

# Sri Lanka Journal of Indigenous Medicine

# SLJIM



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# Sri Lanka Journal of Indigenous Medicine (SLJIM)

Volume 01 Number 01 Page 01 - 50 June 2011

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## Cover story

The figure on the cover page shows twig of *Santalum album* L (Sandalwood) of the family Santalaceae. It is indigenous to Mysore state in India and grows in the dry regions in India especially in Karnataka, Kerala and Tamilnadu and also in Eastern Java, Timor and Island of Malay Archipelago. It is cultivated in Sri Lanka.

It is a small to medium sized, ever green semi parasitic tree with slender branches reaching up to 18 meters in height and 2-4 meters in girth. Bark: dark grey or nearly black, rough, with deep vertical cracks on old trees. Leaves: glabrous, thin elliptic ovate or ovate-lanceolate. Flowers: straw-coloured, brownish purple, reddish purple or violet, unscented, in terminal axillary peniculate cymes.

Sandalwood oil derived from the root and heartwood of the tree is a viscous, yellowish liquid having a peculiar, heavy, sweet and very lasting odour. The main constituents of sandalwood oil is santalol. This primary sesquiterpene alcohol from more than 90% of the oil and is present as a mixture of two isomers,  $\alpha$ -santalol and  $\beta$ -santalol, the former predominating. The characteristic odour and medicinal properties of sandalwood oil are mainly due to the santalol. The higher the santalol contents of the oil, the greater its value. Oils of best quality contain over 94% of santalol.

Sandalwood has anti-inflammatory, anti-bacterial, anti-viral, anti-pyretic, blood purifying, and immune enhancing properties. Sandalwood oil acts as disinfectant on the mucus membranes of the genito-urinary and bronchial tracts; is applied externally in the form of paste with water to scorpion stings, inflamed swellings, prickly and skin eruptions, and on temples in headache, fever and also in skin diseases to allay itching, inflammation and heat.

The review article on page 44 describes the scientific studies of Rathakalka of which sandalwood is one of the major ingredients.

## **From the Director**

It is indeed a great pleasure and honour for me to write a message on the occasion of the release of first issue of the *Sri Lanka Journal of Indigenous Medicine (SLJIM)*, June 2011.

The system of Indigenous Medicine contains uncountable number of popular and effective medical formulations, their combinations with dosages, methods of usage and dietary and other restrictions in various texts.

The Institute of Indigenous Medicine of University of Colombo being the premier higher educational institution that provide instructions in Ayurveda, Unani and traditional systems of medicine in university education in Sri Lanka, has done a tremendous work to explore the scientific base of these information. It is also a prime duty and the responsibility of a university to impart evidence based knowledge for both health care seekers and the providers to meet their needs and challenges. This *SLJIM* is one such effort. The release of volume 01 of it is much applaudable because it is the first ever peer reviewed journal on Ayurveda in Sri Lanka. The information contained in this Journal will undoubtedly be useful for the Undergraduate and Postgraduate students who engage in research activities in relation to indigenous system of medicine.

I congratulate the Editor-in-Chief, Prof. A. P. G. Amarasinghe and the editorial board for their untiring effort to bring this task to reality with huge success. I also thank the authors for the articles, their valuable contribution, to promote health through indigenous medicine.

I wish all the success.

**Dr. R. A. Jayasinghe**

DAMS, DFMS, MCL(Delhi),  
Attorney-at-Law (SL)

*Director*

*Institute of Indigenous Medicine,  
University of Colombo,  
Sri Lanka*

## **From the Editor**

It is a great pleasure and privilege for me to have the opportunity of introducing the first volume of *Sri Lanka Journal of Indigenous Medicine (SLJIM)* in this form. This historical event fills a great lacuna in the field of Ayurveda and indigenous system of medicine in Sri Lanka.

The main purpose of this refereed journal is to provide a wide platform for the Sri Lankan scholars and researchers mainly in the field of Ayurveda and indigenous system of medicine to meet their counterparts in the other parts of the world in order to exchange new knowledge.

The first issue of *SLJIM* comes in this form due to the fullest cooperation received from the authors, referees, board of editors and advisors, and also continuous encouragement and guidance of the Director and the Higher Degrees Committee of the Institute of Indigenous Medicine.

Further, the day of coming out of the first issue of the Journal coincides with the 82<sup>nd</sup> anniversary of the Institute of Indigenous Medicine which is very important and memorable. Finally, I wish to bestow my best wishes for the future success of the Journal.

**Prof. A. P. G. Amarasinghe**

*Editor-in-Chief*

*SLJIM*

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# A preliminary study of the oral hypoglycaemic activity of the ethanol and water extracts of *Munronia pinnata* in the healthy Wistar rats

S D Hapuarachchi<sup>1</sup>, T S Suresh<sup>2</sup>, W T P S K Senerath<sup>3</sup>

## Abstract

This study was conducted to determine the oral hypoglycaemic activity of different doses of ethanol and a single dose of water extracts of *Munronia pinnata* in healthy adult Wistar rats. The ethanol extract (MPEt) was prepared using soxhlet apparatus and water extraction (MPW) was prepared according to the conventional/ traditional method used in Sri Lanka using whole plants. Healthy adult male Wistar rats 8 weeks of age and weighing 175.0 -225.0 g were used and they were divided randomly with six rats ( $n=6$ ) in each group for these experimental studies. The selected doses of MPEt (50.0 mg, 100.0 mg and 200.0 mg/kg body weight) and a single dose (1.6 g/ kg body weight) of MPW were given orally via a Sondi needle. After an overnight fast, fasting serum glucose concentration was determined and a glucose challenge was performed for the selected doses of MPEt (50.0 mg, 100.0 mg and 200.0 mg/kg body weight) and a single dose of MPW. Blood was drawn after 90 minutes following glucose (3.0 g/kg body weight) administration. Serum glucose level was measured by the glucose-oxidase method. Both extractions (MPEt and MPW) exerted statistically significant hypoglycaemic effects. All three selected doses were elicited comparatively less mean serum glucose concentrations ( $5.2 \pm 0.24$ ,  $5.1 \pm 0.26$  and  $4.2 \pm 0.34$  mmol/L) compared with the control group ( $5.4 \pm 0.22$  mmol/L) after 2nd hour. The maximum hypoglycaemic effect was recorded in 3rd hour of the 200.0 mg/kg dose (26.7%). The selected single dose of both extracts showed more effective hypoglycaemic activity ( $4.9 \pm 0.1$  mol/L of MPW and  $4.6 \pm 0.35$  mmol/L of MPEt) when compared with the control group ( $5.1 \pm 0.19$  mmol/L) respectively. The reduction given by the MPEt was significantly lower when compared with the control in paired t test ( $p \leq 0.001$ ). The MPW also showed a statistically significant ( $p \leq 0.01$ ) effect compared with control group.

## Introduction

Diabetes mellitus is the most common endocrine metabolic disorder. It has affected several millions in

different populations all over the world. This clinical syndrome is characterized by hyperglycaemia due to a deficiency or diminished effectiveness of insulin. Although there are number of widely accepted synthetic allopathic drugs that are used to control this metabolic syndrome, some are ineffective in many patients. Further, the controls of Non Insulin Dependent Diabetes Mellitus patients (NIDDM) are managed with long term treatment of allopathic drugs which can produce serious side effects. There are many plant species which are known in traditional or folk medical systems in Sri Lanka which are used for the treatment of diabetic mellitus due to their hypoglycaemic property [1]. The different parts of the plants as well as whole plant have been used for the treatment of diabetes. There are various forms of medicine such as *kwatha* (decoctions), *vati* (pills), *churna* (powders), *kalka* (pastes) and *arishta/asva* (fermented preparations) with the combination of herbal or herb-mineral drug preparations have been utilized in the Sri Lankan ayurveda/ traditional medical system. The evaluation of these plants and their natural principles is a logic way of searching for new drugs to treat this disease. Though, Sri Lanka is the smallest island in the Indian Ocean today it is considered one of the most biodiversity areas in South Asia. The island's natural flora has been enriched with 1500 medicinal plant species including 180 endemic plant species. Many of these plants were introduced from places as far off as China, Ethiopia and Arabia by travelers who visited the country during a period of at least two thousand years [2]. The plant *Munronia pinnata* (Wall) Theob (Meliaceae) locally called "Binkohomba" is a valuable and rare medicinal herb in Sri Lanka is not an endemic plant but now it is a threatened plant due to over exploitation. About six species of this plant restricted to tropical Asia and subtropical China eastwards to Timor in drier forests up to about 900.0 m. Presently *M. pinnata* plant has been distributed in Sri Lanka, Southern and Northeastern India, China, Vietnam, Burma, Thailand and Timor etc. In Sri Lanka this plant can be identified naturally

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over 700.0 m to 900.0 meter hilly areas. Ayurvedic and traditional physicians use this plant as a substitute for *Swertia chirata*, Family - Gentianaceae which is named as *kiratha* or *kiratha thiktha* in Sanskrit.

This plant is not available in Sri Lanka and also is prohibited to be exported from India [3]. Further, *M. pinnata* has been extensively used for fever, dysentery, diabetes and skin diseases in the form of powder and decoction due to its bitter taste.

The *M. pinnata* whole plants are uprooted from the wild and dried for commercial purposes [4]. Sri Lanka is the only country where this plant is used for medicinal purposes. However, traditional physicians claim that *M. pinnata* has been used in folk medical practice in Sri Lanka for hundreds of years. But there are no reports of any experimental or clinical studies of the biological activities of this plant. Therefore, the present study was carried out as an analytical interventional study.

## Materials and Methods

**Plant material:** *Munronia pinnata* whole plants were collected from the medicinal plant nursery at Haldummulla, Department of Ayurveda, Sri Lanka between the periods of November - December 2010. They were used for the preparation of extractions. *M. pinnata* plant was taxonomically identified and authenticated by the National Herbarium, Department of National Botanical Garden, Peradeniya, Sri Lanka where a voucher specimen was deposited (PDA/ MP 01). The air dried herb was coarsely powdered and used for the preparation of extractions.

**Experimental animals and their care:** Healthy out-bread male Wistar rats (175.0 g - 225.0 g) purchased from Medical Research Institute, Colombo, were used in this study. The study was conducted at the Department of Biochemistry and the Animal House of the Faculty of Medical Sciences, University of Sri Jayewardenepura. Rats were housed individually in rat cages in a well-ventilated room at an ambient temperature of  $29 \pm 2^\circ\text{C}$  at the Animal House. The standard WHO recommended diet was given and water was supplied *ad libitum*. The experimental procedures and animal care were conducted according to international laws and guidelines [5].

### Ethical clearance

A project protocol was submitted to the Ethical Review Committee of Faculty of Medical Sciences, University of Sri Jayewardenepura and ethical approval was obtained (No: 474/09).

### Preparation of extracts

The ethanol extract was prepared by using the Soxhlet extraction. The air dried coarse powder of *M. pinnata* (30.0 g) was run in Soxhlet apparatus using 300.0 ml of ethanol for  $30 \times 20$  minute cycles. The ethanol was evaporated in rotary evaporator at  $40^\circ\text{C}$  and a sticky

dark brown material was obtained and it was dissolved in 20.0 ml of distilled water. The supernatant was filtered and dried in a water bath. Finally, a brown colour material was obtained (MPEt) and was used for the hypoglycaemic study in rats.

The aqueous extract of *M. pinnata* was prepared according to the conventional/ traditional method used by traditional medical practitioners in Sri Lanka. The air dried coarsely powdered *M. pinnata* 60.0 g (12 kalan) was mixed with 8 parts/*patha* (1920.0 ml) of water in an earthen vessel and boiled over moderate heat and reduced to 1/8th part (240 ml). The dose is 240.0 mL per day (MPW) for adult human.

**Dose response curve of the ethanol extract (MPEt) of *M. pinnata* on the blood glucose level in the healthy rats:** Four groups of six rats ( $n=6$ ) in each were divided according to weight and fasting serum glucose concentrations. All four groups were fasted overnight with free access to water. To detect the most effective dose, three doses of the ethanol extract (50.0 mg, 100.0 mg and 200.0 mg/kg body weight) were administered orally via Sondi needles to each animal in the three groups. The animal dose was corresponded to the normal therapeutic dose administered to adult humans as calculated on the basis of relative surface areas of humans and rats [6]. The control group was treated with 2.5 ml of distilled water. After 30 minutes, a glucose load of 3.0 g/ kg body weight was given. Blood (0.1 ml) was drawn from the lateral tail vein of rats under light anesthesia with diethyl ether 90 minutes after the glucose administration. Blood samples were centrifuged (3000 ppm  $\times$  20 min.) and serum was separated. The serum glucose concentration was measured by the glucose-oxidase method [7] using BIOLABO reagent kits.

**Time course of the ethanol extract (MPEt) of *M. pinnata* on the blood glucose level in the healthy rats:** To determine the optimal time of activity two groups of rats ( $n=6$ ) were divided as test and control. After an overnight fast, fasting serum glucose concentration was determined and a glucose challenge was performed. The test group received 2.5 ml of MPEt as a single dose 200.0 mg/kg of and 2.5 ml each of distilled water was given to control group. After the administration of glucose (3.0 g/ kg), blood was drawn for the estimation of glucose at 1, 2, and 3 hrs.

**Comparison of the oral hypoglycaemic effect of a single dose of aqueous (MPW) and ethanol (MPEt) extracts of *M. pinnata* on the blood glucose level in the healthy rats:** Healthy adult Wistar rats were divided in to three groups according to body weight. Fasting serum glucose levels were determined as above.

According to the results of dose curve 200.0 mg/kg of MPEt and 1.68g/kg of MPW (as determined previously) were given to the rats in the test group. The control group

was treated with 2.5 ml of distilled water and the glucose challenge test were performed as described above.

### Statistical analysis

Statistical analysis was done by the help of student's t-test and presented as mean  $\pm$  S.D and a p value of  $\leq 0.05$  was taken as significant.

## Results and Discussion

The effects of different doses of ethanol extract of *M. pinnata* on the fasting serum glucose concentrations of healthy Wistar rats are shown in table 1. All three selected doses (50.0 mg, 100.0 mg and 200.0 mg/kg body weight) were elicited comparatively less mean serum glucose concentrations ( $5.2 \pm 0.43$ ,  $5.1 \pm 0.26$  and  $4.2 \pm 0.34$  mmol/L) compared with the control group ( $5.4 \pm 0.22$  mmol/L) after 3rd hour. The dose 200.0 mg/kg was recorded the lowest

mean serum glucose concentration ( $4.1 \pm 0.33$ ) in 3rd hour after the administration of MPET to the rats. Further, the maximum percentage of reduction of serum glucose concentration (26.7%) was also elicited from the same dose when compared with its control group ( $p \leq 0.001$ ).

The table 2 describes the results of the effect of a single dose of water and ethanol extractions of *M. pinnata* on the serum glucose concentration in the healthy rats.

Accordingly both extracts of *M. pinnata* showed more effective hypoglycaemic activity when compared with the control groups ( $4.9 \pm 0.04$ ,  $4.6 \pm 0.12$  and  $5.1 \pm 0.19$  mmol/L respectively).

