l Maxima at, nm	280

Table-8. TLC fingerprinting data

Sl.No.	Technical details	I	II
1.	Layer	Silica gel GF.	Silica gel GF
2.	Solvent system	n-butanol-acetic acid-water (3:15:5.5, v/v)	Benzene-Ethyl Acetate (4:1, v/v)
3.	No. of spots	04	04
4.	h Rf. Values of visualised spots	16.4, 59.7, 67.2 and 97.0	5.8, 28.3, 75.0 and 79.2

present at certain places adjacent to endodermis; vascular bundles in stele amphivasal and collateral, collateral and closed more frequent, surrounded by lignified fibre sheath; xylem vessels lignified with simple pits and retuclate thickening, fibres lignified, surround the phloem; phloem with usual elements; ground tissue of stele parenchymatous, some of the cells of epidermis, hypodermis and cortex filled with dark brown tanniferous content; simple, oval starch grains present in the cells of cortex and tissue of stele. The other parameters studied compliment the pharmacognostical study for ensuring the purity and strength.

Acknowledgement

The author is deeply indebted to late Dr. M.R. Sharma, former head of botany department, D.A.V. (PG) College, Deharadun, under whose able guidance he had privilege to work.

References

Anonymous, 1950. The Wealth of India (Raw Materials), Vol. II (C). C.S.I.R., New Delhi.

Anonymous, 1966. Pharmacopoeia of India. Manager of Publications, Govt. of India, New Delhi.

Anonymous, 1981. National Formulary of Unani Medicine, Part I, Govt. of India, Ministry of Health & Family Welfare, New Delhi.

Anonymous, 1976 The Medicinal Plants of India, Vol. I, I.C.M.R., New Delhi.

Chopra, R.N., Chopra, I.C., Handa, K.L. and Kapoor, L.D., 1958. Chopra's Indigenous Drugs of India. U.N. Dhur & Sons Pvt. Ltd., Calcutta.

Chopra, R.N., Nayar, S.L. and Chopra, I.C., 1956. Glossary of Indian Medicinal Plants, C.S.I.R., New Delhi.

Cromwell, B.T., 1955. In Modern methods of plant analysis Peach, K. and M.V. Tracy Vol. 4. Springer – Verlag, Heidelberg.

Chunekar, K.C., 1972. Glossary of vegetable drugs in Brahattrayi. Chowkhamba Sanskrit Series office, Varanasi.

Datta, S.C. and Mukerjee, B., 1950. Pharmacognosy of Indian root and rhizome drugs. Manager of Publications, Govt. of India, New Delhi.

Herman, Samuel, 1968. Paxton's Botanical Dictonary comprising the Names, History and culture of all plants known in Britain. Bradurry, Evans & Company., Bouverie, London.

Jackson, B.P. and Snowdon D.W., 1968. Powdered Vegetable Drug. Churchill Ltd., London.

Johansen, D.A., 1940. Plant Microtechnique, Mc Graw Hill Book Co., New York.

Khan, S.U., Roy, S. and Arora, R.B., 1985. Geomedicinal and elementological aspect of herbal drugs used for cardiovascular disease, in Development of Unani Drugs from Herbal sources and the role of elements in their machanism of action, edited by R.B. Arora, pp. 63-69. Hamdard National Foundation, IHMMR., New Delhi.

Kirtikar, K.R. and Basu, B.D., 1933. Indian Medicinal Plants, Vol. 1-4. L.M. Basu, Allahabad.

Nadkarni, A.K., 1954, K.M. Nadkarni's Indian Materia Medica. Vol. I & II. Popular Book Depot., Bombay.

Rayner, R.W., 1970. A Mycological Colour Chart. Commonwealth. Mycological Institute, Kew, Surrey and British Mycological Society London.

Robinson, T., 1963. The organic constituents of Higher plants, Burgus Publishing Co., U.S.A.

Sharma, Rajeev Kr., 1987 Pharmacognostic Studies leading to standardization for identification and authentication of some commercially exploited roots and rhizomes employed as drug in Ayurveda. D. Phil Thesis. Garhwal University, Srinagar-Garhwal.

Shellared, E.J., 1968. Quantitative paper and thin-layer chromatography. Academic Press, London.

Smith, I. and Feinberg, J.G., 1972. Paper chromatography, Thin layer chromatography and Electrophoresis. Longmans, London.

Stahl, E., 1969. Thin-layer Chromatography. A Laboratory Hand book. Translated by M.R.F. Ashworth. Allen and Unwin, London.

Trease, G.E. and Evans, W.C., 1972. Pharmacognosy 10th edn. Edn. Bailliere Tindel, London.

Trease, G.E. and Evans, W.C., 1978. Pharmacognosy 11th edn. Edn. Bailliere Tindel, London.

Watt, G., 1889-93. A Dictionary of Economic Products of India, 6 Vols. (Index 1896). Govt. Printing Press, Calcutta.

Willard, H.H., Merrit, L.L. and Dean, J.A., 1965. Instrumental methods of analysis, 4th edn. Affiliated East-West Press, Pvt. Ltd., New Delhi.

Youngken, H.W., 1951. Pharmaceutical Botany, 7th ed., The Blackistan Company, Toronto.

Pharmacognostical Standardization of a Unani Herbal Drug – Habb-ul-Rashaad (*Lepidium sativum Linn.*)

[1]Sonali Sajwan,
[2]Kunal Sajwan,
[3]R.K. Pawar,
[4]Shamshad Ahmad,
[4]M.K. Siddiqui
and
[5]U.C. Aggrawal

Drug Standardization Research Institute (Unani), CCRUM, PLIM Building, Kamla Nehru Nagar, Ghaziabad, U.P.

[3]PLIM, Sec. 23, Sanjay Nagar, Ghaziabad, U.P.

[4]Central Council for Research in Unani Medicine, 61-65, Institutional Area, Janakpuri, New Delhi

[5]Department of Botany, D.S. College, Aligarh-202001

Abstract

The present study has been taken up to establish certain pharmacopoeial standards of *L. sativum* Linn which may help in correct identification of drug while in crude form. The communication deals with macroscopy, microscopy; powder study important physicochemical studies and HPTLC. These parameter will contribute for the standardization of drug.'

Key Words: Habb-ul-Rashaad, Standardization

Introduction

Lepidium sativum Linn (Family-Cruciferae) is a small herbaceous, glaberous, annual herb, about 15-45 cm high, cultivated throughout India. This plant has been used in compound Unani formulations and constitutes an important drug in pharmacopoeia (Anonymous, 2004). The drug is used as ingredient in compound Unani formulations Raughan-e-Sudab, Habb-e-Khabs-ul-Hadeed etc. It is also used in Ayurvedic formulations, namely, Kasturyadigutika. The drug is used in following diseases – Hikka, Atisara, Vatarakta.

Material and Methods

The plant material for study was collected from PLIM herbal garden Ghaziabad, Khari Baoli, Karol Bagh, New Delhi.

For microscopic study standard procedures were followed (Johanson,1940) The physico chemical studies of drug were carried out according to the methods given in Ayurvedic Pharmacopoea (Anonymous, 2004). For HPTLC a Camacg HPTLC system equipped with a sample applicator Linomat V, automatic Multiple Devloper-2 chamber, TLC scanner 3, Reprostar 3 and Wincats an integrated Software 4.02 (Switzerland).

1. DSRI, CCRUM, PLIM Building, Ghaziabad, U.P.
2. DSRU, CCRUM, Jamia Hamdard, Hamdard Nagar, New Delhi.
3. PLIM, Opp M Block, Sec 23, Sanjay Nagar, Ghaziabad, U.P.
4. Central Council for Research in Unani Medicine, Janakpuri, New Delhi.
5. Dept. of Botany, DS College, Aligarh, U.P.

Observations

Macroscopic: Seeds small, oval shaped, pointed at one end, smooth about 2-2.5 mm long and 1-1.5 mm wide, reddish brown, a furrow present on both surface when soaked in water seeds swells and covered with a transparent colourless mucilage, taste mucilaginous, odour pleasant (plate 1A, B).

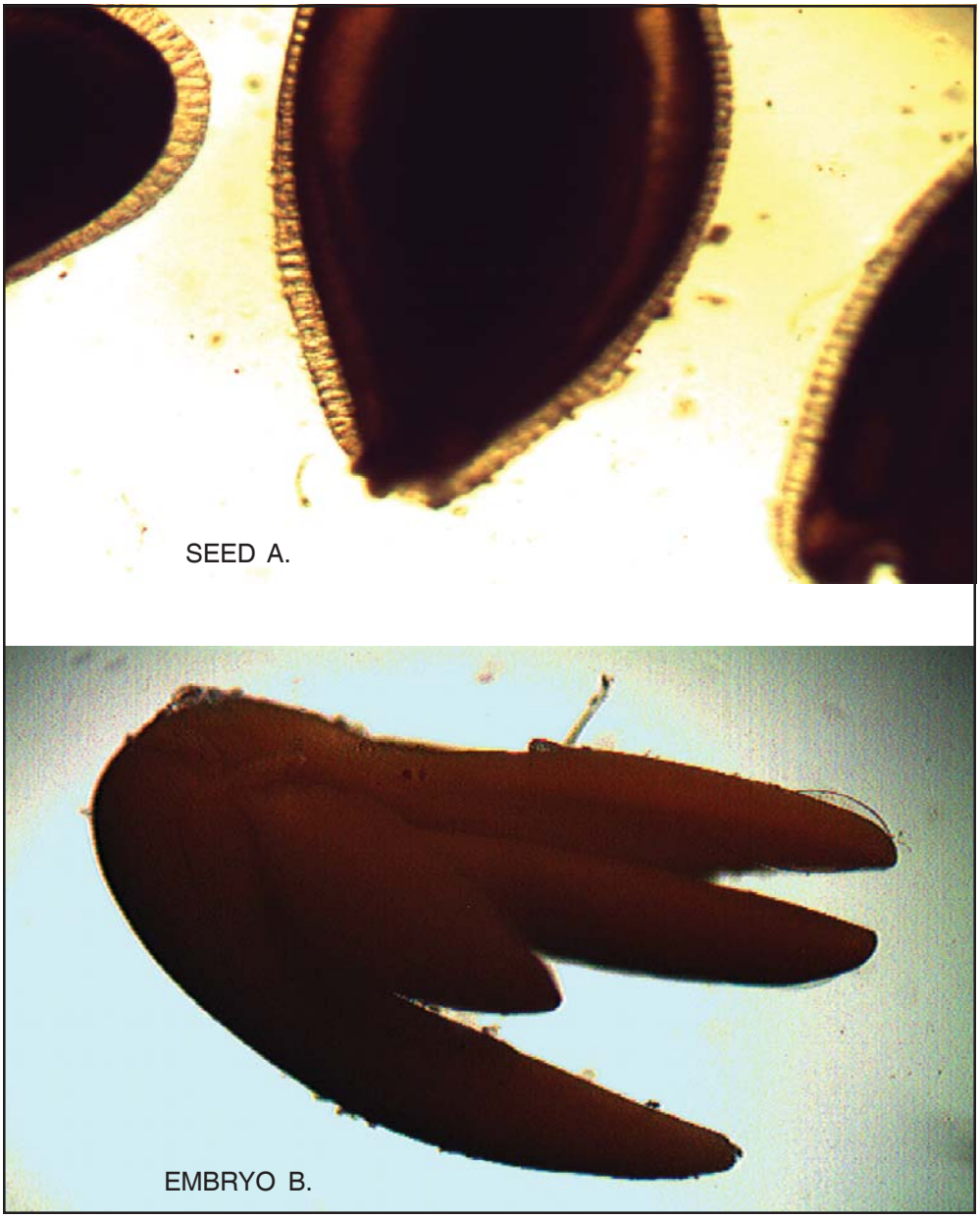
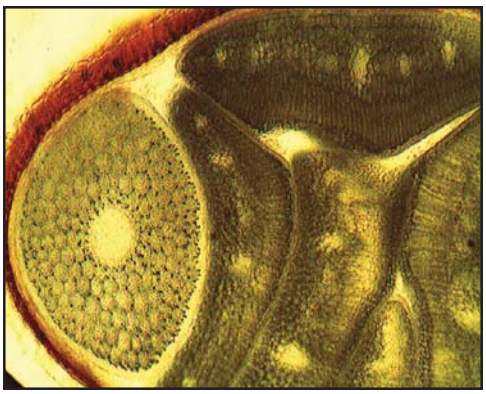


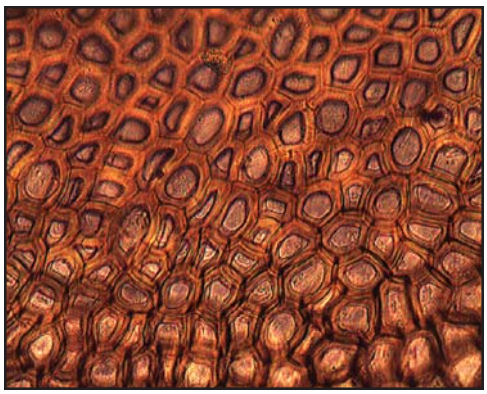
Plate-1. Morphological Features

Microscopic: Seeds in cross section reveal a simple structure, externally lined by thick mucilagenous epidermis which is followed by one celled thick sub-epidermal layer. The inner most layer of seed coat is represented by single layered thick walled cells with U- shaped thickenings. Some cells contain pigment. The endosperm cells are thin walled and filled with oil droplets and aleurone grains. In cross section radicle is shown separately placed at one end of the section with cotyledonary portions. Cells of radicle are in outer region-multilayered oval shaped cells and in inner region- hexagonal small size cells. Parenchymatous cotyledon shows 3- 4 layer of palisade like cells whereas at inner side the remaining cells are polyhedral in shape (plate 2-A, B, C, D).

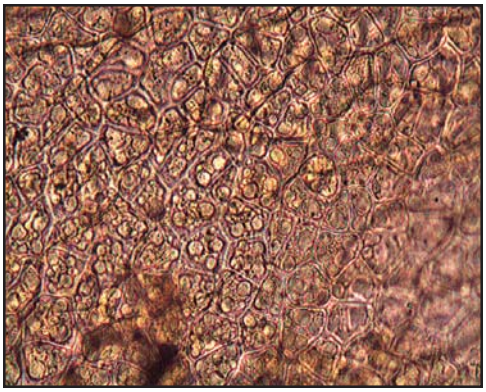
Powder microscopy: Creamish yellow with some reddish brown fragments of seed coats some showing red colouring matter and others with uniformly thick walls, endosperm cells filled with oil and aleurone grains (Plate 2- E, F).



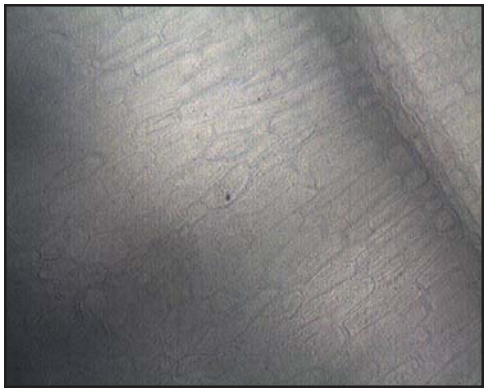
Seed T.S. x 40 A



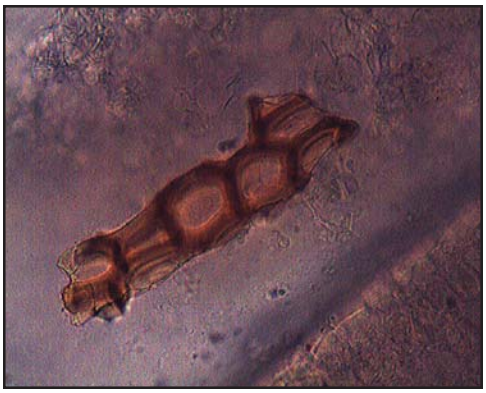
Seed surface view showing seed coat x 40 B



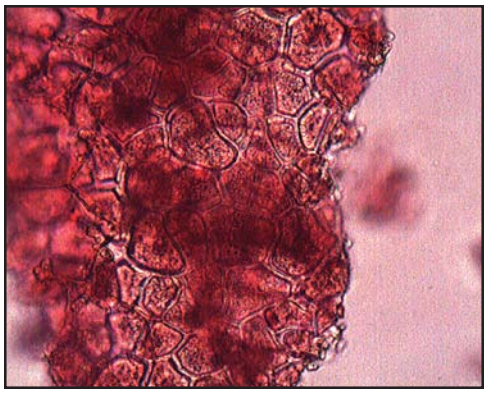
Seed surface view showing endosperm cells x 40 C



Seed T.S. cotyledon cells x 40 D



Powder showing cells of seed coat x 40 F



Powder showing endosperm cells seed coat x 40 E

Plate-2. Microscopical Features

Physico-Chemical Studies	Average
Total ash, (%) w/w	0.51
Acid Insoluble ash, (%) w/w	0.35
Ethanol Soluble extractive, (%) w/w	15.61
Water soluble extractive, (%) w/w	the formation of jelly
Fixed oil, % v/w	9.60

HPTLC Profile

For TLC profile, the solution of the drug was prepared with dissolving 1 mg of the extract in 10 ml methanol.