The reduction given by the ethanol extract of *M. pinnata* was significantly lower when compared with the control group in paired t test ( $p \leq 0.001$ ). The water extract of *M. pinnata* also showed a statistically significant ( $p \leq 0.01$ ) effect compared with control group.

**Table 1: Effect of different doses of (MPET) on the blood glucose level in the healthy rats**

Doses	Serum blood glucose concentrations mmol/L ( n=6)			
	Time			
	Zero hour	1st hour	2nd hour	3rd hour
MPET 50mg/kg	3.3 $\pm$ 0.21	5.7 $\pm$ 0.43 (12.5%)	5.2 $\pm$ 0.24* (3 %)	4.9 $\pm$ 0.07* (12.5%)
MPET 100mg/kg	3.2 $\pm$ 0.24	5.8 $\pm$ 0.27* (10.7%)	5.1 $\pm$ 0.26* (5 %)	4.7 $\pm$ 0.14* (16.1%)
MPET 200mg/kg	3.3 $\pm$ 0.32	5.7 $\pm$ 0.14* (12.3%)	4.2 $\pm$ 0.34* (22.2 %)	4.1 $\pm$ 0.13** (26.7%)
DW 2.5ml (Control group)	3.3 $\pm$ 0.22	6.5 $\pm$ 0.41	5.4 $\pm$ 0.22	5.6 $\pm$ 0.13

Values are expressed as mean  $\pm$  SEM (n=6). Asterisks denoted the significance levels in comparison to control values. \* $p \leq 0.05$  and \*\* $p \leq 0.0001$ .

MPET: ethanol extract of *M. pinnata*; DW: distilled water.

Figures in parenthesis indicate reduction of the glucose percentage after administration of MPET.

**Table 2: Effect of the single dose of aqueous (MPW) and ethanolic (MPET) extracts of *M. pinnata* on the serum glucose concentration in the healthy rats**

Group ( n=6)	MPW	MPET	Control
Mean serum glucose concentration mmol/L	4.9 $\pm$ 0.04*	4.6 $\pm$ 0.12**	5.1 $\pm$ 0.07

Values are expressed as mean  $\pm$  SEM (n=6). Asterisks denoted the significance levels in comparison to control values. \* $p \leq 0.01$  and \*\* $p \leq 0.001$ .

MPW: water extract of *M. pinnata*; MPET: ethanol extract of *M. pinnata*; control: 2.5 ml of distilled water

## Conclusions

This is the preliminary experimental study of *Munronia pinnata* in healthy Wistar rats. Water and ethanol extracts of this plant exert a statistically significant oral hypoglycaemic effect in healthy rats. From the selected doses, the dose of 200.0 mg/kg of ethanol extract markedly pronounced more oral hypoglycaemic effect than the other selected doses. Further, the maximum hypoglycaemic effect (26.7%) was elicited from same dose, 200.0 mg/kg after 3rd hour from the administration of MPEt when compared with its control group ( $p \leq 0.001$ ). The reduction given by the single dose of ethanol extract of *M. pinnata* was significantly lower when compared with the control in paired t test ( $p \leq 0.001$ ). The water extract of *M. pinnata* (1.68 g/kg) also showed a statistically significant ( $p \leq 0.01$ ) effect compared with control group. Therefore, further experimental studies have to be carried out to determine the oral hypoglycaemic activity in diabetic rats.

## Acknowledgements

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## References

1. Thabrew MI, Tissera MHA. Medicinal plants and Ayurvedic preparation containing medicinal plants used in the control of diabetes mellitus. Publication of the Department of Ayurveda, Sri Lanka, 2001, 6-7.
2. Ashton MS, Gunathileke S, Zoysa N, Dassanayake MD, Gunathileke N, Wijesundara S. A Field Guide to the Common Trees and Shrubs of Sri Lanka. WHT Publication (Pvt.) Limited, Sri Lanka, 1997: 1-3.
3. Dassanayake MD, Fosberg FR, Clayton WD. A revised hand book to the flora of Ceylon, Smithsonian Institute and the National Science Foundation, Washington DC, Amerind Co. Ltd., New Delhi, 1995; **ix**: 236-9.
4. Jayaweera DM. Medicinal Plants in Ceylon, National Council of Sri Lanka, 1982; **4**: 59.
5. Research guidelines for evaluating the safety and efficacy of herbal medicines. World Health Organization, Regional Office for the Western Pacific, Manila, 1993.
6. Paget CE, Barnes JM. Evaluation of drug activities. *Pharmacometrics* 1996; **1**.
7. Huggeet A, Nelson DA. use of glucose oxidase, peroxidase and dianisidine in the determination of blood and urinary glucose. *Lancet* 1956; **2**: 368-70.

# Effect of fertilizer and irrigation on growth and yield of *Andrographis paniculata* (Burm.f.) Wall. Ex Nees var. *paniculata*

K S S Sugathadasa<sup>1</sup>, D K N G Pushpakumara<sup>2</sup>, M A N de Silva<sup>1</sup>

## Abstract

*Andrographis paniculata* (Burm.f.) Wall. ex Nees var. *paniculata* which belongs to the family Acanthaceae is an indigenous species in Sri Lanka. This study was designed to identify the effect of fertilizer and irrigation treatments on growth and yield of *A. paniculata*. The experiment was carried out in two agro ecological sites, the wet zone low country (WL1b) and the intermediate zone low country (IL2) of Sri Lanka. Factorial combination of three fertilizer treatments (no fertilizer, organic fertilizer only (1 kg per plot) and in-organic fertilizer only (N: P 80:40 kg /ha) and two irrigation methods (no irrigation and irrigation – twice a day) were used in a split plot experimental design. Growth characters measured showed significant variations between the two different sites. Highest growth was recorded in the wet zone with average dry weight per plant of 83 g compared to 53 g in dry zone. According to the dry matter production, the optimum time for the harvesting of plants were identified as 3.5 – 4 months after planting. Data analysis showed significant differences in the total, shoot and leaf dry weight per plant at different treatment levels. Root dry weights were not significantly different among treatment combinations. Results also revealed that the dry matter partitioning was significantly improved by the combined effect of both fertilizer and irrigation, but the magnitudes of impact are different for the two agroecological regions. Application of organic fertilizer with irrigation was found to be the most suitable combination for cultivation of *A. paniculata* in both zones.

## Introduction

*Andrographis paniculata* (Burm.f.) Wall. ex Nees var. *paniculata* belongs to the family Acanthaceae is an indigenous species to India, Sri Lanka, Malay Peninsula, China, Indo-China and Thailand [1]. The species found to be a weed in semi-shade areas in the moist and dry lowlands and moist mid-country areas in its native places in Sri Lanka. The entire plant is used in ayurvedic system in Sri Lanka and elsewhere. The plant is reported to posses

bitter, astringent, anodyne, alexiphamic and tonic properties [2]. It is suggested to be useful in liver disorders, jaundice, dysentery, cholera, consumption, influenza and bronchitis [3]. The plant has been used against AIDS, cancer and a variety of bacterial and virally induced diseases. *A. paniculata* is used as a substitute for *Swertia chirata* Buch.-Ham. of the family Gentianaceae which is not found in Sri Lanka. In India, *Andrographis echoides* is used as an adulterant or substitute for *A. paniculata*. This plant is mostly cultivated in China and India [4].

A number of diterpenes from the aerial parts of *A. paniculata* have been isolated where the most important diterpenes are diterpene glycoside andrographolide and andropanoiside [5]. The leaves contain the maximum active principle content while in the stem it is in lesser amount. The presence of dehydroandrographolide succinic acid mono ester, derived from andrographolide, and has been found to inhibit the human immunodeficiency virus (HIV) *in-vitro* [6]. The effect of agronomic practices used in cultivation of *A. paniculata* and its processing on chemical constituents has not been studied. According to the statistics available in year 2000 in Sri Lanka, the national demand for *A. paniculata* was estimated at about 12 mt/year [7] and it has been locally collected from wild although it has been suggested that *A. paniculata* can be easily cultivated by seeds [8].

There is no information on the effect of agronomic practices used in cultivation of *A. paniculata* and its processing on its biomass and chemical constituents in Sri Lanka or elsewhere. Accordingly, the objective of this study was to investigate the effect of fertilizer and irrigation treatments on growth and yield of *A. paniculata*.

## Materials and Methods

### Seed collection and their germination

*A. paniculata* seeds were collected from the plants grown at the garden of Bandaranaike Memorial Ayurvedic Research Institute (BMARI), Nawinna. Only healthy plants were selected to collect seeds. Seeds were freshly collected from the dried pods and used for germination. Seeds were first soaked in water for 12 hrs and then placed in seed trays filled with sand. Seed trays were kept in a

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plant house under the shade and watered twice a day. The number of seeds germinated was counted and recorded at two-day intervals after the emergence of the first seedling approximately 7 days after sowing.

#### **Selection of sites for trial, preparation of land and experimental design**

Two sites were selected: Herbal garden at Ayurveda Hospital, Meegoda to represent wet zone low country (WL1b) with a mean annual rainfall of less than 2,800 mm/year which contain red yellow podzolic soil (Site 1) and Herbal Garden at Girandurukotte to represent intermediate zone low country (IL2) with mean annual rain fall less than 1600 mm/ year which contain reddish brown earth soil (Site 2). Harrowing and ploughing of the land was done using a tractor. The land was leveled to prepare blocks and plots for planting. The area under the experiment was about 115.85 square meters and it was divided into 3 blocks of  $7.8 \times 3.9$  m size. One block was divided into 18 plots of  $1.2 \times 1.2$  m sizes which were arranged in two rows.

Factorial combination of three fertilizer treatments and two irrigation methods (Table 1) were as used and arranged in a split plot design where fertilizer treatments were replicated 6 times within a block and irrigation treatments were replicated 3 times.

**Table 1: Fertilizer treatments and irrigation methods**

Treatment	Levels
Fertilizer	1. No fertilizer 2. Organic fertilizer only (1 kg/plot) applied 1 week before planting 3. In-organic fertilizer only (N:P 80:40 kg/ha) applied 1 week before planting
Irrigation (started only a month after establishment)	1. No irrigation 2. Irrigation – Twice a day

#### **Establishment of plants**

Four centimetre tall plants were transferred into the small holes dug at  $30 \times 30$ cm distance within a plot. Daily watering was done twice a day for well establishment of plants in the field and after one month of growth, watering was done only for 9 plots within a block. *A. paniculata* was established in the two sites in two different seasons considering the rainfall pattern of the area. Plants were established at the end of March 2006 in Site 1 with the onset of inter monsoon before the southwest monsoon and mid September 2007 in Site 2 during inter monsoon before northwest monsoon.

#### **Measurement of growth parameters**

Growth was monitored during a 5 month period. First growth measurements were taken one week after the field establishment. It was repeated once a week for 2 months and once a month after 2 months over a total period of 5 months.

During this period plant height (cm), number of leaves, number of primary branches, days to 50% flowering, days to harvest, dry weight (g), leaf characters (leaf lamina length (cm)/ leaf lamina width (cm)/ petiole length (cm)/ petiole width (cm)), leaf shape, leaf surface, days to maturity of the capsule, fruit characters (capsule length (cm)/ capsule width (cm) /no. of seeds per capsule), capsule shape, flower characters, pest and diseases were measured and recorded. Considerable variation was observed in the growth of plants from the beginning. Immediately after field establishment, the growth of *A. paniculata* seedlings was found to be very slow, which recovered later.

#### **Soil analysis**

Soil analysis was done before adding fertilizer to the soil. Twenty five soil samples each of 100 g was taken randomly from a depth of 5 cm uppermost soil layer into the polythene bags using small fork and spade. These samples were mixed separately to obtain single compound sample, crumbled the fresh samples by hand and spread it on aluminium trays. Trays were kept in an oven ( $40^{\circ}\text{C}$ ) for 3-4 days. The dried soil samples were sieved using 2 mm sieve. Nitrogen (N), phosphorus (P), potassium (K) content, pH, organic carbon and organic matter percentage were measured for each soil sample. This was done for two sites separately [9].

### **Results and Discussion**

#### **Site conditions**

Soil analysis revealed that soil fertility status was different at the two sites. Soil pH of site 1 is acidic (5.64) compared to the value of site 2 (7.13). N content and percentage of organic carbon and organic matter in the site 1 is higher than that of site 2. P and K content recorded from the site 2 are higher than the site 1 (Table 2).

#### **Seed germination**

Seed germination was started 7 days after sowing and continued till 21 days. The seed germination rate of *A. paniculata* was high ( $90\% \pm 5.24$ ) after 21 days. All seedlings that emerged from the germinated seeds were healthy and after they reached 4 cm in height they were transferred to the field, with three different fertilizer treatments.

#### **Growth and yield**

These plants particularly in the two sites were morphologically different (Tables 2 and 3). These morphological differences mainly appeared due to the differences in the soil type, temperature and the rainfall

pattern of the two sites. Plants grown at Site 1 contained dark green fleshy leaves with broadly ovate-lanceolate shape compared to the pale green coriaceous leaves of ovate-lanceolate observed in Site 2.

The differences in growth of plant parts seem to be the combined effect of both fertilizer and irrigation.

The results also showed that height and number of leaves varied widely among the different fertilizer treatments and higher results were recorded from Site 2 compared to the Site 1 (Tables 5 and 6). Plants grown under irrigation with the application of organic fertilizer gave higher yields in both sites.