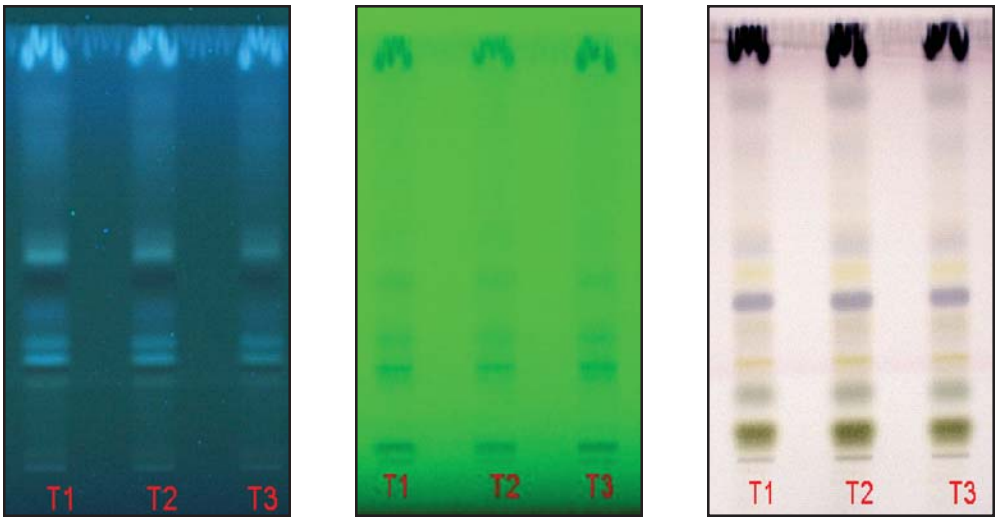
Extracts of the drug was applied on a pre-coated silica gel TLC plates (E. Merck) of uniform thickness with the help of Desaga HPTLC applicator and the TLC was developed in solvent system n- Butanol: Acetic acid: Water (4:1:5) upper layer in the development chamber and the photograph was taken with the help of Video documentation system. The photographs were taken with the help of Desaga Video documentation unit. Under UV 254 nm four spots were observed (R_f 0.05, 0.23, 0.29 and 0.43 all dark grey), under UV 366 nm six spots were observed (R_f 0.19 light blue, 0.23 black, 0.25 & 0.29 both blue, 0.43 black and 0.48 light blue) and after derivatization when the plate was sprayed with Anisaldehyde sulphuric acid reagent and heated at 105^0C for 10 minute in oven, eight spots were observed (R_f 0.05 yellowish green, 0.14 dark grey, 0.23 brownish yellow, 0.29 grey, 0.36 violet, 0.43 yellow and 0.82 light violet) (Plate 3 A,B,C).

Results and Discussion

Lepidium sativum L. is one of the highly efficacious herbal drugs in Unani and Ayurvedic System of medicine. The study assumes great significance as it will facilitate detection of adulterants and other wasteful matter in the drug available commercially. When the three samples of drug were compared. It was found that all three samples were almost same.

Acknowledgment

The authors are thankful to the Director, Central Council for Research in Unani Medicine (CCRUM), New Delhi and Director PLIM, Ghaziabad, for providing facilities and constant encouragement.



UV-366nm A UV-254nm B After Derivatization C

Plate-3. TLC Profile
Lepidium sativum Linn.

Solvent system: Toluene: Ethyl Acetate (9:1)
Spray reagent: vanillin Sulphuric acid

Refrences/Selected Reading

Alexander, C. Martin, Seed identification manual. Oxford and IBM publishing Co., New Delhi.

Anonymous, 1981. National Formulary of Unani Medicine, Part I, Ministry of Health & Family Welfare, Govt. of India.

Anonymous, 2005. Wealth of India, Vol I, CSIR, New Delhi, p. 71.

Anonymous, 1999. The Unani Pharmacopoeia of India, Ministry of Health & Family Welfare, New Delhi, Part I, First Edition, pp. 82-83.

E.J.H., Corner, 1976. The seeds of Dicotyledons Vol. I, Univ Press, Cambridge, pp. 111.

Johanson, D.A. 1940. Plant Microtechniques. Mc. Graw hill Bookcompany, New York.

Anonymous, 2004. The Ayurvedic Pharmacopoeia of India, Ministry of Health & Family Welfare, New Delhi, Part I, Vol. I, pp. 28.

Microscopical and Chemical Standardization of a Poly Herbal Drug – Jawarish-e-Hazim

[1]Rampratap Meena,
[1]S. Mageswari,
[1]D. Ramasamy,
[2]Shamshad Ahmed Khan,
[2]Shamsul Arfin
and
[1]Gowher Sultana

[1]Regional Research Institute of Unani Medicine (CCRUM), Royapuram, Chennai-13, Tamil Nadu

[2]Central Council for Research in Unani Medicine, 61-65, Institutional area, Janakpuri, New Delhi-58

Abstract

According to the World Health Organization (WHO), 80% of the developing world's rural population depends on traditional medicines for its primary health care needs. This underlies the urgent need for investing in the standardization and to develop the SOP's for traditional medicines, particularly for Unani system of medicine. With a view to lay down pharmacopoeial standards and SOP's, three batch samples of Jawarish-e-Hazim were prepared at laboratory scale at Drug Standardization Research Unit. In order to develop the pharmacopoeial standards and also to evaluate the quality, safety and efficacy of this drug the pharmacognostic, thin layer chromatography, microbial contamination, aflotoxin, pesticide residue and heavy metal studies were carried out. The data evolved from this study will be helpful in laying down SOP's and pharmacopoeial standards for Jawarish-e-Hazim.

Key Words: Microscopy, Chemical, TLC studies, Standardization.

Introduction

Jawarish-e-Hazim is a Unani Poly herbal compound formulation listed under the category of Majooniath in the Unani Formulary of India, Part-II. The drug is used in the ailment of incontinence of urine and dyspepsia. It is also very effective tonic for retentive, vesicular and digestive (Anonymous, 2007). According to the formula composition, this drug consists of four plant ingredients. In order to lay down SOP's and pharmacopoeial standards, the drug was prepared in three different batches at laboratory scale and subjected to analysis for micoscopical, physico-chemical, microbial, thin layer chromatography, aflotoxin, pesticide residue and heavy metal studies. The present paper describes the salient features of preparation, microscopical characters, physico-chemical and thin layer chromatography data of Jawarish-e-Hazim.

Material and Methods

The ingredients Nankhwah (Fruit), Kundur (Resin), Maweez (Fruit) and Qand Safaid (Sugar) were procured from local raw drug dealers with the help of Unani physician and identified botanically (Anonymous, 1990-2006, 2007) using pharmacognostical methods. The Jawarish-e-Hazim was prepared as per the formulation composition in the National Formulary of Unani Medicine, Part-II at the Drug Standardization Research Unit, Regional Research Institute of Unani Medicine, Chennai.

Formulation composition

1.	Nankhwah API-I	*Trachyspermum ammi* (L.) Sprague.	Frt.	125g
2.	Kundur API-IV	*Boswellia serrata* Roxb.	Res.	125g

3.	Maweez UPI-IV	*Vitis vinefera* Linn.	Frt.	250g
4.	Qand Safaid	Sugar	–	1.375kg

Method of Preparation

All the ingredients were taken of pharmacopoeial quality. Ingredients number 1 and 2 of the formulation composition were cleaned, dried, powdered and passed through sieve number 80. The powder of ingredient number 2 was mixed with the powder of ingredient number 1 and kept separately. Ingredient number 3 was cleaned and made into paste in mortar and pestle and kept separately. Specified quantity of sugar as per composition was dissolved in 1250ml of water on slow heat, at the boiling stage 0.1% of citric acid was added, mixed thoroughly and filtered through muslin cloth and prepared the quiwam of 78% consistency. The vessel was removed from the fire. While hot condition the paste of ingredient number 3 was added and mixed thoroughly. Then added the mixed powders of ingredient number 1 and 2 along with 0.1% sodium benzoate and mixed thoroughly to prepare the homogenous product. Allowed to cool to room temperature. Packed in tightly closed container to protect from light and moisture.

Chemical analysis

The samples of Jawarish-e-Hazim were carried out for chemical analysis. Physico-chemical studies like foreign matter, total ash, acid insoluble ash, solubility in alcohol and water, loss on drying at 105ºC, microbial load, aflotoxin, pesticide residue and heavy metal were carried out as per the WHO guidelines (Anonymous, 1998). The bulk density, sugar estimation and *pH* values for 1% and 10% aqueous solution were also carried out (Anonymous, 1987).

Microscopy

5g of the sample was weighed and mixed with 50ml of water in a beaker with gentle warming, till the sample was completely dispersed in water. The mixture was centrifuged and decanted the supernatant. The sediment washed several times with distilled water, centrifuged again and decanted the supernatant. A few mg of the sediment was taken and mounted in glycerine and observed for the following characters. Camera lucida drawings were done for the salient features of the drug (Johansen, 1940).

Preparation of extracts for TLC

2g of drug samples were soaked in chloroform and alcohol separately for 18 hours, refluxed for ten minutes on water bath and filtered. The filtrates were concentrated on water bath and made upto 5ml in a standard flask separately. These extracts were used for the thin layer chromatography analysis (Wagner *et. al.*, 1984).

Results and Discussion

Jawarish-e-Hazim is pale brown, semi solid with pleasant smell and sweetish bitter in taste. The drug did not show any filth, fungus or objectionable extraneous matter when the sample was spread in petri dish.

Microscopic observations

Nankhwah: Papillose epidermal cells in surface view with club shaped simple unicellular trichomes and trichome bases, vittae entire or broken pieces upto 250µ width and tapers towards the ends, endosperm cells in surface view with moderately thick walled parenchyma cells contain fixed oils and aleurone grains with micro rosette crystals (Fig. 1).

Maweez: Epidermal cells in surface view with reddish brown content, mesocarpic parenchyma cells in surface with irregular margin, vessels with spiral and reticulate thickening upto 25µ, fibres thick walled upto 800µ length and 20µ breadth with narrow lumen (Fig. 2).

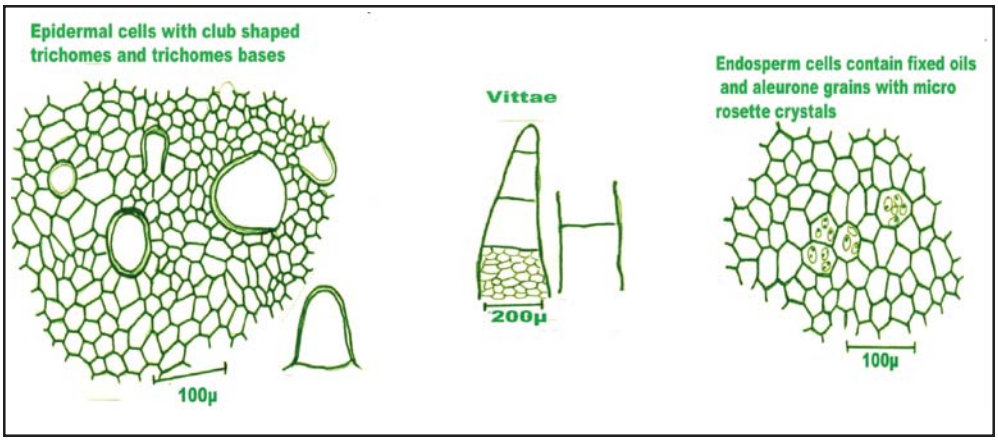


Fig. 1. Nankhwah

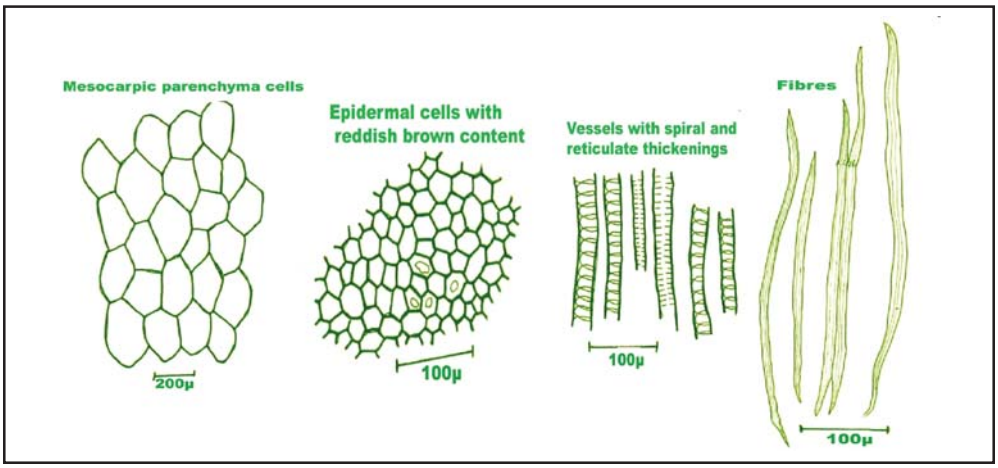


Fig. 2. Maweez

Kundur: Fibres thick walled upto 30µ width with numerous pits on lateral walls surrounded by a crystal sheath with prism of calcium oxalate from the debris, very few cork cells (very few) in surface view, (Fig. 3).

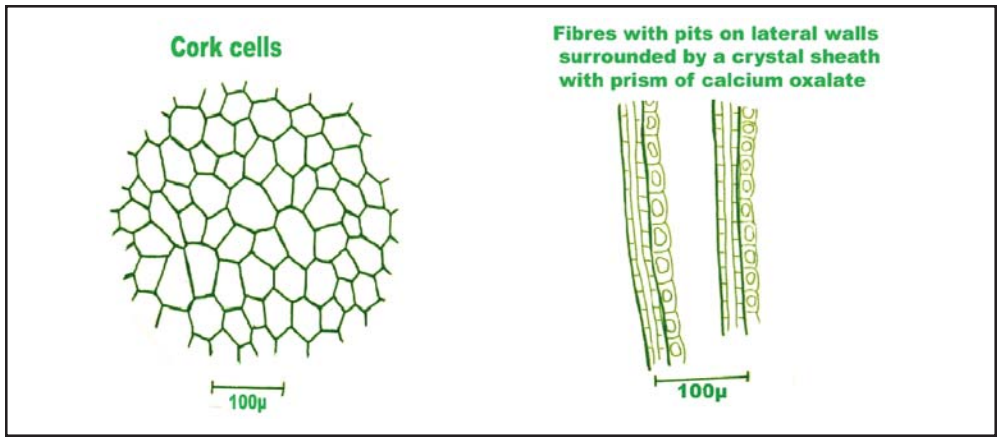


Fig. 3. Nankhwah

Chemical analysis

The physico-chemical data of the drug are shown in Table-I. The low value of acid insoluble ash of the drug indicates that the drug is free from siliceous matter. Alcohol soluble extractive content 61.83% might be due to the extraction of polar chemicals constituents. The water soluble extractives 70.66% indicates the presence of inorganic constituents. The content 21.84% is loss on weight at 105ºC shows the presence of moisture in the drug. The results of total bacterial count, total fungal counts of the microbial studies are with in the permissible limit and the other parameters were found to be absent in the drug. The analysis of aflotoxin and pesticide residue studies shows that the drug is free from aflotoxins and pesticide residues. The content of heavy metals such as lead and mercury are present with in the permissible limit, arsenic and cadmium were found to be below detection limit. The results observed for microbial load, aflotoxin level, pesticide residue and heavy metals are shown in Table II, III, IV and V respectively.