**Table 2: Soil pH, N, P, K content and organic carbon (OC) and organic matter (OM) recorded from two sites**

Site	pH	N mg/g	P mg/g	K mg/100g	OC%	OM%
Meegoda (Site 1)	5.64 ± 0.27	1.79 ± 0.21	25.17 ± 5.38	0.24 ± 0.02	1.98 ± 0.25	3.41 ± 0.44
Girandurukotte (Site 2)	7.13 ± 0.47	1.12 ± 0.21	36.34 ± 6.21	13.10 ± 2.84	0.75 ± 0.24	1.29 ± 0.31

**Table 3: Mean of leaf length, leaf width, petiole length and petiole width of *Androgaphis paniculata* under different treatments on final harvest at the herbal garden at Ayurveda Hospital, Meegoda**

Treatment		Leaf length (cm)	Leaf width (cm)	Petiole Length (cm)	Petiole Width(cm)
Inorganic	Irrigated	11.33 ± 0.614	3.64 ± 0.231	4.15 ± 0.583	2.024 ± 0.194
	Non irrigated	10.16 ± 0.809	3.09 ± 0.178	3.30 ± 0.307	2.05 ± 0.20
Organic	Irrigated	10.75 ± 0.897	3.22 ± 0.273	4.06 ± 0.520	2.125 ± 0.263
	Non irrigated	10.71 ± 0.556	2.6 ± 0.263	3.3 ± 0.280	1.96 ± 0.187
Control	Irrigated	10.04 ± 0.503	2.86 ± 0.209	3.04 ± 0.307	2.0 ± 0.20
	Non irrigated	9.64 ± 0.547	2.69 ± 0.204	3.28 ± 0.344	1.93 ± 0.43

**Table 4: Mean of leaf length, leaf width, petiole length and petiole width of *Androgaphis paniculata* under different treatments on final harvest at the herbal garden, Girandurukotte.**

Treatment		Leaf length (cm)	Leaf width (cm)	Petiole Length (cm)	Petiole width(cm)
Inorganic	Irrigated	9.84 ± 1.045	2.76 ± 0.204	4.25 ± 1.181	1.85 ± 0.30
	Non irrigated	9.57 ± 1.384	2.56 ± 0.462	4.67 ± 1.423	1.6 ± 0.469
Organic	Irrigated	9.90 ± 1.628	2.75 ± 0.453	4.13 ± 0.585	1.85 ± 0.191
	Non irrigated	7.58 ± 0.371	1.9 ± 0.324	3.18 ± 0.147	1.25 ± 0.262
Control	Irrigated	9.47 ± 1.263	4.09 ± 0.205	4.38 ± 0.784	1.85 ± 0.208
	Non irrigated	8.83 ± 0.479	2.35 ± 0.172	4.23 ± 0.806	1.58 ± 0.434

**Table 5: Mean height, mean number of primary branches and mean no. of leaves of *A.paniculata* at final harvesting stage under different treatments at the herbal garden, Meegoda**

<i>Treatment</i>		<i>Height (cm)</i>	<i>No. primary branches</i>	<i>No. of leaves</i>
Inorganic	Irrigated	47.88±3.88	54±2.88	297±26.62
	Non irrigated	47.89±2.96	38±1.87	222±22.01
Organic	Irrigated	60.08±3.60	79±2.26	423±29.94
	Non irrigated	56.95±2.64	37±1.50	345±25.36
Control	Irrigated	46.98±3.44	57±1.72	231±24.90
	Non irrigated	46.31±2.97	36±1.37	196±20.66

**Table 6: Mean height, mean number of primary branches and mean no. of leaves of *A.paniculata* at final harvesting stage under different treatments at the herbal garden, Girandurukotte**

<i>Treatment</i>		<i>Height (cm)</i>	<i>No. primary branches</i>	<i>No. of leaves</i>
Inorganic	Irrigated	61±3.755	24±2.06	949±63.10
	Non irrigated	61.25±2.217	19±2.98	763±49.92
Organic	Irrigated	64.75±3.795	22±1.74	905±57.8
	Non irrigated	59.25±2.872	21±1.20	827±41.37
Control	Irrigated	59±2.449	24±0.5	726±45.69
	Non irrigated	56.75±2.722	19±0.93	867±33.70

**Table 7: Mean leaf area (LW), dry weight of partition to leaves (DWL), shoot (DWS), root (DWR), total dry weight (TDW) of plant at final harvesting under different treatments at the herbal garden, Meegoda**

<i>Treatment</i>		<i>LA</i>	<i>DWL</i>	<i>DWS</i>	<i>DWR</i>	<i>TDW</i>
Inorganic	I	5506.67 ±866.87	27.34 ±2.17	40.1 ±3.28	2.92 ±0.21	70.36 ±5.14
	NI	4517.5 ±307.97	18.70 ±0.66	27.92 ±1.90	2.56 ±0.29	49.18 ±2.24
	I	5656.67 ±1172.37	31.65 ±2.37	48.54 ±5.18	3.18 ±0.30	83.37 ±7.13
	NI	5001.67 ±291.19	21.56 ±2.29	41.84 ±7.46	2.88 ±0.35	66.28 ±9.72
Organic	I	3087.17 ±295.82	12.48 ±1.16	19.12 ±2.63	1.21 ±0.19	32.8 ±3.43
	NI	3064.17 ±406.17	14.37 ±1.01	21.13 ±1.32	1.57 ±0.30	37.07 ±2.35
	I					
	NI					
Control	I					
	NI					

**Table 8: Mean leaf area (LA), dry weight of partition to leaves (DWL), shoot (DWS), root (DWR), total dry weight (TDW) of plant at final harvesting under different treatments at the herbal garden, Girandurukotte**

<i>Treatment</i>		<i>LA</i>	<i>DWL</i>	<i>DWS</i>	<i>DWR</i>	<i>TDW</i>
Inorganic	I	4272.5	19.57	30.75	3.29	53.6
		$\pm 701.62$	$\pm 2.70$	$\pm 11.13$	$\pm 0.48$	$\pm 13.56$
	NI	3195	17.48	36.55	3.42	57.44
		$\pm 355.65$	$\pm 2.53$	$\pm 4.90$	$\pm 0.71$	$\pm 7.61$
Organic	I	3840	15.04	35.09	3.33	53.46
		$\pm 321.64$	$\pm 0.62$	$\pm 6.70$	$\pm 0.27$	$\pm 7.24$
	NI	3042.5	17.33	29.72	2.29	49.33
		$\pm 362.57$	$\pm 1.98$	$\pm 4.34$	$\pm 0.34$	$\pm 5.69$
Control	I	2997.5	13.5	30.11	3.20	46.81
		$\pm 668.44$	$\pm 2.6$	$\pm 10.39$	$\pm 0.94$	$\pm 13.51$
	NI	2840	14.19	23.88	3.02	41.09
		$\pm 409.16$	$\pm 1.62$	$\pm 4.02$	$\pm 0.21$	$\pm 5.11$

Organic and inorganic fertilizer at the rate applied here was sufficient for good growth. Use of organic fertilizer was only possible at the planting stage. Subsequent application of two types of fertilizers was not needed comparing the harvesting time and growth. However, the two types of fertilizer produced plants with different compositions of plant parts. Higher root yield at site 2 (herbal garden at Girandurukotte) was also due to the better soil conditions (Tables 7 and 8).

Although studying the effect of fertilizer and the irrigation on increasing the dry matter yield was the intension of this experiment and the changes in the composition of plant parts could be an important consideration.

Greater yield of leaves was important in this crop and methods of improving shoot growth with more branches were the main concern. The data showed that when the application of organic fertilizer with irrigation dry matter yield of all parts and leaf area were greater mainly in the Site 1.

The overall leaves, shoot and root dry matter yield in all experimental plots was high compared to control. A higher per plant yield was obtained from the plants grown under organic fertilizer with irrigation (Tables 7 and 8). Yield from the site 2 was poor when compared to the harvest obtained from site 1 where the initial nitrogen level was high (Table 3).

Use of fertilizer was very important for the successful growth of these plants. Higher yields per plant could be obtained by using fertilizer. Response to fertilizer was clear

from the seedlings stage onwards. Both organic fertilizer and inorganic fertilizer were effective in improving the growth. Application of organic fertilizer before planting and inorganic fertilizer used here showed a continuous increase in growth in height during the 4-5 months of study and gave the highest dry matter yield. Using organic fertilizer must have had its physical advantages too. Better yields also can be expected by using higher levels of organic fertilizer without using inorganic fertilizer which is not recommended for medicinal plant cultivations. Although the plants in the irrigated blocks produced higher dry matter yields when compared to non-irrigated.

#### Harvesting time

Harvesting at the proper time in medicinal plants is very important. When plants started flowering after 3 1/2 months of growth they continually produced flowers and fruits. The percentage composition of plant parts also changes with the age of plants. It was noted that when plants are not harvested at correct time plants having flowers with more fruits and most of leaves started to yellow. The best harvesting time for *A.paniculata* is at the flowering stage. Harvesting at an earlier stage of 2 months yielded plants with a balanced composition of plant parts but with low dry matter yield. Older plants (5 months) mostly contained flowers and capsules compared to 4 months old plants with more leaves with flowers. It was very clear that when the plants were kept for a longer period they reached senescence with more capsules.

Therefore, it seems that harvesting at 4 months of growth is more acceptable when 4 cm seedlings were the

planting materials. At this stage the entire plant can be uprooted and air dried. The observations in these experiments showed that 3 1/2 – 4 months old plants were of the best quality (visually) with good proportions of leaves, shoots and flowers.

#### Pests and diseases

During the study period no pests or diseases were observed at any stage. Plants started to die with maturity of fruits at the age of 5-6 months in the field.

#### Conclusions

Experiments carried out to study the effect of irrigation and inorganic and organic fertilizer on the growth of plants raised from seeds revealed that organic fertilizer had significant effects on the plant growth. Inorganic fertilizer at the rate applied also gave good growth. The plants were harvested by up rooting at the end of 3 1/2 – 4 months of growth under organic fertilizer with an average yield of 9,500 kg and 6,000 kg /ha at Site 1 and 2, respectively.

#### References

1. Cramer LH, Dassanayake MD, Clayton WD (eds). A Revised Handbook to the Flora of Ceylon, Oxford and IBH Publishing Co. Pvt. Ltd., New-Delhi, 1998; **XII**: 97.
2. Jayaweera DMA. Medicinal Plants (Indigenous & Exotic) Used in Ceylon, The National Science Council of Sri Lanka, Colombo, Part I, 1981, p.07.
3. Kirtikar KR, Basu BD. Indian Medicinal Plants, International Book Distributors, Dehradun, India, 1987; **III**: 1738.
4. Sharma PC, Yelne MB, Dennis TS. Database on medicinal plants used in Ayurveda, Central Concil for Research in Ayurveda & Siddha, Department of ISM & H, Ministry of Health & Family Welfare, Rakmo Press Pvt. Ltd, New Delhi, 2002; **4**: 34.
5. Medicinal and Poisonous Plants. Ed. De Pauda LS, Bunyapraphatsara and Lemmens RHMD1, No. 12(I), Backhuys Publishers, Leiden, 1999.
6. Husain A, Virmani OP, Popeli SP, Misra LN, Gupta MM, Srivastava GN, Abraham Z, Singh AK. Dictionary of Medicinal Plants, Central Institute of Medicinal and Aromatic Plants, Lucknow, India, 1992.
7. Abeywardana N, Hettiaratchi LJK. Statistics on the National Demand for Medicinal Plants, Consultants report, Sri Lanka Conservation and Sustainable Use of Medicinal Plants Project, 4, Woodland Avenue, Kohuwala. The World Conservation Union, Sri Lanka, 2001.
8. De Silva G. Determination of germination period and optimum temperature for seed certification of medicinal plants. *Samarpana*, Department of Ayurveda, Nawinna 1997; **2**: 12-17.
9. Van Ranst E, Verloo M, Demeyer A, Pauwels TM. Manual for the Soil Chemistry and Fertility Laboratory, Analytical Methods for Soils and Plants Equipment and Management of Consumables, 1999, p.21-85.

# A clinical survey to evaluate the role of diet, lifestyle and stress as etiological factors in pathogenesis of type 2 diabetes mellitus

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## Abstract

Diabetes is a global epidemic with devastating human, social and economic consequences. For the first time, a non-infectious disease has been seen as posing as serious a global health threat as infectious epidemics such as HIV/AIDS. The prevalence of type 2 diabetes is rising at alarming rates worldwide because of increased urbanization, high prevalence of obesity, sedentary lifestyles and stress. Survey of 151 type 2 diabetes mellitus patients revealed that rice, potato, bajra, curd, milk, krishara, ghee are most potent followed by banana, paratha, and butter, bhajiya, puri, chiku, mango, masha etc to cause the disease. Most of the patients indulged in sedentary life style like abstinence of physical and mental work, excessive sleep, and day sleep. These diabetic subjects were feeling unhappy and depressed, constantly under strain, lost sleep over worry, feeling of not overcoming difficulties, loss of concentration, incapable of making decisions, inability to face problems, loss of confidence, cannot enjoy day-to-day life. On brief psychiatry rating scale, psychological factors affected at various levels include; tension, somatic concern, anxiety, depressed mood, suspiciousness, guilt feeling, emotional withdrawal. The data reflects that defective diet and lifestyle including stress and obesity play an important role in aetiopathogenesis of diabetes mellitus.

## Introduction

Diabetes mellitus (DM) is a chronic disease marked by elevated blood glucose levels. It affects 5-6% of the global adult population. Type 2 diabetes prevalence is rising at alarming rates worldwide because of increased urbanization, high prevalence of obesity, sedentary lifestyle and stress, among other factors. Up to 80% of type 2 diabetes is preventable by adopting a healthy diet and increasing physical activity. High status of life, less labour, stressful jobs, junk-frozen-fried foods, irregular meals and distorted life style together have made a fatal cocktail named diabetes mellitus. Every 10 seconds a person dies from diabetes-related causes. Every 10 seconds two people develop diabetes. Seven of the 10

countries with the highest number of people living with diabetes are in the developing world. India has the largest diabetes population in the world with an estimated 41 million people, amounting to 6% of the adult population. India is the kingdom of diabetes, having more than 5 crore diabetic patients. Among them 11.8% Indian diabetics are residing in Gujarat which is a matter of great concern.

The pathogenesis of type 2 diabetes has been attributed variably to defects in insulin action or to defects in insulin secretion and it is likely that both defects are usually involved [1]. When insulin secretion is sufficient to meet insulin requirements, whether large or small, normal glucose homeostasis will be maintained and glucose intolerance will be avoided. An area of active research in cell culture and transgenic mouse models suggests that abnormalities in the insulin receptor signaling pathway, in both pancreatic beta-cells and muscle cells, may be involved [2]. For the glucose intolerant, lifestyle changes can also prevent the onset of diabetes through weight loss [3]. Physical activity is recommended for diabetics because of its importance in weight loss management and due to its acute and chronic effects on glucose controls [4, 5]. It reduces the risk for developing Type 2 diabetes through the years [6]. Even non vigorous physical activity such as bowling, gardening, and house hold work has been shown to reduce insulin resistance [7]. Physical activity has been shown to reduce hyperinsulinemia and improve insulin peripheral activity in 65 year old subjects [8], which shows that even at this age, chronic diseases can be fought through a better lifestyle. As central obesity is a major contributor to insulin resistance, reduction of former is of utmost importance. Even without weight loss, physical activity reduces abdominal fat in men [9].

When combined with weight loss, physical activity reduces insulin resistance. A recent meta-analysis showed that exercise reduces HbA1c levels by an amount that is expected to reduce diabetic complications, without a mean effect on body weight [10].

Stress is a potential contributor to chronic hyperglycemia in diabetes as it has major effects on metabolic activity. Energy mobilization is a primary result

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of the fight or flight response. Stress stimulates release of various hormones which can result in elevated blood glucose level. In diabetes, as a result of relative or absolute lack of insulin, stress-induced increases in glucose cannot be metabolized properly. Furthermore, regulation of these stress hormones may be abnormal in diabetes. In contrast, more consistent evidence supports the role of stress in type 2 diabetes. Although human studies on the role of stress in the onset and course of type 2 diabetes are few, a large body of animal study supports the notion that stress reliably produces hyperglycemia in this form of disease. Furthermore, there is mounting evidence of autonomic contributions to the pathophysiology of this condition in both animals and humans [11].

Anger (krodha), grief (shoka), and anxiety (udvega) are described amongst the etiological factors of Pittaja and Vataja Prameha in the Ayurvedic classics [12,13]. The objective of this study was to evaluate the role of diet, life style and stress as etiological factors in pathogenesis of type 2 diabetes.

## Materials and Methods

Total 151 patients of type 2 diabetes, attending the O.P.D./I.P.D. of Institute for Post Graduate Teaching and Research in Ayurveda Hospital, Gujarat Ayurved University, Jamnagar, were selected irrespective of their sex, caste for survey study. The sample is from the Saurashtra region of Gujarat in India.