Thin Layer Chromatography analysis

Chloroform extract was applied on TLC plate. The plate was developed using Toluene : Ethyl acetate (9 : 1) as mobile phase. After development the plate was allowed to dry in air and examined under UV (254nm). Major spots were observed at R_f 0.96, 0.81, 0.40, 0.31 and 0.11 (Pink). Under UV (366nm). Major spot was observed at R_f 0.21 (Light blue). The plate was dipped in vanillin-sulphuric acid reagent followed by heating at 110° for about 5 min and observed under visible light. The plate showed major spots at R_f 0.96 (Dark blue), 0.81 (Yellowish green), 0.77 (Violet), 0.74 (Light blue), 0.58 (Yellowish grey), 0.54, 0.49 (Violet), 0.44 (Blue),

Table-I. Physico-chemical parameters

Parameters	Batch Number					
	I	Mean value	II	Mean value	III	Mean value
Alcohol soluble matter (% W/W)	61.24 61.36 61.52	61.37	62.24 62.44 62.56	62.41	61.56 61.72 61.88	61.72
Water soluble matter (% W/W)	69.36 69.96 70.16	69.82	70.60 70.76 70.96	70.77	71.16 71.44 71.64	71.41
Total ash (% W/W)	1.05 1.11 1.18	1.11	1.11 1.15 1.24	1.16	1.18 1.23 1.26	1.22
pH values 1% Aqueous solution	5.60 5.70 5.80	5.70	5.70 5.80 5.90	5.80	5.50 5.60 5.70	5.60
pH values 10 % Aqueous solution	4.70 4.80 4.90	4.80	4.50 4.60 4.80	4.63	4.50 4.60 4.70	4.60
Sugar estimation Reducing sugar (% W/W)	28.54 28.56 28.76	28.62	28.58 28.62 28.84	28.68	28.81 28.96 29.45	29.07
Non reducing sugar (% W/W)	6.30 6.31 6.33	6.31	6.08 6.25 6.30	6.21	5.84 5.96 6.43	6.07
Moisture (% W/W)	21.92 21.99 22.05	21.98	21.76 21.83 21.87	21.82	21.65 21.71 21.80	21.72
Bulk Density	1.3015 1.3085 1.3125	1.3075	1.3146 1.3166 1.3188	1.3166	1.3138 1.3146 1.3156	1.3146

Table-II. Microbial load

S.No.	Parameter Analyzed	Results	Permissible Limits as per WHO
1	Total Bacterial Count	8600 CFU / gm	10^5 CFU / gm
2	Total Fungal Count	Nil/gm	10^3 CFU / gm
3	Enterobacteriaceae	Absent / gm	10^3 CFU / gm
4	Salmonella	Absent / gm	Nil
5	Staphylococcus aureus	Absent / gm	Nil

Table-III. Aflatoxin level

S.No.	Parameter Analyzed	Results	Detection limits
1	B1	Not detected	0.05 ppb
2	B2	Not detected	0.05 ppb
3	G1	Not detected	0.05 ppb
4	G2	Not detected	0.05 ppb

Table-IV. Pesticide residue

S.No.	Parameter Analyzed	Results	Limits
1	Organo Chlorine Group	Not detected	0.01 ppm
2	Organo Phosphorus Group	Not detected	0.01 ppm
3	Alachlor	Not detected	0.02 mg / kg
4	Aldrin	Not detected	0.05 mg / kg
5	Chlordane	Not detected	0.05 mg / kg
6	DDT	Not detected	1.0 mg / kg
7	Endosulfan	Not detected	3.0 mg / kg
8	Heptachlor	Not detected	0.05 mg / kg
9	Lindane	Not detected	0.6 mg / kg
10	Malathion	Not detected	1.0 mg / kg

Table-V. Heavy metals

S.No.	Parameter Analyzed	Results	Permissible Limits as per WHO
1	Arsenic	Below detection limit	10 ppm
2	Cadmium	Below detection limit	0.30 ppm
3	Lead	1.2 ppm	10 ppm
4	Mercury	0.16 ppm	1.0 ppm

0.38 (Pale yellow), 0.28 (Greenish yellow), 0.24 (Light pink) and 0.15 (Yellowish green).

The alcohol extract was applied on TLC plate. The plate was developed using Toluene : Ethyl acetate (1 : 1.5) as mobile phase. After development the plate was allowed to dry in air and examined under UV (254nm). It showed major spots at R_f 0.32 and 0.27 (Pink). The plate was dipped in vanillin-sulphuric acid reagent followed by heating at 110° for about 5 min and observed under visible light. The plate showed major spots at R_f 0.96 (Dark blue), 0.84 (Greenish yellow), 0.79 (Dark pink), 0.67 (Violet), 0.61 (Light grey), 0.54 (Blue), 0.45 and 0.35 (Violet). TLC studies with chloroform and alcohol extract, all the three batch samples showed similar spots. The plates were developed using Vannilin-Sulphuric acid and heated at 105º till colored spots appeared (Fig. 4 & 5).

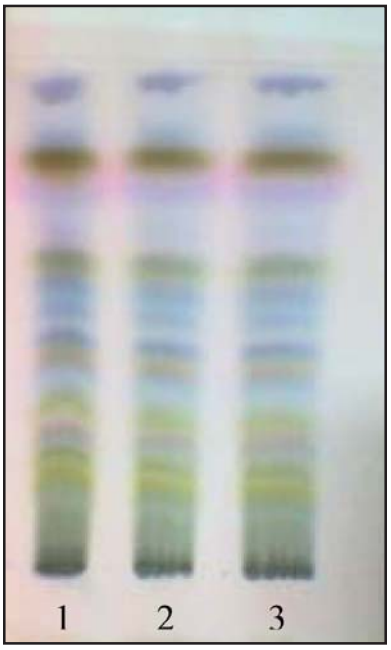


Fig. 4. Chloroform Extract
Track 1. Batch - I
Track 2. Batch - II
Track 3. Batch - III

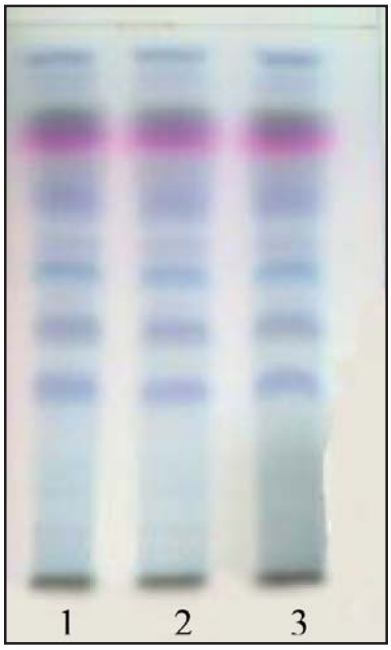


Fig. 5 . Alcohol Extract
Track 1. Batch - I
Track 2. Batch - II
Track 3. Batch - III

Acknowledgement

The authors are extremely thankful to the Director, CCRUM, New Delhi, for his valuable guidance, encouragement and providing necessary research facilities.

Reference

Anonymous, 1987. Physico-chemical standards of Unani Formulations Part – II, CCRUM, Min. of Health & Family Welfare, New Delhi.

Anonymous, 1998. Quality Control Methods for Medicinal Plant Materials. World Health Organisation, Geneva, p. 25-28.

Anonymous, 1990-2006. Ayurvedic Pharmacopoeia of India, Vol I – V, Min. of Health & Family Welfare, Govt. of India, New Delhi.

Anonymous, 2007. The Unani Pharmacopoeia of India, Vol - IV, Min. of Health & Family Welfare, Govt. of India, New Delhi.

Anonymous, 2007 (New Edition). National Formulary of Unani Medicine, Part-II (English Edition), Govt. of India, Min. of Health & Family Welfare, New Delhi.