**Inclusion Criteria:** Patients of type 2 diabetes fulfilling the standard diagnostic criteria of World Health Organization (W.H.O.) for diabetes mellitus. Symptoms of diabetes mellitus plus random blood glucose > 200 mg/dl or fasting blood glucose > 126 mg/dl or two-hour blood glucose > 200 mg/dl during an oral glucose tolerance test.

The detailed examination of patients was done on the basis of specially prepared proforma incorporating classical symptoms; daily dietetics with their frequency and quantity approximately to assess the Hetu Skandha (causative factors).

**Table 1: Scoring pattern adopted for assessing the dietary habit of the patients [14]**

Frequency of consumption	Score
Never consumes the item	0
Very occasionally (Two or three monthly once)	1
Monthly once	2
Monthly twice or thrice (Seasonal consumption)	3
Weekly once	4
Weekly twice, thrice or more	5
Daily once	6
Daily more than once	7

Quantity of Consumption	Score
Regular quantity	add 0
Slightly over in quantity	add 1
Significantly over in quantity	add 2
In much more quantity	add 3

**Mean score of consumption (M.S.C.):** The total score of consumption is done by summing up both the scores of frequency and quantity.

## Correction of M.S.C. [14]

Quantity of Consumption	Score
The classical reference or indirect classical reference for hetutva in Prameha	+1
Satmya (congenial) but harmful if consumed in bulk for long duration	-1
Pathya and Aharya but not Vyadhivirodhi	-2
Hitatama and Vyadhivirodhi also	-3

*Likely etiological factor (L.E.F.) = corrected M.S.C.  
% of patients consuming the Nidana*

Highly Significant L.E.F. = >350

Significant L. E. F. = between - 250 - 350

Mild L.E.F. = between - 150 - 250

## Observation and Results

Data of 151 type 2 diabetic patients surveyed revealed that maximum number of patients (40.4%) belonged to the age group of 50-60 yrs. Majority of patients (58.94%) belonged to middle class followed by upper middle class (19.87%). Positive family history of diabetes was reported in 48.34% patients. Family history of obesity was positive in 51% of patients. The 41.06% patients were addicted to tea, tobacco (30.12%) smoking (27.71%), beetle (7.23%), beetle nut (4.82%), Alcohol (2.41%). The 31.79% patients were secondary educated followed by primary (18.54%) whereas 19.2% patients were uneducated. Maximum numbers of patients (43.71%) were housewives followed by service men (24.5%) and businessmen (17.88%).

**Symptoms reported includes:** Polyuria (62.25%), polyphagia (9.27%), polydypsia (48.34%), burning sensation in palm and sole (50.33%), numbness in hand

and leg (59.60%), fatigue (51.65%), weakness (37.69%), Leg cramps (50.33%), excessive sweating (43.05%), dryness in mouth (28.48%).

The data on dietary habits revealed that majority of the patients (83.44%) were vegetarian. The 78% patients were afflicted towards Madhura rasa followed by Katu rasa (49.67%), Amla rasa (47.68%) and Lavana rasa (27.15%). As far as Agni is concerned maximum number of patients (36.42%) had Vishamagni followed by Mandagni (23.84%) and Tikshnagni (11.28%). Maximum patients (47.02%) had habit of Adhyashana followed by Ajirnashana (41.72%) and Vishamagni (25.16%).

**Highly significant L.E.F. group:** Rice (630), Potato (503), Bajra (491), Curd (471), Milk (432), Krishara (425), Ghee (403).

**Significant L.E.F. group:** Green leafy vegetables (281), Banana (275), Parotha (268).

**Mild L.E.F. Group:** Buttermilk (239), Bhajiya (224), Puri (207), Chiku (203), Butter (195), Bread (192), Mango (191), Pavbhaji (162), Masha (161).

The data on life style revealed that most of the patients indulged in sedentary life style like not doing any exercise (83.44%), Tyakta Chinta (33.11%), habit of day sleep (84.1%). Among them 37.09% patients were sleeping for 1 hr., 28.48% patients for 1-2 hrs. in day time. The 29% patients had disturbed sleep whereas 17.22% had excessive sleep.

To determine the influence of stress on an individual as per General Health Questionnaire (GHQ) – 12; significant level of stress (score  $\geq 3$ ) was observed in 24.50% of patients. Among them maximum number of patients (40%) were feeling unhappy and depressed, constantly under strain (26%), lost sleep over worry (19%), feeling of not overcoming difficulties (19%), loss of concentration (19%), incapable of making decisions (16%), inability to face problems (16%), loss of confidence (12%), cannot play useful part in the things (11%), cannot enjoy day-to-day life (10%) more than usual or much more than usual which can be taken as acute stress. While evaluating GHQ it was observed that patients had loss of confidence (73%), feeling of not overcoming difficulties (70%), feeling of worthlessness (70%), constantly under strain (66%), feeling unhappy and depressed (49%) same as usual which shows this trait in their nature. Though intensity is less they are not at all healthy feelings rather chronic in nature so can be taken as chronic stress (n=151).

The 20% patients had overweight and 70% patients were obese. Among them 58% patients had grade II obesity followed by grade I (11%) and grade III (1%) obesity (n=100). Serum cholesterol, serum triglyceride, serum LDL and serum VLDL levels were found abnormally high in 39.22%, 62.75%, 73.53% and 36.27% of patients, respectively while S.HDL level was low in 30.39% of patients (Tables 2 and 3).

**Table 2: Blood and urine sugar level**

FBS (n=121)			PPBS (n=127)			Urine Sugar (n=102)		
Range (mg %)	No. of pts.	%	Range (mg %)	No. of pts.	%	Range	No. of pts.	%
>200	44	36.36	>275	46	36.22	++++	8	7.84
125-199	57	47.11	200-275	38	29.92	+++	19	18.63
105-129	14	11.57	140-199	36	28.35	++	14	13.73
<105	6	4.96	<140	7	5.51	+	11	10.78
						Trace	15	14.71
						Nil	35	34.31

**Table 3: Lipid profile of 102 patients**

S.Cholesterol		S. Triglyceride		S. LDL		S. VLDL		S. HDL	
Range (mg %)	%	Range (mg %)	%	Range (mg %)	%	Range (mg %)	%	Range (mg %)	%
>300	1.96	>500	3.92	160-189	58.82	>140	1.96	<40	30.39
240-299	8.82	200-499	31.37	130-159	11.76	90-140	1.96	40-60	65.69
200-239	28.43	150-199	27.45	100-129	2.94	40-90	32.35	>60	3.92
<200	60.78	<150	37.25	<100	26.47	<40	63.73		

## Discussion

Majority of patients (87.42%) were above 40 yrs with chronicity of >1 yr. (60.26%) which shows maturity onset nature of disease. Maximum patients were male. "The female is dominant sex at Global Platform but for India it is M>F" (API, 1999). Majority of patients belonged to middle class and upper middle class. Once known luxuries now with advancing technologies they have converted into necessities due to which physical activity has lessen at significant level and struggling for gaining these facilities middle class has invited stress in their lives which has a great impact on initiation, progression and exacerbation of the disease. Positive family history for DM (48.34%) showed genetic background of the disease. Majority of patients were addicted to tea, smoking, tobacco chewing which decreases natural immunity and makes person susceptible for any disease. Smoking has been shown to increase the incidence of diabetes in several cohort studies. The magnitude of this effect is moderate; smoking is estimated to increase the incidence of diabetes by 1.5-3 fold [15, 16]. The mechanism of this effect is not known but may be that smoking contributes to upper body obesity, which is associated with the metabolic syndrome of central obesity, insulin resistance, glucose intolerance or overt diabetes, hypertension and dyslipidemia. Moreover smoking increases oxidative stress which antagonize the insulin action and leads to diabetes mellitus. The 2.41% patients were addicted to alcohol. There is some evidence of a 'U' or 'J' shaped curve relating alcohol consumption and the risk of developing diabetes [17,18,19]. This may reflect increased insulin sensitivity [20]. That is, person consuming moderate amounts of alcohol may be slightly less likely than non-drinkers to develop type 2 diabetes, whereas heavy alcohol users are more likely than non-users or moderate drinkers to develop diabetes.

Alcohol may improve insulin sensitivity when consumed in small amounts. At higher levels of intake, alcohol may interfere with insulin-mediated glucose disposal, causing insulin resistance [21]. Majority of the patients were secondary educated followed by primary and uneducated which denotes that they may be less cautious about the causes and complication of the disease therefore not managing their dietary regimen properly. Most of the housewives indulge sedentary life style and have habit of Adhyashana. They are also less cautious about following or managing proper diet and lifestyle regimen due to less education. Servicemen either due to sedentary lifestyle or/and increased level of stress at work place are more prone to develop this disease. Businessmen are also affected more due to sedentary life style and increased level of stress. Most of the patients were indulging sedentary life style like abstinence of physical and mental work, excessive sleep which are direct causative factors of the disease. Disturbed sleep illustrates the disequilibrium of mental faculty due to stress and anxiety or fear. These factors may cause insulin resistance and decreases insulin sensitivity. The present study also

supports the fact that ~90% of people with type 2 diabetes are overweight or obese [22].

Hyperinsulinemia and insulin resistance are pervasive features of obesity, increasing with weight gain and diminishing with weight loss. Insulin resistance is more strongly linked to intra abdominal fat than to fat in other depots [23].

On the basis of uncontrolled FBS (95.04%) and PPBS (94.49%) it can be said that the disease cannot be controlled only with oral antihyperglycemic agents until their dietary habits are corrected and disturbed psychological factors are brought to physiological limits. Positive urine sugar (65.69%) in diabetic patients can be correlated with the Ayurvedic term '*Mutre Abhidhavanti Pipilika*'. In patients of type 2 diabetes abnormal lipid profile like: high level of S. cholesterol (39.22%), S. Triglyceride (62.75%), S. low density lipid (73.53%), S. very low density lipid (36.27%) and low level of S. high density lipid (30.39%) showed the *Bahu* (excessive) and *Abaddha* meda is the main *dushya* in Prameha as well abnormally high level of lipid in blood stream because of stress induced lipolysis.

## Conclusions

This study establishes that excessive consumption of potato, rice, curd and other milk products like ghee, oily foods like puri - paratha, lack of exercise, day sleep and chronic stress leading to anxiety and depression play a significant role in causation of the disease. Physical inactivity is the result of a progressive shift of lifestyle towards more sedentary patterns leading to obesity coupled with genetic back ground is responsible for type 2 diabetes with disturbance in blood glucose and lipid profile. Hence avoidance of such dietary factors, sedentary lifestyle and stress can contribute significantly for prevention of disease as well as promotion of health of diabetic subjects.

## References

- Lebovitz HE. Type 2 diabetes – an overview. *Clinical Chem* 1999; **48**: 1339-45.
- Sreenan SK, Zhou YP, Otani K, et al. Calpains play a role in insulin secretion and action. *Diabetes* 2001; **50**: 2013-20.
- Tuomilehto J, Lindstrom J, Eriksson JG, et al. Prevention of type 2 diabetes mellitus by changes in life style among subjects with impaired glucose tolerance. *N Engl J Med* 2001; **344**: 1343-50.
- American College of Sports Medicine Position Stand. Exercise and type 2 diabetes. *Med Sci Sports Exer* 2001; **32**: 1345-60.
- American Diabetes Association. Diabetes mellitus and exercise (position statement). *Diabetes Care* 2000; **24**: S51-S55. See also [www.diabetes.org](http://www.diabetes.org), Clinical Practice Recommendation, accessed in Sept 2001.
- Manson JE, Rimm EB, Stampfer MJ, et al. Physical activity and incidence of non-insulin dependent diabetes mellitus in women. *Lancet* 1991; **338**: 774-8.

7. Mayer-Davis EJ, D'Agostino R, Karter AJ, et al. Intensity and amount of physical activity in relation to insulin sensitivity. *JAMA* 1998; **279**: 669-74.
8. Kirwan JP, Kohrt WM, Wojta DM, et al. Endurance exercise training reduces glucose stimulated insulin levels in 60 to 70 year old men and women. *J Gerontol* 1993; **48**: M84-90.
9. Ross R, Dagnone D, Jones PJH, et al. Reduction in obesity and related co morbid conditions after diet induced weight loss or exercise induced weight loss in men. *Ann Intern Med* 2000; **133**: 92-103.
10. Boule NG, Haddad E, Kenny GP, et al. Effects of exercise on glycemic control and body mass in type 2 diabetes. *JAMA* 2001; **286**: 1218-27.
11. Department of Psychiatry, Duke University Medical Center, Durham, North Carolina 27710. doi: 10.2337/diacare.15.10.1413. *Diabetes Care* 1992; **15**: 1413-22.
12. Agnivesha, Charaka samhita, Nidana sthana 4/24, edited by Vaidya Yadavji Trikamji Acharya, Chaukhambha Surbharati Prakashan, Varanasi, India, 2008, p. 214.
13. Agnivesha, Charaka samhita, Nidana sthana 4/36, edited by Vaidya Yadavji Trikamji Acharya, Chaukhambha Surbharati Prakashan, Varanasi, India, 2008, p. 215.
14. Shrirang Galgali, R R Dwivedi. A clinical study of Caraka Nidana sthana with special reference to Prameha. M.D. (Ayu) thesis; I.P.G.T. & R.A., Jamnagar, India, 2000.
15. Kerr D, Sherwin RS, Pavalkis F, et al. Effect of caffeine in the recognition of and responses to hypoglycemia in humans. *Ann Intern Med* 1993; **119**: 799-804.
16. Rimm EB, Chan J, Stampfer MJ, et al. Prospective study of cigarette smoking, alcohol use, and the risk of diabetes in men. *BMJ* 1995; **310**: 555-59.
17. Bergrem H, Kalo I, Staehr Johansen K. The St Vincent Declaration activities. *The SVD Newsletter* 10th Anniversary 1999; **14**: 8.
18. Krans HMJ, Porta M, Keen H, Staehr Johansen K. Diabetes care research in Europe: St Vincent Declaration action programme. Copenhagen: WHO Office, EUR/ICT/CLR 055/3; 1995.
19. Krans HMJ, Porta M, Keen H, Staehr Johansen K. Diabetes care research in Europe: St Vincent Declaration action programme. Implementation document. *Giornale Italiano di Diabetologia* 1995; **15**: 1.
20. Rosenquist U. Implementation of the St Vincent Declaration in Stockholm country, Sweden. *Giornale Italiano di Diabetologia* 1993; **13**: 75-6.
21. Backlund LB, Algvere PV, Rosenquist U. New blindness in diabetes reduced by more than one third in Stockholm Country. *Diabet Med* 1997; **14**: 732-40.
22. Dr. Ajay Gopalani G, Dr. Bhushana. Obesity, Sarmandal, Kottakkal Ayurveda Series - 67, Vaidyaratnam P.S.Varier's Arya Vaidya Sala, Kottakkal, First edition, India, 2007, p. 84.
23. Ascaso JF, et al. *Eur J Intern Med* 2003; **14**: 101-6.