Johansen, D.A., 1940. Plant Microtechnique. Mc. Graw Hill Book Company Inc. New York and Londan, p. 181-186.

Wagner, H., Bladt, S. and E.M. Zgainski, 1984. Plant Drug Analysis, A Thin Layer Chromatography Atlas (2nd Edition). Springer-Verlag, Germany.

Folk Medicinal Plants of Lansdowne Forest Division Kotdwar (Pauri Garhwal), Uttarakhand

Zaheer Anwar Ali
and
Sarfraz Ahmad

Survey of Medicinal Plants Unit,
Regional Research Institute of
Unani Medicine (CCRUM),
Post Box 70, Aligarh-202001 (U.P.)

Abstract

A total of 26 species of plants belonging to 20 families of angiosperms, used as folk drugs in the Lansdowne forest division, Kotdwar of Pauri Garhwal, Uttarakhand is described in this report based on ethnobotanical field study. For each species the correct botanical and prevalent local names, the part used, claimed medicinal use(s) and mode of administration are given. It lists many new and interesting phyto-therapeutic uses.

Key Words: Ethnobotanical survey, Folk medicine, Lansdowne, Pauri Garhwal, Uttarakhand.

Introduction

The Garhwal Himalaya is spread over a large area in the state of Uttarakhand. It has significant intact tracts of natural forests and tribal population. Plants still continue as one of the major source of drugs in traditional medicine throughout the region. This was the reason that explorations undertaken by ethnobotanists and other investigators have yielded a large number of useful information regarding the folk medicinal uses of plants as evident from published reports (Bhatt, 1994; Bhatt and Panwar, 1994; Bist and Badoni, 1990; Chandra and Pandey, 1983; Dhasmana, 1986a, 1986b, 1987; Gargya *et al*., 1997; Gaur *et al*., 1983; Joshi *et al*., 1982; Maheshwari and Singh, 1984; Mehrotra, 1979; Naithani, 1973; Nautiyal, 1981; Negi *et al*., 1985, 1992, 1999; Puri, 1983; Purohit *et al*., 1983; Rajwar, 1983; Rana and Dutt, 1997; Rao *et al*., 2002; Samant *et al*., 1996; Sharma *et al*., 1979; Singh, 2003, 1997; Singh *et al*., 1984, 1997, Singh and Ali, 1998; Tiwari and Pande 2004; Uniyal, 1987; Uniyal *et al*., 2002). But no separate list exists for the Lansdowne forest division Kotdwar. Hence, an ethnobotanical survey was conducted in the area. The present paper communicates information on some folk medicinal plants obtained during the fieldwork. After perusal of available literature on medicinal plants and folk medicines of the country (Anonymous, 1948-1976, 2001; Chopra *et al*., 1956; Jain, 1991; Kirtikar and Basu, 1935; Nadkarni, 1954), it has been found that uses of some medicinal plants were similar to those already published. But, their methods of drug preparation and mode of administration differ in majority of cases. For other plants therapeutic uses reported herein seem to be new or less-known and enrich our existing traditional knowledge.

The study area forms a part of North Kumaon Forest Circle of Uttarakhand and lies between 29º 37"- 30º 08' North latitude and 78º 10' 35"- 78º 43' East longitude in the lower Himalayan ranges of Pauri Garhwal district. It is rich in vegetation as well as wildlife and inhabited by the tribes Bhoxa and Vangujjar. The selected tribal settlements viz. Bagnala, Kohluchor, Saneh, Dhandyanwala, Haldukhatta, Gularjhala, Mollapuri, Motadhak, Nayagaon, Methiberi, Sattapuri, Gumakhal, Morakhal,

Sendikhal, Pokhal were visited which are located in Dogadda, Kohtri, Kotdwar, Laldhang, and Lansdowne forest ranges (Fig. 1).

Methodology

Fieldwork was carried out in November 2006 and information on folk medicinal uses of plants was obtained through direct field interviews with local medicine men and other knowledgeable villagers. Data on the common name of the plant or crude drugs, medicinal use(s), part used, other ingredients added (if any), method of drug

Fig. 1. Map showing areas of study in Lansdowne forest division Kotdwar, Uttarakhand

preparation, mode of application, dosage and duration of treatment were recorded for each claim. Plant specimens were collected with the help of informants and later identified by the authors. "*The Flora of British India*" (Hooker, 1872-1897), "*The Flora of the Upper Gangetic Plain and of the Adjacent Siwalik and Sub-Himalayan Tracts*" (Duthie, 1903-1922) and "*A forest flora for Kumaon*" (Osmaston, 1926) were consulted for botanical identification. All voucher specimens were prepared and deposited in the Herbarium of the Survey of Medicinal Plants Unit, Regional Research Institute of Unani Medicine, Aligarh (U.P.), India.

Observations

In the following listing, plants are arranged in alphabetical order by their botanical name together with respective family between parentheses, local name (in italics), locality and voucher specimen number, followed by habit and claimed use(s).

Achyranthes aspera L. (Amaranthaceae), *Ultakanta*, Morakhal, (*SMPUA7562*).

Root and fruits of this coarse weed are used to treat loosening of teeth and bronchitis. (a) Long tender piece of the root is made into a toothbrush and used daily in the morning to strengthen gum and teeth. (b) Dried fruits are burnt to ashes and about 1g of this is mixed with honey and taken twice a day, for 10-15 days.

Ageratum conyzoides L. (Asteraceae), *Phulghass,* Bagnala (*SMPUA7001*).

Fresh leaf juice of this softly hairy herb is poured on sharp cut and wounds to stop the bleeding.

Asparagus adscendens Roxb. (Liliaceae), *Jhirna*, Kohtri (*SMPUA7568*).

A sub-erect spiny shrub with tuberous roots. For treating sty, only a touch of spine with the sty is recommended.

Boerhavia diffusa L. (Nyctaginaceae), *Patharchata*, Saneh (*SMPUA7598*).

A diffusely branched herb used to dissolve and expel small stones from kidney and urinary bladder. Root is cut into small pieces and ground to a fine paste. 10 g of this paste are given three times a day, for one month.

Bombax ceiba L. (Bombacaceae), *Simbal*, Nayagaon (*SMPUA7627*).

It is a tall deciduous tree. But, tap root of the young plant in the form of paste is valued as a general tonic.

Butea monosperma (Lam.) Taub. (Fabaceae), *Dhak*, Kohluchor (*SMPUA7580*).

Stem bark of this moderate-sized tree is used to treat toothache and loosening of gum and teeth. Stem bark is boiled, strained and cooled. It is used as mouthwash twice a day, for 10-20 days.

Caesalpinia bonduc (L.) Roxb. (Caesalpiniaceae), *Karaunj*, Paniyali (*SMPUA7585*).

Seeds of this large rambling climber are bitter and used in worm infestation and to give up breast feeding in cases of children. (a) In cases of worm infestation, about 1g powder of seed mixed with honey is given three times a day, for 5-7 days. (b) It is advised to apply paste of the seed on breasts of lactating mother to give up feeding.

Cassia fistula L. (Caesalpiniaceae), *Amaltas*, Morakhal (*SMPUA7558*).

The sweetish pulp round the seed of this moderate-sized tree is used to treat constipation. About 15-20 g dried pulp is soaked in water and given orally with little *'misri'* (crystalline sugar) at bedtime, for 3-5 days.

Catharanthus roseus (L.) G. Don (Apocynaceae), *Sadabahar*, Saneh (*SMPUA7597*).

Flowers of this cultivated shrub are used for controlling diabetes. Five to seven freshly crushed flowers of white flowered variety are given once daily in the morning.

Cuscuta reflexa Roxb. (Cuscutaceae) *Amarbel,* Dogadda (*SMPUA7499*).

A leafless parasitic herb, with glabrous greenish-yellow twining stems, is used for abdominal swelling. Fresh stems are collected and ground to obtain a fine paste. It is applied externally on abdomen, for 7-10 days.

Dalbergia sissoo Roxb. (Fabaceae), *Shisham*, Mithiberi (*SMPUA7587*).

Wood oil of this fairly large tree is used to treat scabies. The oil, obtained by heating the small pieces of heart wood in an earthen pot, is applied locally on affected parts.

Dioscorea bulbifera L. (Dioscoreaceae), *Gitti,* Nayagaon (*SMPUA7637*).

The small potato-like bulbils of this twining herb are dried and made into chips. These are cooked and taken for indigestion.

Euphorbia thymifolia L. (Euphorbiaceae), *Dudhi*, Dogadda (*SMPUA7590*).

A small herb used to treat malaria fever. Whole plants are crushed and squeezed to obtain the juice. One to two spoonful of this juice are given 2-3 times a day, for 5-7 days.

Grewia optiva Drumm. *ex* Burret (Tiliaceae), *Bhimal,* Pokhal (*SMPUA7527*).

Fresh paste of the stem bark of this small tree is used to wash hair for healthy growth. *Holarrhena pubescens* (Buch.-Ham.) Wall. *ex* G. Don (Apocynaceae), *Kura*, Morakhal (*SMPUA7505*).

The bark of this small deciduous tree is used as febrifugal in cases of cattle.

Holoptelea integrifolia (Roxb.) Planch. (Ulamaceae), *Kanju* or *Papri*, Gularjhala (*SMPUA7638*).

Leaf of this large deciduous tree is used for treating ringworms. Leaf is ground with little *'mattha'* (liquid left after extraction of butter from curd) to make a paste and applied externally once only.

Justicia adhatoda L. (Acanthaceae), *Basing*, Dogadda (*SMPUA7522*).

A dense evergreen shrub with a fetid smell used to treat cough and cold. Shaded dried leaves are burnt and 1g ash is given with lukewarm water three times a day, for 3 consecutive days.

Launaea procumbens (Roxb.) Ramayya and Rajgopal (Asteraceae), *Churi,* Laldhang (*SMPUA7643*).

A glabrous herb with yellow juice is used for flatulence. The paste of 10 g leaves obtained by grinding in water is taken once every other day.

Litsea glutinosa C.B. Robinson (Lauraceae), *Maida*, Bagnala (*SMPUA7599*).

The inner mucilaginous stem bark of this medium-sized tree is ground with '*tara'* (seeds of *Eruca vesicaria* (L.) Cav. ssp. *sativa* (Mill.) Thell.) and plastered around the fractured limb after setting the bone right. Splints and bandage are used to hold the bones and plaster in position.

Mallotus philippinensis (Lam.) Muell.-Arg. (Euphorbiaceae), *Rohini*, Morakhal (*SMPUA7543*).

The red resinous powder from the dried fruits is collected. It is mixed with fodder and fed to cattle for worm infestation.

Oroxylum indicum (L.) Vent. (Bignoniaceae), *Tantia*, Laldhang (*SMPUA7649*).

Bark of this small soft-wooded tree is used for diarrhoea of cattle. About 1 Kg of fresh stem bark is ground to a fine paste and fed to cattle twice a day, for 3-5 days.

Premna mucronata Roxb. (Verbenaceae), *Bokar* or *Gandeela*, Laldhang (*SMPUA7624*)

A small tree used to treat herpes, locally called '*makri'*. Fresh pieces of the stem bark are crushed and squeezed to obtain the juice. It is applied externally 3-4 times a day till the cure is obtained.

Pyrus pashia Buch.-Ham. ex D. Don (Rosaceae), *Mehal,* Lansdowne (*SMPUA7621*).

Over ripe fruits of this medium-sized tree are chewed for stomatitis.

Sida cordifolia (Willd.) Miers (Malvaceae), *Khurenti,* Bagnala (*SMPUA7605*).

An erect under-shrub used to treat jaundice: 2-3 teaspoonful of the juice of fresh leaves are given, once a day in the morning on empty stomach, for 7-10 days.

Tinospora cordifolia (Willd.) Miers (Menispermaceae), *Guruch* or *Giloh,* Haldukhatta (*SMPUA7633*).

A soft-wooded succulent climber used to treat fever of cattle. Fresh stem-bits mixed with fodder are given 2-3 times a day till the cure is obtained.

Tridax procumbens L. (Asteraceae), *Bhangaraya*, Paniyali (*SMPUA7667*).

A procumbent herb used to stop the bleeding of sharp cut. Leaf juice is applied externally.

Discussion

The present study on Lansdowne forest division Kotdwar (Pauri Garhwal), Uttarakhand has led to the documentation of folk medicinal uses of some 26 plant species belonging to 20 different families of angiosperms. The information was gathered from the reliable informants. Folk medicines are generally used by elderly people who repose deep faith in the healing properties of medicinal plants whereas the younger generations usually do not show interest in plant remedies. The knowledge of medicinal plants in the area is at the risk of disappearing because of acculturation of indigenous societies and increasing access to Primary Health Centers and above all the apathy of younger people. Therefore, it is pertinent to undertake surveys of other ethnobotanically unexplored areas of this region before most of such information is forgotten forever.

Uses of folk medicinal plants in the listing are based on ancestral knowledge and empiric experience. Such information may provide access to researchers in development and search of new pharmaceuticals. As many potent drugs, in western medicine used throughout the world, have been discovered through follow up of traditional knowledge (Cox, 1994).

Acknowledgements

We are highly grateful to Dr. Mohammad Khalid Siddiqui, Director, Central Council for Research in Unani Medicine, New Delhi for providing necessary facilities for carrying out this investigation. We would also record our gratitude to the informants who graciously provided the information presented herein.

References

Anonymous, 2001. Medicinal Plants in folklores of Northern India. CCRUM, New Delhi.

Anonymous, 1948-1976. The Wealth of India (Raw Materials), Vol. I-XI. CSIR, New Delhi.

Bhatt, B.P. and Panwar, M.S., 1994. Plants with fertility regulating potential of Garhwal Himalayas. *J. Econ. Bot. & Phytochemistry* 1(1): 33-34.

Bhatt, V.P., 1994. Ethnomedicine of Pilgrims of Badrinath the holy shrine of Hindus. *Fourth Int. Cong. of Ethnobiology* (NBRI), Lucknow, P. 144.

Bist, M.K. and Badoni, A.K., 1990. Araceae in the folk life of the tribal populace in Garhwal Himalaya. *J. Econ. Bot. & Phytochemistry* 1: 21-24.

Chandra, K., and Pandey, H.C., 1983. Collection of plants around Agora Dodital in Uttarkashi district, Uttar Pradesh, with medicinal values and folklore claims. *Int. J. Crude Drugs Res.* 21: 21-28.

Chopra, R.N., Nayar, S.L. and Chopra, I.C., 1956. Glossary of India Medicinal Plants, CSIR, New Delhi.

Cox, P.A., 1994. The ethnobotanical approach to drug discovery: strength and limitations. In: Chadwick, D.J. and Marsh, J. (Eds.) Ethnobotany and the search for new drugs. John Wiley & Sons, England. Pp. 25-41.

Dhasmana, H., 1986a. Medicinal plants of Pauri town (Garhwal) and adjacent forest region (Part: family-Labiatae). *J. Sci. Res. Pl. Med*. 4(1-4): 52-60.

Dhasmana, H., 1986b. Medicinal plants of Pauri town (Garhwal) and adjacent region, family Compositae. *J. Sci .Res. Pl. Med*. 7(1-4): 45-49.

Dhasmana, H., 1987. Some medicinal plants of Pauri town and adjacent forest regions (Pauri Garhwal). *J. Sci. Res. Pl. Med.* 8(1-4): 1-8.

Duthie, J.F., 1903-1922. Flora of the Upper Gangetic Plain and of the Adjacent Siwalik and Sub-Himalayan Tracts. Vol. I-II (Repr.1960). BSI, Calcutta.

Gargya, G.R., Vasistha, H.B. and Gaur, R.D., 1997. Medicinal importance of some legumes of Mandakini Valley in Garhwal Himalaya. *Van Vigyan35*: 172-177.

Gaur, R.D., Semwal, J.K. and Tiwari, J.K., 1983. A survey of high altitude medicinal plants of Garhwal Himalaya. *Bull. Med. Ethnobot. Res*. 4(3-4):102-116.

Hooker, J.D., 1872-1897. The Flora of British India. Vol.1-VII. L. Reeva and Co. London.

Jain, S.K., 1991. Dictionary of Indian Folk Medicine and Ethnobotany. Deep Publications, New Delhi.