## **Anal manometry study in guggulu based kshara sutra in the management of fistula in ano**

**A A J Pushpakumara<sup>1</sup>, D J Anthony<sup>2</sup>**

### **Abstract**

Anal manometry can be regarded as single most important investigation to study ano-rectal sphincter mechanism. In the Ayurvedic text, fistula in ano has been described as "Bhagandara". Sushruta (800 B.C.) has adopted surgical, para surgical and conservative measures for the fistula in ano. Under the para surgical management 'Ksharsutra' also known as a medicated thread had been introduced. There is growing evidence that even division of the internal anal sphincter, leaving the puborectalis and external sphincter undisturbed, may cause impaired continence after successful elimination of the fistula. These conflicting objectives pose a challenge for the colorectal surgeon. In one hand recurrent sepsis and fistulation must be avoided, and on the other hand continence must be preserved. The present study was conducted at the Ano-rectal Clinic at Gampaha Wickramarachchi Ayurveda Teaching Hospital. 100 patients participated in the present study – 50 patients of fistula in ano treated by Guggulu based kshara sutra and 50 patients under the control group for the anal pressure recording by using anal manometer. Pre-treatment, post-treatment and follow up pressure were recorded in the study group. In this study in the patients treated in kshara sutra there were no significant reduction of the resting anal pressure ( $P>0.05$ ) observed either post treatment or follow up, but the squeeze anal pressure was found to be significantly reduced ( $P<0.01$ ) after treatment and it insignificantly reduced ( $P>0.05$ ) in the follow up for high anal fistula.

### **Introduction**

The anorectal disorders have been known to man from its inception of evolution of Medical science. Anorectal disorders are usually not life threatening. They cause a lot of symptoms and social embarrassment through clinical features such as fecal incontinence. The diseases of fistula in ano is a condition therefore which requires in depth study so as to ensure better status of life. In the Ayurvedic text, fistula in ano has been described

as "Bhagandara". *Sushruta* (800 B.C.) elaborately described various ano-rectal disorders for the first time in the history among which fistula in ano is a prominent one [1].

*Susrutha* has adopted surgical, para surgical and conservative measures for the fistula in ano. Under the para surgical management 'kshara sutra' also known as a medicated thread that had been introduced.

Surgical treatment of fistula in ano is associated with significant risk recurrence and high risk of impaired continence. There is growing evidence that even division of the internal anal sphincter, leaving the puborectalis and external sphincter undisturbed, may cause impaired continence after successful elimination of the fistula [2]. Nevertheless, unless all the secondary tracks are also attended to, there is a risk of recurrent sepsis and fistulation. These conflicting objectives pose a challenge for the colorectal surgeon. In the one hand recurrent sepsis and fistulation must be avoided on the other hand continence must be preserved.

Anal manometry [3] can be regarded as single most important investigation to study ano-rectal sphincter mechanism. The balloon system was a convenient method to use, pressure was reproducible and easy to estimate and motility details were well shown [4] was adopted in this study.

The goal of kshara sutra treatment of fistula in ano by virtue of the properties of its content which has necrolytic action on tissues. During application of kshara sutra there is a continuous drainages of fistulous track and ingredients used in the thread help in healing. There is no doubt about the efficacy of kshara sutra treatment in fistula in ano which has been almost confirmed by the modern surgeons [5].

At present no studies have been pursued for regarding the assessment of anal continence and pressure profile of anal canal after the kshara sutra therapy. Therefore factors associated with incontinence have not been assessed. Thus this study focuses on the assessment of the reliability of kshara sutra as a mode of treatment for the fistula in ano.

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The main advantage of Guggulu based kshara sutra was its uniform thickness (non beaded shape), enhanced binding of drugs to the thread less hygroscopic nature and causation of significantly less pain and burning sensation to the patient.

The patients were treated by kshara sutra consisting high anal cases with recurrence having high internal opening or an abscess cavity above the levator ani muscle and supra sphincteric with supra levator abscess. High transsphincteric [6], multiple track and high horse fistula. This type of fistulae invariably ends up with complication after surgery in the form of injury to the anal sphincter leading to incontinence. In this study it has been observed that there are no significant pressure reduction either resting anal pressure or squeeze anal pressure in both post-treatment and follow up.

## Materials and Methods

The present study was conducted at the Ano-rectal Clinic at Gampaha Wickramarachchi Ayurveda Teaching Hospital. Thus clinical study was planned on the patients of bhagandara attending the Ano-rectal Clinic. The study was conducted in 100 cases, out of this 50 patients of fistula in ano and 50 patients under the control group (without evidence of anorectal disorders).

The patients were divided into 02 groups of 50 each.

Group - I 50 cases of controls as healthy volunteers.

Group - II 50 cases of fistula in ano treated by kshara sutra treatment.

### A. Selection of controls:

The patients coming to hospital, surgical out patient department without any evidence of ano-rectal disorder were taken as controls. A thorough questionnaire was made to rule out symptoms of ano-rectal disorder. Any neurological disease was also ruled out.

All patients underwent detailed examination of the abdomen and perianal area including per rectal examination, proctoscopy and anal manometry. These patients were further divided into groups according to sex and age.

### B. Selection of cases:

Patients coming to hospital surgical out-patient department from March, 2009 to March, 2010 with symptoms of fistula in ano were taken as cases in the study groups. All patients were given the questionnaire to evaluate symptoms and their degree. The cases selected for study groups only had chronic or cryptoglandular origin. All had history of previous perianal suppuration drained either surgically or spontaneously. Patient with superficial fistula associated with fissure, inflammatory bowel disease hematologic malignancy preoperative incontinence and those who underwent primary fistulostomy at time of draining the access were not included.

## Anal incontinence was assessed by using anal manometry and questionnaire from the Cleveland clinic, Oliveira 1996

Incontinence type	Never	Rarely (a) (< 1 month)	Sometimes (b) (> 1 month < 1 week)	Usually (c) (> 1 week < 1 day)	Always (d) (< 1 day)
Solid	0	1	2	3	4
Liquid	0	1	2	3	4
Flatus	0	1	2	3	4
Use of pad	0	1	2	3	4
Lifestyle alteration	0	1	2	3	4

- (a) Less than once a month.
- (b) More than once a month, less than everyday.
- (c) More than once a week, less than everyday.
- (d) Everyday.
  - Score '0' – Perfect continence
  - Score '20' – Complete incontinence

## **Anal manometry reading**

### **Distance from analverge Resting Squeeze Pressure**

- 1 cm
- 2 cm
- 3 cm
- 4 cm
- 5 cm

## **Internal sphincteric pressure**

### **External sphincteric pressure**

All patients were examined in lithotomy position. Fistula was classified according to the criteria of parks et al. Anal manometry in both controls and in patients with fistula in ano was performed by the following technique:

### **Instrument design and anal pressure recording (anal manometry)**

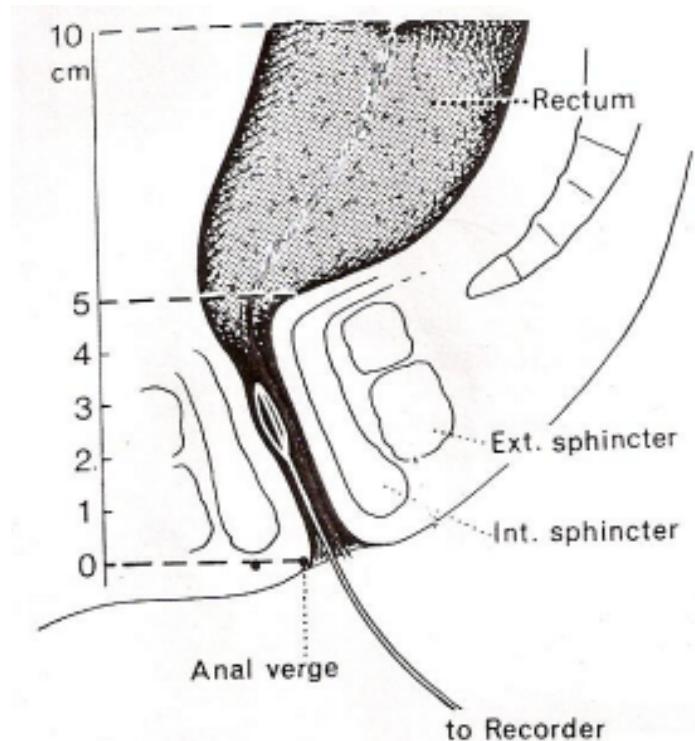
Pressures in the anal canal were measured with a small water filled latex balloon prepared from condom, of approximately one centimeter in diameter. The device was somewhat similar to that, used by Lusnniss et al [7]. This balloon was connected to a "U" tube mercury manometer

via a low compliance circuit. The circuit was filled with water and made air free to avoid variations in pressure recordings due to compressibility of air. The probe thus constructed was marked in centimeters to know the proper position of balloon in the anal canal.

All manometric measurements were done without any prior preparation. Patient was kept in left lateral position and after anal inspection to see any external pathology probe with balloon at the tips was inserted up to 1 cm from anal verge with small amount of non anaesthetic lubricating jelly.

After stabilization, i.e. after about 30 seconds, pressure was recorded at 2 cm, 3 cms, and 4 cms, above anal verge. The maximum pressure recorded at any of these positions was taken as maximum resting anal pressure. Then with balloon positioned in the anal canal patient was asked to squeeze the anal canal as if he or she wants to postpone the defecation. The pressure, thus recorded was taken as maximum squeeze pressure. These steps were repeated at least twice and maximum recorded pressure obtained in these steps was taken as maximum squeeze pressure.

Patients were followed up and the symptom analysis as well as manometric evaluation was done after 60 days of kshara sutra treatment.



**Diagram of the pressure recording units in situ with the help of a small balloon**

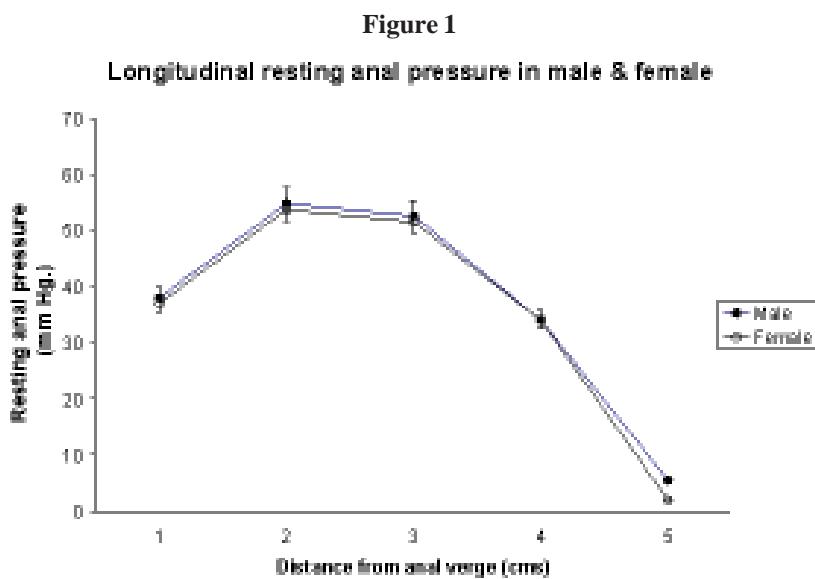
## Observation and Results

**Table 1: Mean  $\pm$  SD value of anal manometric pressure profile (mm Hg) of controls (group-I)**

	Overall (n=50)	Male (n=32)	Female (n=18)
Mean maximum resting pressure	54.40 $\pm 2.87$	54.78 $\pm 2.80$	53.72 $\pm 2.95$
Mean maximum squeeze pressure	116.82 $\pm 5.23$	117.81 $\pm 5.42$	115.06 $\pm 4.49$

The longitudinal resting anal pressure profile of controls by station pullout technique is shown in Figure 1.

Resting anal pressures have been found almost equal at all the stations in both males and females, except at 5 cm from anal verge, where resting anal pressure in females was significantly lower than that in males at same distance.



**Table 2: Comparison of pre-manometric anal pressure profile in control (group I) and study groups (group II)**

Group	Maximum resting pressure (mmHg) mean $\pm$ SD	Maximum squeeze pressure (mmHg) mean $\pm$ SD
Group I control (n=50)	54.40 $\pm 2.87$	116.82 $\pm 5.23$
Group II (n=50)	56.72 $\pm 4.20$	117.80 $\pm 8.78$

Maximum resting anal pressure pre-treatment value have been marginally increased in group II as compared to the control group. This may be due to the inflammatory process of the disease but maximum squeeze anal pressure has not been followed.

In 30 patients of the low anal fistula in group IV whose pre value for maximum resting anal pressure was  $(55.83 \pm 7.54)$  slightly found to be reduced to  $(54.70 \pm 5.02)$  in post treatment. This difference was not statistically significant. Maximum squeeze anal pressure was also found to be reduced from pre-value  $(111.70 \pm 5.17)$  to post value  $(110.20 \pm 6.05)$ . This difference was statistically not significant (Table 3).

**Table 3: Maximum resting anal pressure and squeeze pressure in response to kshara sutra treatment (n = 30). (within the group comparison low anal fistula – group II)**

<i>Maximum resting pressure (mmHg) mean ± SD</i>			<i>Maximum squeeze pressure (mmHg) mean ± SD</i>		
<i>Pre-treatment</i>	<i>Post-treatment</i>	<i>t=test (pre - post)</i>	<i>Pre-treatment</i>	<i>Post-treatment</i>	<i>t=test (pre - post)</i>
55.83	54.70	t=0.89	111.70	110.20	t=1.24
±7.54	±5.02	p>0.05	±5.17	±6.05	p>0.05
		N.S.			N.S.

**Table 4: Maximum resting anal pressure and squeeze pressure in response to kshara sutra treatment (n=20). (within the group comparison high anal fistula – group II)**

<i>Maximum resting anal pressure (mmHg) mean ± SD</i>					<i>Maximum squeeze pressure (mmHg) mean ± SD</i>				
<i>Pre Treatment</i>	<i>Post Treatment</i>	<i>Follow up</i>	<i>t = test (pre-post)</i>	<i>t = test (pre-follow)</i>	<i>Pre Treatment</i>	<i>Post Treatment</i>	<i>Follow up</i>	<i>t = test (pre-post)</i>	<i>t = test (pre-follow)</i>
54.89	52.65	53.10	t=1.21	t=0.2	110.75	107.50	109.70	t=3.53	t=0.99
±5.02	±6.21	±9.51	p>0.05	p>0.05	±7.47	±7.42	±8.14	p<0.01	p>0.05
			N.S.	N.S.				H.S.	N.S.

20 cases of high anal fistula in group II the maximum resting anal pressure was slightly found to be reduced from pre-value ( $54.89 \pm 5.02$ ) to post-value ( $52.65 \pm 6.21$ ). The pressure value for follow up was  $53.10 \pm 9.51$ . Pressure reduction in both the post-treatment and follow up are statistically not significant (Table 4).

Consequently the maximum squeeze anal pressure was also found to be reduced from pre-value ( $110.75 \pm 7.47$ ) to post-value ( $107.50 \pm 7.42$ ).

**Table 5: Comparison of the unit cutting time in the group II**

<i>Group</i>	<i>Unit cutting time (U.C.T.) (Days)</i>
Group II	5.10

Unit cutting time (U.C.T.) is defined as the number of days required for the excision of unit (1 cm) of the fistulous track.

$$\text{U.C.T.} = \frac{\text{Total no. of days}}{\text{Initial length in cm}}$$

**Table 6: Incontinence and recurrences in kshara sutra group**

<i>Group</i>	<i>Procedure</i>	<i>Patients number</i>	<i>Recurrence number</i>	<i>%</i>	<i>Incontinence</i>	<i>%</i>
Kshara sutra group (group II)	Kshara sutra	50	01	1	-	-

1% recurrence was observed in this study but no patient was found to be incontinence in kshara sutra series.

## Discussion

Several authors have tried to define manometric values for the normal anal canal. Significant pressure variations have been described in normal subjects chiefly with regard to gender and age.

In this study we didn't get significant difference between the anal pressures for male and female (Table 1).

In this study the resting anal pressure in males at 5cm from anal verge was significantly higher than in females (Figure 1). This indicate shorter anal canal length in females. Though in this study the exact length of anal canal could not be measured which is more precisely measured by perfusion pressure recording system.

There is no significant pressure reduction observed in low anal fistula. So the low anal fistula could be successfully managed without risk because the low anal fistulae the track that does not extend above the level of the anal crypts and indeed usually is formal to be open at this level to the anal canal.

In group II the patient treated in kshara sutra, no significant reduction of the resting anal pressure has been observed either post-treatment or follow up but the squeeze anal pressure was found to be significantly reduced after treatment and it insignificantly reduced in the follow up for high anal fistula.

The high rectal fistulas are the most difficult varieties. It denotes the type of fistula having exceptionally long tracks and includes high anal and anorectal varieties. The high anal fistula lies above the middle belly of external sphincter and the ano rectal varieties lies above the levatorani muscle [8].

In these cases a vast area of multilication is required during operative procedures resulting in significant fall in resting and squeeze anal pressures and dreadful complications.

The high anal fistulae the track rises to a higher level and is in relation to the upper parts of the anal sphincters, it may extend close to the anorectal ring.

High transsphincteric fistula track passes through both the internal and external sphincters before exiting to the skin and also pass dangerously close to the anorectal ring before it enters the ischiorectal fossa and the perineum. According to the study of Belliveau et al falling in resting pressure to less then 50% of the pre-operative value in patient having trans sphincteric fistulectomy [9].

The patients consist of group II treated by kshara sutra consist high cases with recurrence having high internal opening or an abscess cavity above the levator ani muscle and supra sphincteric with supra levator abscess. High transsphincteric, multiple track and high horse fistulas. This type of fistulae invariably ends up with complication after surgery in the form of injury to the anal sphincter leading to incontinence. In this study it

was observed that there are no any significant pressure reduction in resting anal pressure in both post-treatment and follow up. In the post-treatment a significant reduction of squeeze anal pressure in both groups was observed. It is transient and may be perhaps related to the stage of cutting through the anal sphincters by the thread, which subsequently become complete during the follow up.

Faecal incontinence is mainly caused as a result of direct injury to anal sphincter and also due to the spinal cord injury. There were no incontinence cases observed in the patients treated in kshara sutra of group II.

## Conclusions

There is no age or sex related difference in maximum resting anal pressure and squeeze anal pressure. Patients in fistula in ano had marginally higher maximum resting anal pressure compared to the control group, this may be due to the inflammatory process of the disease.

Kshara sutra appear to be the best option for the management of high anal fistula where there were no post-treatment incontinence and least recurrence rate. Further there were no significant reduction of resting or squeeze pressure at the time of follow up.

## References

1. Sushruta: Sushruta Samhita, 5th Edition, Chikitsa sthana and nidana sthana of Bhagandara, Motilal Banarasidas, New Delhi, 1994; 18th Ch. p. 421-5.
2. Hancock BD, Smith. The internal anal sphincter and Lord's procedure for haemorrhoids. *British Journal of Surgery* 1975; **62**: 833-6.
3. Belliveau P, Thomson JPS, Parks AG. Fistula in ano – a manometric study. *Disease Colon Rectum* 1983; **26**: 152-4.
4. Cali RL, Blatchford GJ, Perry RE, et al. Normal variation in anorectal manometry. *Diseases Colon Rectum* 1992; **35**: 1161-4.
5. Deshpande PJ, Sharma KR. Successful nonoperative treatment of high rectal fistula. *American Journal of Proctology* 1976; **39**: 132-4.
6. Christensen A, Nilas L, Christiansen J. Treatment of trans-sphincteric anal fistulas by the seton technique. *Disease Colon Rectum* 1986; **29**: 454-5.
7. Lusnniss PJ, Armstrong P, Barker PG, et al. Magnetic resonance imaging (MRI) of anal fistulae. *Lancet* 1992; **340**: 394-6.
8. Milligan ETC, Morgan CN. Surgical anatomy of the anal canal with special reference to anorectal fistula. *Lancet* 1934; **2**: 1150.
9. Tudor RG, Keighley MRB. Impaired sphincter function after fistulectomy for low fistula in ano. *British Journal of Surgery* 1986; **73**: 1042.

## Study of microbial quality on different drug formulations widely used in Ayurvedic system of medicine

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### Abstract

World Health Assembly (WHA) has given the following limitations for herbal preparations. Aerobic bacteria  $1 \times 10^5$ /g. for internal use and  $1 \times 10^7$ /g. for topical dosage forms. Fungi  $1 \times 10^4$ /g., *E-coli*  $1 \times 10^2$ /g, and *Salmonellae* – none for internal and topical dosage forms. This study was carried out to study the microbial load, micro-organisms, anti microbial effects of compound preparations. Powders (Choorna), Pastes (Kalka), Pills (Vatika), Fermented decoctions (Arista and Asawa), Oils (Thaila) and Local applications (Lepa) preparations were tested. Microbial load of some of the Choorna, Lepa and Kalka were exceeded the WHA limitations. Some of the Kalka, Arista and Asawa, Vatika, Thaila and Lepa preparations were within the acceptable limits. Isolated pure cultures were identified using morphological and biochemical tests and slide culture techniques. Most of the bacterial cultures belong to the genus *Bacillus*. The isolated fungi were identified as *Penicillium* Sp, *Aspergillus* Sp, *Mucor* Sp, *Rhizopus* Sp, *Fusarium* Sp. Some of the Thalisadi Choorna and Chandra Kalka preparations were positive for *Salmonellae* and *E-Coli*. It was noted that Hinguastaka Choorna and Manibadra Choorna had anti microbial effect on *Salmonellae typhi*. Thripaladi Choorna had effect on *Pseudomonas aeruginosa*. Sarvavisadee Oil was active against *Pseudomonas aeruginosa*, *Staphylococcus aureas*, *Salmonellae typhi*, and *klebsiella*. Buddharaja Kalka was active against *Pseudomonas aeruginosa*, *Staphylococcus aureas* and *Bacillus cereus*.

Hazard Analysis and Critical Control Point Analysis was done on the Thalisadi Choorna and found implementation of Good Manufacturing Practice and HACCP principals could contribute to microbiologically safe end products. The steam treatment method was found to be an effective method to reduce the microbial load. Studies on the antimicrobial activity justify the use of these compound preparations in common infective conditions.

### Introduction

Herbal medicine has been in practice for several thousand years. In developed countries, the popularity of herbal medicine is continuing to grow. Further medicinal plants are important sources for the biopharmaceutical industry. Hence, medicinal plants and herbal medicines account for a significant percentage of the biopharmaceutical market. All these materials contain a natural inherent microbial flora and may be contaminated during harvesting, processing, preparation and storage. Considering these facts the World Health Assembly (WHA) in its resolutions WHA 31:33, WHA 40:33, WHA 42:43 has emphasized the need to develop and ensure the microbial quality standards of medicinal plant products using modern techniques and applications of suitable standards [1].

### Aims and objectives

This study was carried out to assess the microbial load on the final preparations, identify the specific micro-organisms, study the ways and means to control and prevent contamination, establish good manufacturing practice, define an acceptable microbiological quality standards in the final preparation as envisaged by WHA standards and study the anti-microbial activity of the compound preparations.

### Materials and Methods

#### Study on microbial load

The compound preparations of various formulations (Choorna, Kalka, Vatika, Arista, Thaila, and Lepa) of different manufacturers were subjected to the analysis of microbial load of bacteria and fungi. Samples were collected from open market from fifteen different drug manufacturers. Tested Choorna (Powders) are Dathri Choorna, Thripaladi Choorna, Thalisadi Choorna, Hinguastaka Choorna and Manibadra Choorna. Tested Kalka (Pastes) are Buddharaja Kalka, Nawaratna Kalka, Chandra Kalka, Sarkaradi Kalka and Desadun Kalka. Tested Vatika (Pills) are Seetharama Vati, Suranvidura Vati and Jeevananda Vati. Tested Arista (Fermented decoctions) are Dasamoola

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Arista, Saraswatharista, Draksarista Arista, Pipalyadi Asawa and Arawindasawa. Tested Thaila (Oil) are Pinda Thaila, Narayana Thaila, Sarvavisadee Thaila, Visarpahara Thaila and Wathavi-duranga Thaila. Tested Lepa (Topical application) are Dasanga lepa, Sothahara lepa and Rogan e khas [2].

Three different samples in each drug of manufactures were taken for the study. Studies on microbial load was done using a dilution series up to  $10^0$  to  $10^{-3}$  in sterile distilled water. Oil preparations were directly applied on agar plates to study the microbial load. Nutrient agar and potato dextrose agar were used as common culture media for bacteria and fungi respectively. Pour plate technique was used on nutrient agar and spread plate technique was used on potato dextrose agar. Colony forming units were taken after 24 hours and 72 hours for bacteria and fungi respectively. It was assumed that each colony was formed by a single organism.

#### **Morphological and biochemical tests**

Isolated microbial colonies from above compound preparations were purified by re-streaking in the same agar medium and stock cultures were prepared and stored at 4°C until biochemical tests were done for identification. Periodically cultures were renewed to maintain the viability of the microorganism. Morphological study and identification of bacteria were done through biochemical tests and fungi were identified through slide culture techniques [3]. Stock cultures were sub cultured on Nutrient agar plates by using streak plate technique. Culture plates were used for morphological and biochemical tests within 24 hours.

The following bio chemical tests were done on these cultures: Grams stain, Spore stain, Motility test, Catalase activity, Oxidase activity, Acid production from Glucose, Oxidative and fermentative activity tests for Carbohydrate utilization, Methyl red test, Voges proskauer reaction, Indole test, Starch hydrolysis, Growth in 7% Sodium chloride, Growth in 65°C, Growth in 45°C, Gelatin hydrolysis and Casein hydrolysis.

#### **Tests for *Salmonellae***

Buffer peptone was used as non-selective enrichment media. One gram of the solid preparation or one ml of the liquid preparations was added to 20 ml of the buffer peptone and kept on gentle shaking position for 24 hours (at the ratio of 25 grams in 475 ml).

Selanite broth and tetra thionate broth were used as selective media (these media were prepared according to the specifications of the Oxoid-manual). Within 48 hours of incubation period in the selective media one loop of these test solutions were streaked on Bismuth Sulphite agar (B/S Agar) and Brilliant Green agar (B/G Agar) plates separately (Bismuth Sulphite agar and Brilliant Green agar were prepared according to the specifications of Oxoid manual). Black colonies on B/S agar and pink colonies on

B/G agar was considered as positive for *Salmonellae* organisms. These specific colonies were picked and streaked on already prepared nutrient agar plates and incubated for 24 hours to obtain pure cultures. Within 24 hours these pure cultures were biochemically tested for *Salmonellae*. These stock cultures also identified during the tests done for identification of the other micro-organisms. Urease test, Indole test, and Hydrogen sulfide production tests were done as biochemical confirmation tests for *Salmonellae* [4].

#### **Test for coliforms**

One gram or one ml of each of the samples was dissolved in 10 ml of sterile distilled water. Using this stock solution series of dilutions up to  $10^{-3}$  was made. One ml of this solution was transferred into 09 ml of single strength sterile MacConky broth tubes which contain a Durham tube. Mix the tubes by gentle rotation. These tubes were incubated at 37°C for 48 hours. The tubes which gave yellow colour with gas production were assumed as positive for Coliforms. These positive tubes were sub cultured into sterile Brilliant Green Bile broth tubes which also contained a Durham tube in two sets. One set was kept at 37°C and another was kept at 44°C for 48 hours. The tubes which showed gas production at 37°C were considered as positive for Coliforms and the tubes which showed gas production at 44°C were considered as positive for faecal Coliforms. *E.coli* was confirmed by Indole test [4].

#### **Test for anti microbial activity**

Compound preparations below two years of manufacturing date were selected. Minimum adult dose of Choorna and Kalka were dissolved in 15 ml of sterile distilled water and kept in a shaker at 100 r.p.m. continuously for four hours in order to get the maximum soluble water extract of these preparations. 0.1 ml of these extracts was used to the antimicrobial assay. In case of oils, 0.1 ml of oil was used directly. 50 ml of the Nutrient broth was prepared, taken into five metal capped tests tubes and sterilized. These tubes were inoculated separately using sterile inoculating needle with pure American Type Culture Collection (ATCC test cultures).

The test organisms were ATCC cultures of *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonellae typhi*, *Klebsiella*, *Bacillus cereus* and *Staphylococcus aureus*. Nutrient agar plates were prepared and kept in an incubator at 37°C for 24 hours in order to exclude contamination and to reduce the moisture content of these plates. These agar plates were seeded with above ATCC test cultures separately using spread plate technique. Sterilized metal cylinders (assay cup) were placed in each sector using sterile forceps. The cylinders were pressed gently until the edge of the cylinders sink into the agar to keep it firmly in place. 0.1 ml of the drug extract was placed into these cylinders and plates were sealed. In case of oil 0.1ml of oil was placed into these cylinders and plates

were sealed. Within 24 hours the growth of the seeded organisms were noted. The clear zones around the assay cups were measured and recorded. Width of the zones was measured and considered as the efficacy of the drugs against the test organism [4].

#### HACCP analysis

Analysis was done at the Nawinna Ayurvedic Drugs Corporation. The sites which were subjected to identify any critical points are main drug stores, pre preparation site, drug manufacturing site, packaging site, labeling site and stores of the finish product. Product Thalisadi Choorna was selected as a test product on the basis of the test results of the microbial load. A flow chart of the product was prepared. Possible critical points were identified. A test preparation was prepared according to identified critical points in order to minimize the contamination and to reduce the microbial load. Microbial load was repeated in the test product.

#### Methods tested to control microbial load on compound preparation "Thalisadi Choorna"

1. Heat treatment. 100g of the above sample was subjected to heat treatment in a hot air oven at 80°C for 10 minutes for three consecutive days.
2. Ultra violet radiation at 256 wave length continuously for 24 hours (100 g).
3. 100 g of the sample was steamed under atmospheric pressure in a closed container up to 10 minutes for three consecutive days.

Then the study of microbial load was thereafter repeated in every sample. Then Thin Layer Chromatographic (TLC) patterns and the volatile oil content of the steamed and original samples were done on sample

which showed relatively low microbial load and the original sample which was not subjected to any pretreatment methods.