Joshi, D.N., Sah, B.C.L. and Suri, R.K., 1982. Some medicinal plants of Rudranath Bugyal (Dist. Chamoli), U.P. *Bull. Med. Ethnobot. Res.* 3(1): 27-42.

Maheshwari, J.K. and Singh, J.P., 1984. Contribution to the ethnobotany of Boxa tribe of Bijnor and Pauri Garhwal districts (U.P.). *J. Econ. Tax. Bot*. 5: 251-259.

Mehrotra, B.N., 1979. Survey of medicinal plants around Kedarnath Shrine of Garhwal Himalaya. *Indian For.*105: 788-801

Naithani, B.P., 1973. Medicinal plants of western Garhwal. *Khadi Gramoudyog*19(5): 269-278.

Nautiyal, S., 1981. Some medicinal plants of Garhwal hills-A traditional use. *J. Sci. Res. Pl. Med*. 2(1-2): 12-18.

Negi, K.S., Gaur, R.D. and Tiwari, J.K., 1999. Ethnobotanical notes on the flora of Hari-ki-Doon (District Uttarkashi) Garhwal Himalaya, Uttar Pradesh, India. *Ethnobotany* 11(1-2): 9-17.

Negi, K.S., Tiwari, J.K. and Gaur, R.D., 1985. Economic importance of some common trees in Garhwal Himalayas: An ethnobotanical study. *Indian J. Forestry* 8(4): 276-289.

Negi, K.S., Tiwari, J.K., Gaur, R.D. and Pant, K.C., 1992. Notes on ethnobotany of five districts of Garhwal Himalaya, Uttar Pradesh. *Ethnobotany* 5: 73-81.

Osmaston, A.E., 1926. A forest flora for Kumaon. Periodical Experts Book Agency, Delhi.

Puri, H.S., 1983. Low altitude medicinal plants of south Garhwal (Garhwal Himalaya). *Bull. Med. Ethnobot. Res*. 4(1-2): 1-13.

Purohit, V.P., Silas, R.A. and Gour, R.D., 1985. Ethnobotanical studies on some medicinal plants used in skin diseases from Raath (Pauri) Garhwal Himalaya. *J. Sci. Res. Pl. Med*. 6: 39-47.

Rajwar, G.S., 1983. Low altitude medicinal plants of South Garhwal. *Bull. Med. Ethnobot. Res*. 4(1-2): 14-28.

Rana, T.S. and Datt, B., 1997. Ethnobotanical observation among Jaunsar-Bawar, Dehradun (U.P.), India. *Int. J. Pharmacog*. 35: 371-374.

Rao, K.S., Maikhuri, R.K., Nautiyal, S. and Saxena, K.G., 2002. Ethnobotany of tolcha sect of Bhotiya community of Garhwal Himalaya, India. In: V.K. Singh, J.N. Govil and G. Singh (Eds.) *Recent Progress in Medicinal Plants.* Vol. II. *Ethnomedicine and Pharmacognosy*. SCI TECH Publishing LLC, USA, Pp. 9-32.

Samant, S.S., Dhar, U. and Rawat, R.S., 1996. Natural resources use by some natives within Nanda Devi biosphere reserve in West Himalaya. *Ethnobotany* 8: 40-50.

Sharma, P.K., Dhyani, S.K. and Shankar, V., 1979. Some useful and medicinal plants of the district Dehradun and Siwalik. *J. Sci. Res. Pl. Med*. 1(1): 17-43.

Singh, H., 2003. Herbal recipes for spermatorrhoea by Bhoxa tribe of Uttaranchal. *Ethnobotany* 15(1-2): 115-117.

Singh, K.K., 1997. Studies on native medicine of Jaunsari tribe of Dehradun district, Uttar Pradesh, India. *Int. J. Pharmacog*. 35: 105-110.

Singh, V.K., Ali, Z.A. and Siddiqui, K.M., 1997. Folk medicinal plants of Garhwal and Kumaon forests of Uttar Pradesh, India. *Hamdard Medicus* 40: 35-47.

Singh, V.K., and Ali, Z.A., 1998. Herbal drugs of Himalaya: Medicinal Plants of Garhwal and Kumaon regions of India. *Aspects of Plant Science*. Vol.15, Today & Tomorrow's Printers and Publishers, New Delhi.

Singh, V.K., Anis, M. and Khan, A.M., 1984. Folk medicinal claims of Chakrata forests, Uttar Pradesh, India. *J. Pl. Nature* 1(2): 16-21.

Tiwari, L. and Pande, D.C., 2004. Traditional veterinary practices in South-eastern part of Chamoli district, Uttaranchal, India. *Indian J. Trad. Knowledge* 3: 397-406.

Uniyal, M.R., 1987. Medicinal flora of Garhwal Himalaya. Shree Baidyanath Ayurved Bhawan Pvt. Ltd. Nagpur.

Uniyal, S.K., Awasthi, A. and Rawal, G.S., 2002. Traditional and ethnobotanical uses of plants in Bhagirathi Valley (Western Himalaya). *Indian J. Trad. Knowledge* 1(1): 7-19.

HIPPOCRATIC JOURNAL OF UNANI MEDICINE

Instructions to contributors

1. The paper(s) should be submitted in duplicate. Submission of a paper will be taken to imply that it is unpublished and is not being considered for publication elsewhere.

2. Papers should be written in English language and typed with double spacing on one side of A-4 size paper leaving top and left hand margin at least 1" (One inch) wide. Length of the paper should not exceed 12 pages.

3. Papers should be headed by a **title,** the initial(s) and surname(s) of author(s) followed by address.

4. Each paper should bear **abstract,** 2 to 5 **keywords, introduction, methodology, observations, results and discussion** followed by **acknowledgements and references.**

5. In all studies of plants or animals proper identification should be made as to the materials used.

6. While submitting the paper(s) for publication, Author(s) should decode the drugs specially in case of clinical studies.

7. Bibliographical reference should be listed in alphabetical order of the author at the end of the paper. Authors should be cited in the text only by their surname(s) but their initial(s) should be shown in the bibliography.

8. References to periodicals should include the name(s) and initial(s) of author(s), year of publication, title of the book, periodical, title of the article, volume number (Arabic numerals), issue number where appropriate, first and last page number. Reference to books should include name(s) and initial(s) of the author(s), year of publication, exact title, name(s) of publisher, place of publication, page number.

9. Reference should be cited in the text in parentheses by the name(s) of author(s) followed by the year of publication, e.g. "(Jain, 1991)" except when the author's name is part of the sentence, e.g. "Jain (1991) has reported that –". If there are more than two authors it is in order to put *"et al."* after the first name, e.g. Khan *et al.,* 1981.

10. Each table should be typed on a separate sheet of paper. Tables should be numbered consequently in Arabic numerals e.g. "Table 1, Table 2" etc., and attached to the end of the text. Tables should be provided with headings and kept as simple as possible and should be referred to in the text as "table 1" etc.

11. Figures (including photographic prints, line drawings on strong white or transparent paper, and maps) should be numbered consequently in Arabic numerals, e.g. “Fig. 1 etc.” and attached to the text behind the tables. Graphs and diagrams should be large enough to permit reduction to a required size. Legends for figures should be listed consequently on a separate sheet of paper. Photographs should be on glossy printing paper.

12. The editors reserve the right to refuse any manuscript submitted, whether on invitation or otherwise, and to make suggestions and modifications before publication.

13. Paper accepted by the editorial board will become the property of the CCRUM. No article or any part thereof may be reproduced in whatever form, without the written permission of the editor.

14. The editors and publisher are not responsible for the scientific content and statements of the authors of accepted papers.

R.N.I. Registration No. DELENG/2006/18866

CENTRAL COUNCIL FOR RESEARCH IN UNANI MEDICINE
Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy (AYUSH)
Ministry of Health & Family Welfare, Government of India
61 - 65 Institutional Area, Janakpuri, New Delhi - 110 058, India
Tel.: +91-11-28521981, 28525982, 28525983, 28525831/52/62/83/97, 28520501, 28522524 • Fax: +91-11-28522965
Web site: www.ccrum.com • E-mail: unanimedicine@gmail.com & ccrum@rediffmail.com