#### Study on thin layer chromatographic patterns

Steamed in an open container, steamed in a closed container and original sample of Thalisadi Choorna was subjected to this study.

Moisture content of the 3 gram above samples was measured. 3 gram of the sample and water content of the samples was taken for extraction. Extraction with 50 ml of Ethanol and 50 ml of water were taken separately. Extracts were concentrated and dissolved in 3ml of same extracted solvent and using a sterilized micropipette. 1-2 micro liter of this solution was used to spot the TLC plates. Different solvent systems were used to study the TLC. Using Vanilline + Conc-Sulphuric acid spray reagent the bands were fixed and noted under the UV lamp (254 and 365 nm). Number of bands, R.f values, distance of the solvent front from the spot and the distance of the bands from the spot also recorded.

#### Study on the volatile oil content

Steamed in an open container, steamed in a closed container and original sample were taken for the study. 80 gram of each drug samples was taken with 250 ml of distilled water. "Dead and Stark Distilled Arm" apparatus was used to measure the volatile oil content. Each sample was refluxed in hot water bath for 2-3 hours. Volatile oil collected at the upper end of the distillery arm was measured and recorded.

#### Results and Discussion

Results of the microbial load on different formulations were summarized as below (Table 1).

**Table 1**

	<i>Range for bacterial counts /gr</i>	<i>Range for fungi counts /gr</i>
<b>Choorna (Powders)</b>		
Dathri Choorna	$2.0 \times 10^4$ to $1.1 \times 10^6$	$4.7 \times 10^4$ to $1.7 \times 10^6$
Hinguastaka Choorna	$9.8 \times 10^4$ to $4.0 \times 10^6$	$2.0 \times 10^4$ to $4.5 \times 10^4$
Manibadra Choorna	$8.4 \times 10^4$ to $4.2 \times 10^7$	$2.0 \times 10^3$ to $1.5 \times 10^5$
Thalisadi Choorna	$3.0 \times 10^3$ to $6.2 \times 10^7$	00 to $1.9 \times 10^4$
Thripaladi Choorna	$2.0 \times 10^5$ to $1.2 \times 10^7$	00 to $1.5 \times 10^4$
<b>Kalka (Pastes)</b>		
Buddaraja Kalka	00 to $7.5 \times 10^3$	00 to $5.0 \times 10^2$
Chandra Kalka	$1 \times 10^2$ to $6.1 \times 10^4$	00 to $1.3 \times 10^3$
Desadun Kalka	00 to $3 \times 10^2$	00 to $1.8 \times 10^4$
Nawaratna Kalka	$6.0 \times 10^3$ to $1.2 \times 10^7$	$4.8 \times 10^5$ to $2.0 \times 10^6$
Sarkaradi Kalka	$1.0 \times 10^3$ to $1.7 \times 10^6$	00 to $4.0 \times 10^4$

(Contd)

	<i>Range for bacterial counts /gr</i>	<i>Range for fungi counts /gr</i>
<b>Vatika (Pills)</b>		
Seetharama Vati	00 to $7 \times 10^3$	00 to $1.5 \times 10^4$
Jeevananda Vati	00	00
Suranvidura Vati	00	00
<b>Lepa (Local applications)</b>		
Dasanga Lepa	$9.0 \times 10^2$ to $2.1 \times 10^5$	80 to $1.3 \times 10^4$
Sothahara Lepa	00	00
Rogan E khas	00	00
<b>Thaila (Oil)</b>	<i>Range for Bacterial counts /ml</i>	<i>Range for Fungi counts /ml</i>
Pinda oil	00 to 59	00 to $10^5$
Narayana oil	00 to 23	800 to 54
Sarvavisadee oil	00 to 26	00 to 26
Visarpahara oil	00 to 41	00 to 71
Vathaviduranga oil	00 to 17	00 to 47
<b>Arista (Fermented decoctions)</b>		
Dasamoola Arista	00 to $3.4 \times 10^3$	00 to $4.8 \times 10^2$
Pippalyadi Asawa	$4 \times 10^1$ to $1.3 \times 10^4$	00 to $1 \times 9$
Aravindasava	00 to $1.6 \times 10^4$	00 to $4.8 \times 10^2$
Sarasvatharista	00 to $2.7 \times 10^5$	00 to $3.5 \times 10^1$
Draksa Arista	00 to $1.1 \times 10^5$	00 to $4.5 \times 10^1$

### Choorna (Powders)

WHA limitations for microbial contamination – Total aerobic bacteria =  $10^5$ , yeast and moulds maximum  $10^3$  per gram. All the powder preparations exceeds the WHA limitations for the microbial load. Colony forming units (CFU) per gram for bacteria varies from  $3 \times 10^3$  to  $6.2 \times 10^7$ . Colony forming units for fungi varies from  $1 \times 10^0$  to  $1.7 \times 10^6$ .

### Kalka (Paste)

According to Table 1, only Buddharaja Kalka is below the WHA limitations in both bacterial and fungal counts. Chandra Kalka and Desadun Kalka were under the limits in bacterial counts but exceeds in fungal counts. Nawaratna Kalka and Sarkaradi Kalka preparations exceeds the WHA limitation for both bacterial and fungal counts. Nawaratna Kalka shows the highest range of microbial load. The microbial load in confections for bacteria per gram varies from  $1 \times 10^0$  to  $7.5 \times 10^3$ . The microbial load for fungi in confections varies from  $1 \times 100$  to  $1.2 \times 10^7$ .

### Thaila (Oil)

All these Oil preparations were below the WHA

limitations. In oil samples the colony forming units for bacteria per ml was  $1 \times 10^0$  to  $5 \times 10^1$  and fungi was  $1 \times 10^0$  to  $10^2$ .

### Vatika (Pills)

According to the above range Jeevananda Vati and Suranvidura Vati were totally free from microbial contaminations. Only Seetharama vati exceeds the microbial load in fungal count.

### Lepa (Topical applications)

Lepa preparations are topical dosage forms. The WHA limitations for bacteria and fungi differ from the internal dosage forms. Aerobic bacteria maximum –  $10^7$  per gram and yeast and moulds, maximum  $10^4$  per gram. According to this range all three Lepa preparations were below the WHA limitations for bacterial counts. But Dasanga Lepa preparation was exceeds the fungal counts. The microbial load for Bacteria on Dasanga lepa varies from  $1 \times 10$  to  $2.1 \times 10^5$  per gram. Fungi varies from  $8 \times 10^1$  to  $1.3 \times 10^4$  per gram Sothahara Lepa and Rogan e ghas preparations does not show any growth.

**Arista (Fermented decoction)**

According to the above table Dasamoola Arista, Pipalyadi Asawa and Aravinda Asawa were below the WHA limitations in the microbial load. Saraswatha Arista and Draksa Arista are exceeding the WHA limitations in bacterial counts but both were below the WHA limitations in fungal count. The microbial load varies from  $1 \times 10^0/\text{ml}$  to

$2.7 \times 10^5/\text{ml}$  in Nutrient agar and  $1 \times 10^0/\text{ml}$  to  $5 \times 10^2/\text{ml}$  in potato dextrose agar.

**Results of the biochemical tests on the different formulations**

Most of the bacterial stock cultures belonged to the following *Bacillus* species (Table 2).

**Table 2: Results of the biochemical tests on the different formulations**

Thalisadee Choorna	<i>Bacillus brevis, Bacillus megaterium, Bacillus pumilus</i>
Dathree Choorna	<i>Bacillus brevis, Bacillus laterosporus, Bacillus cereus, Bacillus pumilus, Bacillus licheniformis, Kurthia, Bacillus firmus</i>
Hinguastaka Choorna	<i>Bacillus cereus, Bacillus brevis, Bacillus firmus, Entrobacter spp, Bacillus licheniformis</i>
Manibadra Choorna	<i>Bacillus megaterium, Bacillus brevis, Bacillus pantothenticus, Bacillus pumilus</i>
Thriphaladi Choorna	<i>Bacillus firmus, Bacillus brevis</i>
Buddharaja Kalka	<i>Bacillus brevis, Entrobacter spp</i>
Chandra Kalka	<i>Bacillus pantothenticus, Bacillus brevis, Bacillus firmus</i>
Desadun Kalka	<i>Bacillus pantothenticus, Flavobacterium</i>
Nawaratna Kalka	<i>Bacillus Megaterium, Entrobacteria, Pseudomonas, Entrobacter spp</i>
Sarkarade Kalka	<i>Bacillus firmus, Bacillus brevis, Bacillus Megaterium, Flavobacterium</i>
Narayana Oil	<i>Bacillus pantothenticus</i>
Pinda Oil	<i>Bacillus firmus, Bacillus brevis, Bacillus pumilus</i>
Sarvavisadee Oil	<i>Bacillus brevis</i>
Vathaviduranga Oil	<i>Bacillus pantothencius, Bacillus firmus, Streptococcus</i>
Visarpahara Oil	<i>Bacillus pantothenticus</i>
Dasamoola Arista	<i>Bacillus firmus</i>
Pippalyadi Asawa	<i>Bacillus firmus</i>
Draksa Arista	<i>Bacillus brevis, Bacillus firmus</i>
Aravinda Asawa	<i>Bacillus firmus, Bacillus brevis</i>
Saraswatha Arista	<i>Bacillus firmus</i>
Dasanga Lepa & Sothahara Lepa	<i>Staphylococcus, Bacillus brevis</i>

According to the macroscopic and microscopic appearance the isolated fungi were identified as Penicillium Sp, Aspergillus Sp, Mucor Sp, Rhizopus Sp, Fusarium Sp.

### **Results of the *Salmonellae* tests**

None of the drug samples was positive for *Salmonellae*. Except three samples of Thalisadi Choorna and two samples of Chandra kalka preparations.

### **Results of the Coliform tests**

None of the Choorna, Kalka, Arista, Vatika and Thaila gave positive results for Coliforms in presumptive test. However, some of the Dasanga lepa preparations gave positive results in presumptive tests and test for faecal coliform confirmation tests.

### **Results of the antimicrobial activity**

Choorna (Powders), Kalka (Pastes), and Thaila (Oil) preparations were subjected to this study. Powder preparations of Hinguastaka, and Manibadra showed anti bacterial effect on *Salmonellae typhi*. Thripaladi Choorna showed anti bacterial effect on *Pseudomonas aeruginosa*. None of the tested powder preparations showed anti-bacterial effect on *E-Coli*. The Oil preparation of Sarvavisadee is active against *Pseudomonas aeruginosa*, *Staphylococcus aureas*, *Salmonellae typhi*, and *Klebsiella*. The preparation of Buddaraja kalka was active against *Pseudomonas aeruginosa*, *Staphylococcus aureas*, and *Bacillus cereus*.

### **Results of the HACCP analysis**

According to the microbiological studies carried out on products, the product of Thalisadi Choorna of every manufacturer relatively contained more microbial load and other contaminations. A flow chart of the product was prepared and the following critical points, i.e. C.P. 01 – Out dated or wrong raw materials, C.P. 02 – Impurities (Soil particles etc.), C.P. 03 – Adulterants and C.P. 04 – Improper Washing of raw materials and packaging materials were identified. A test sample was prepared according to identified critical points in order to minimize the contamination and to reduce the microbial load. Again the microbial load was studied in the test product.

It was revealed that the microbial load was very low in the test product. This study clearly indicates that the implementation of good manufacturing practice regulations will lead to microbiologically safe end products.

### **Results of the quality assurance testing**

Steam treatment for powder preparations for a short time period consecutively for three days showed a very low microbial load. Same time it does not bring a major effect on quality of the product.

## **Conclusions**

The above study reveals that most of the powder preparations of every manufacturer were above the acceptable limits of microbial load. Adopting good manufacturing practice can reduce the microbial load and exclude the contaminations. Most of the Oil preparations and Vatika preparations are within the acceptable limits. Most of the Kalka preparations (except the Buddharaja Kalka) were also above the acceptable limits of microbial load. The individual data analysis shows the Dasamoola Arista, Pippalyadi Asawa and Arawinda Asawa preparations were below the acceptable limits. The microbial load on Lepa preparations were below the acceptable level. The positive results for specific micro organisms may be due to poor GMP. HACCP analysis confirmed improving the GMP will ensure the microbiologically safe end products. Steam treatment may be an effective method to reduce the microbial load. Studies on the antimicrobial activity highlight the effectiveness of these preparations in common infective conditions.

## **Recommendations**

1. Educational programmes on G.M.P for the factory workers,
2. Educational programme should be repeated periodically,
3. Separate building sites for pre preparation stores and drug manufacturing sites,
4. These sites should be restricted for other workers in the factory,
5. Quality check on raw materials should be performed before sending for production,
6. Quality checkup on factory water resources should be performed periodically,
7. Preparation of raw materials for drug manufacture should be monitored by a medically qualified trained person,
8. Quality control laboratory with basic infra structure facility should be established in every commercial drug manufacturing establishment,
9. Quality check-up should be performed in between the production line,
10. Packaging materials should be subjected to quality check-up prior to packing,
11. Batch re-call facility is necessary between the production line and from the market [5, 6].

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**References**

1. World Health Organization, Geneva - 1988, Quality control methods for medicinal plant materials, WHO Library cataloguing in publication data, ISBN 92 4 - 15 45 100.
2. Department of Ayurveda, Ayurveda Pharmacopoeia, Volume 1, published by the Ministry of Health and Indigenous Medicine, Department of Ayurveda, Sri Lanka, 1998: 123-261.
3. Cowan ST. Manual for the Identification of Medical Bacteria, Second Edition, Cambridge University Press, Cambridge, London New York, New Rochelle, Melbourne Sydney, 1990; **42**: 124.
4. International Standards, ISO 9308- 2, First edition 1st, Ref. No ISO - 9308-2, 1990 (E); **1**: 14.
5. World Health Organization. Guidelines for the Appropriate use of Herbal Medicine 1948, 1998, Region Office for the Western Pacific Manila, 1990; **1**: 184.
6. World Health Organization. Good manufacturing practice, updated supplementary, guidelines for the manufacture of herbal medicines, 2005; **1**: 184.

# Evaluation of the Yava-Kshara Taila Uttar Basti in the management of tubal blockage

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## Abstract

Tubal blockage is very common and difficultly managed factor of female infertility. Its management available is beyond the reach of the common people of developing countries. Ayurveda does not possess a data-based management of this problem till date. Hence, this study was carried out to establish a data-based treatment regimen of tubal blockage through Ayurveda. Total 14 patients with 50% unilateral and 50% bilateral tubal blockage were registered for the present study, which aimed to find out the effect of intra uterine Uttar Basti of Yava-Kshara Taila on tubal blockage. The total period of the treatment was 2 consecutive cycles and Uttar Basti was given for 6 days in one cycle with an interval of 3 days in between. The dose for one Uttar Basti was 5 ml, and the procedure was carried out with proper antiseptic care. The criteria of assessment for the results were the reports obtained from hysterosalpingography (HSG) performed during and after treatment. The results were highly significant, as blockage was removed in 85.71% of patients. 7.41% patients conceived within the follow up period and no patient reported any feature of complication during the treatment or follow up period.

## Introduction

Tubal blockage is the second most common factor responsible for female infertility with a prevalence of 25-35% [1]. It is very difficult to manage, because the treatment choices for it are only tubal reconstructive surgery and in vitro fertilization (IVF). Both of these, being run in very few infertility clinics in developing countries are not easily accessible. Chances of ectopic pregnancy and other complications are also there. On the other hand, there is no established reliable Ayurvedic treatment for the tubal blockage. Though, some Ayurvedic practitioners claim the effect of intra uterine Uttar Basti on tubal blockage, yet data-base treatment

regimen is not available. Keeping this point in view, the present study was carried out as a very preliminary step to find out a reliable and data-based Ayurvedic management of tubal infertility.

## Aims and objectives

The aims and objectives of the study included: i) to find out the effect of Yava-Kshara Taila Uttar Basti in the tubal blockage and ii) to study the complications, if arise, during and after the course of treatment.

## Materials and Methods

Patients attending the O.P.D. of Stree Roga & Prasuti Tantra, I.P.G.T.& R. A., fulfilling the criteria for selection were incorporated into the study irrespective of caste, religion etc. A detailed history regarding infertility, family history, obstetric history, menstrual history, past illness and clinical finding pertaining to Dosha, Dushya, Dushti, Agni, Srotasa etc. were filled up in specially prepared proforma on Ayurvedic guidelines.

All the patients were examined per vaginally to assess any sign of infection or disorder related to tubal blockage or infertility. The present study was a randomized clinical trial.

## Criteria for selection of cases

Patients of child bearing age with complaint of failure to conceive due to tubal factor within one or more years of regular, unprotected coitus were included in the study. Both the patients, having primary and secondary infertility were included for the study after taking consent from them.

Patients having any urogenital infection, patients having history of excessive menstruation and patients suffering from any chronic debilitating disease, sexually transmitted diseases, hepatitis B, contagious diseases etc. were also excluded from the study.

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### Parameters of diagnosis and assessment of results

Patients were selected on the basis of hysterosalpingography (HSG) for the least chances of false reports. Biochemical screening tests for HIV (Human Immunodeficiency Virus), HBsAg (Australia antigen for hepatitis B) and VDRL (Venereal Disease Research Laboratory) were carried out in all the patients before starting the course of treatment. Transvaginal sonography was carried out before treatment to rule out any pelvic pathology. Routine haematological investigations and urine tests were done before and after treatment.

### Selection of drug

Kshara-Taila is mentioned for Stree Roga Adhikar in Bhaishajya Ratnavali. Kshara Taila (Karna Rogadhikar) is being practiced for Intra Uterine Tubal Blockage in some parts of India for its Ushna-Tikshna property. But for present study, only Yava-Kshara was selected to prepare Taila for first group to make the preparation of drug easier.

Yava-Kshara is considered as Garbhaprada (fertility creating) and effective in Artavanasha (amenorrhoea) in Ayurvedic treatises and is indicated for internal administration [2].

Results were found within the prescribed standard limits [3].

**Table 1: Pharmaceutical analysis of Yava-Kshara Taila**

<i>Organoleptic parameters</i>	
Colour	Yellowish
Odour	Not specific
Appearance	Clear
Clarity	Thin, clear
<i>Physico-chemical parameters</i>	
Loss on drying	0.779%
Refractive index	0.516
Specific gravity	1.464
Acid value	2.826(w/w)
Saponification value	301.19(w/w)
Iodine value	99.2(w/w)

### Course of treatment

The present study was carried out in total 14 patients of tubal infertility. The patient was admitted one day after cessation of menstruation and 5 ml intra uterine Uttar Basti was given by for 6 days in a cycle (with the interval of 3 days in between) in each cycle. The course of the treatment was 2 consecutive cycles and the HSG was repeated for the analysis of results after the cessation of menstruation in third cycle.

### Method of Uttar Basti

**Purvakarma (Pre-procedure)**—Snehana of Bala Taila on lower abdomen, back and lower limbs and then Nadi Sveda with the help of water steam for 15 minutes on lower abdomen and back was done in patients before each Uttar Basti. Yoni Prakshalana with Panchavalkala Kvatha [5] was done as aseptic care of the private part.

**Pradhana Karma**—The procedure is carried out in the operation theatre. The oil and instruments are autoclaved. Patient is taken on operation table in dorsal lithotomy position.

The private part (already shaved) is cleaned with antiseptic solution. Vagina and cervix is visualized with the help of Sim's speculum [6] and anterior vaginal wall retractor [7]. The anterior lip of cervix is caught with the help of Allis' forceps [8]. Uterine sounding is done and then Uttar Basti cannula, already attached with 5 ml. syringe filled with medicated oil is passed in uterine cavity after making head low position. The drug is pushed above the level of internal os with constant force, but fast to make the drug reach up to the tubes.

**Pashchat Karma**—The patient is sent to bed and the bed is kept with head low for 2 hours. The lower abdomen is fomented with hot water bag.

### Precautions

The patients were asked to avoid very spicy food during treatment. Coitus was prohibited during the course of Uttar Basti. Proper care was taken for not allowing patients to suffer from constipations.

### Assessment of complications

As the Taila prepared with Ushna-Tikshna Dravya was administered inside the uterus. Possibility of complications can not be neglected totally. Per vaginal bleeding and lower abdominal pain were the most probable complaints during and after procedure. It was considered as complications, only if this was very much troublesome for the patient. Features of any type of urogenital infection during and after procedure was taken as complication.

### Points to stop the treatment

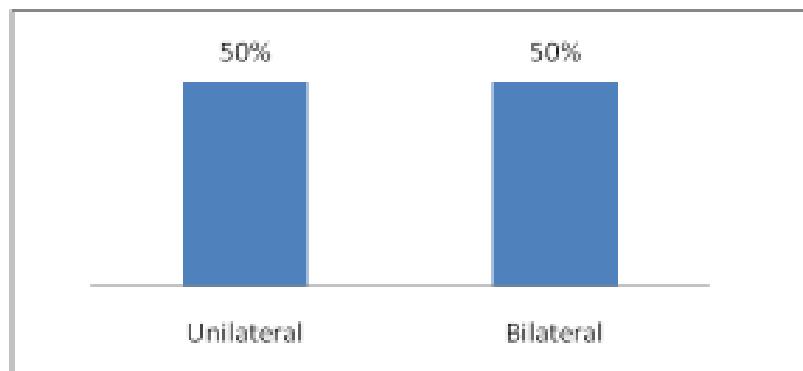
Some points were decided to stop the treatment, if developed during treatment. If the patient conceives in between the course of study. If signs of any type of urogenital infections are observed.

If heavy per vaginal bleeding starts and If there occurs severe abdominal pain, which troubles the patient much.

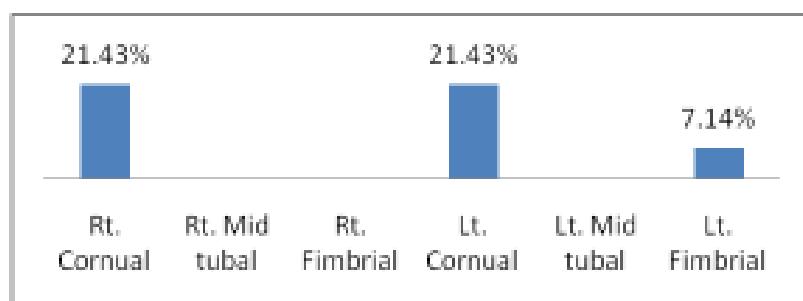
### Follow up study

Follow up study for pregnancy or any late complication was carried out for 2 months after the completion of treatment. Any new complaint emerged during follow up period related to study was also noted.

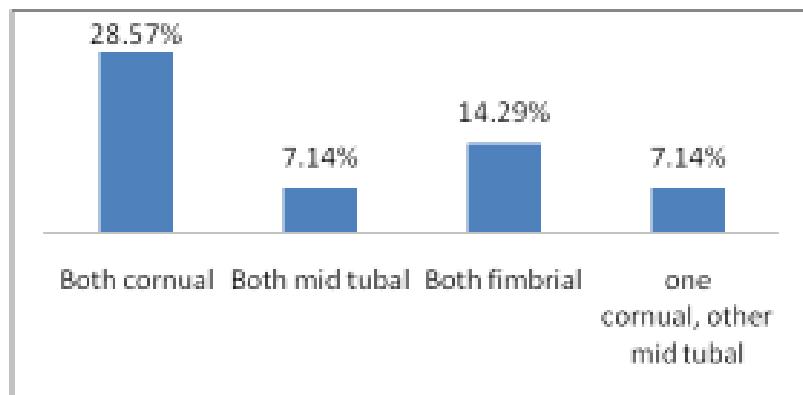
## Observations



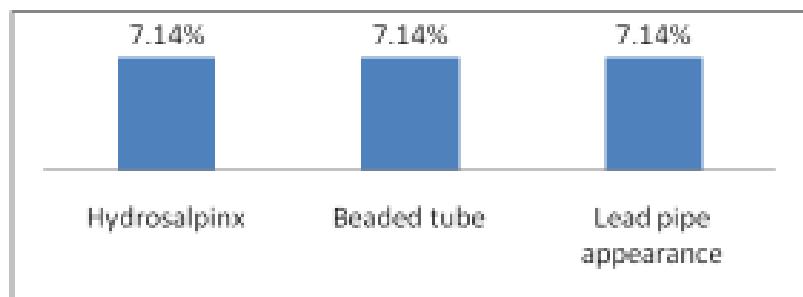
Graph 1. Tubal blockage in 14 patients



Graph 2. Sites of unilateral blockage (n=7)



Graph 3. Sites of bilateral blockage (n=7)



Graph 4. Other tubal anomalies in 14 patients

**Table 2: Observations during and after procedure**

<i>Findings</i>		<i>Number of patients</i>	<i>%</i>
Abdominal pain		08	57.14%
Severity	Tolerable	08	57.14%
	Intolerable	-	-
Duration	< 1 hour	08	57.14%
	>1 hour	-	-
Per vaginal bleeding		09	64.29%
Amount	Spotting	09	64.29%
	More	-	-
Quality	Fresh blood	-	-
	Dark blood	09	64.29%

No complication or any adverse drug reaction was noted during and after procedure.

**Table 3: Total effect of therapy**

<i>No. of patients</i>	<i>Positive results</i>	<i>%</i>	<i>No. of patients, who conceived</i>	<i>%</i>
14	12	85.71%	01	07.14%

**Table 4: Patients, who could not conceive within follow up period after block removal**

<i>No. of patients, in who block was removed but no conception</i>	<i>Patients, in who no other factor could be detected</i>	<i>Patients, in who other factors were involved</i>
11	02	09

**Table 5: Statistical comparison (t-test) of haematological and urinary analysis (before and after treatment)**

<i>Parameter</i>	<i>Mean</i>		<i>S.D.</i>	<i>S.E.</i>	<i>t</i>	<i>P</i>
	<i>B.T.</i>	<i>A.T.</i>				
Hb %	10.92	10.64	0.545	0.15	1.911	>0.05
TC	7657	8042	1251	334.6	1.152	>0.05
N%	61.28	61.07	5.793	1.55	0.138	>0.05
L%	30.14	28.92	5.250	1.4	0.865	>0.05
E %	3.28	2.42	2.248	0.60	1.42	>0.05
B %	2.92	2.92	1.037	0.28	0.00	>0.05
M %	1.71	0.85	2.500	0.534	0.14	>0.05
ESR	27.14	24.64	10.27	2.75	0.910	>0.05
PCV	32.14	31.42	7.040	1.35	0.574	>0.05

## Discussion

The description regarding the prevalence of unilateral and bilateral tubal blockage is not available. The main reason behind it can be that the tubal block is thought to be a very serious problem, only if it is bilateral. It is assumed that if one tube is patent and functioning, it will perform as the channel between peritoneal ovum and sperm. Unilateral blockage is not given importance by modern gynaecologist, and that is why literature regarding its incidence is not available in modern books. Yet unilateral blockage is also important to give due consideration, because, it reduces the possibility of conception. And the condition becomes worse, if another patent tube is not normal physiologically. The incidence of the unilateral and bilateral tubal blockage both, in the present study was 50% (Graph 1). It shows the prevalence of both the conditions high in the society. The most prevalent site of blockage was cornual with 21.43% in right and left both sides (Graph 2). This data supports the already established fact that proximal (corneal) tubal block is the commonest.

Proximal tubal occlusion is mostly due to an inflammatory phenomenon, secondary to an ascending sexually transmitted disease, puerperal infection or septic abortion. It may also be associated to salpingitis isthmica nodosa, endometriosis, polyposis, or other rare causes of endosalpingitis. The other tubal anomalies were also observed in the present study with 07.14% hydrosalpinx, 07.14% beaded tubes and 07.14% lead pipe appearance of fallopian tubes (Graph 3). All these deformities are the manifestation of active tuberculous infection or the after effects of some previous tuberculous infection. The strong antitubercular drugs though, pacify the infection, but can't restore the damage caused by this bacterium, and the damage generates several factors of infertility. Tuberculosis is still, a great problem in front of the population of developing countries like India.

Analyzing the clinical manifestations during procedure, 57.14% patients complained lower abdominal pain (Table 2). The pain was within tolerating capacity in 57.14% patients, and no patient complained pain beyond their tolerating limit. The duration in 57.14% patients was < 1 hour, while no patient suffered from pain for more than one hour after the procedure. The abdominal pain within the tolerating capacity was not considered as complication, because it shows the contractile response of uterus to remove the blockage from the site of obstruction. It was assumed that with the obstruction from the blockage site will be removed with the scraping (Lekhana) property of Ushna-Tikshna drugs and also by the contractile response of uterus. The contractile response was confirmed by the lower abdominal pain, which was a common complaint after procedure. The scraping action of the drugs given by Uttar Basti was proved by the 64.29% patients, who complained vaginal bleeding. All the 64.29% patients had bleeding in the form of spotting of dark colour blood. No patient had excessive or fresh bleeding. It proves the removal of the inner uterine

as well as tubal lining by the Ushna-Tikshna and Lekhana drugs.

The total effect of therapy was very encouraging and highly significant on tubal blockage. The tubal block was removed in total 85.71% patients. Other than that, no complication was noted during and after procedure in any patient. It is highly appreciating that, no drug other than test one was given to patients during treatment. Still, symptoms of genitourinary infection were not reported in patient. It proves the intra uterine Uttar Basti as a safe therapeutic measure against tubal blockage.

7.14% patients conceived within the follow up period of 2 months (Table 3). It seems to be due to the fact that most of the patients, who could not conceive were having one or more factor of infertility other than tubal blockage (Table 4). The tubes were found patent in 12 patients after treatment. One conceived and 11 out of them could not conceive within follow up period. These patients were suffering from other factors of infertility including male factor. Thus, it is not possible to comment on the rate of conception with this study. The present study only suggests the highly encouraging results of tubal block removal with Yava-Kshara Taila Uttar Basti.

### Interpretation of mode of action of intra uterine Uttar Basti on tubal blockage

Action of Uttar Basti on various disorders is by both the ways, local as well as systemic. In case of tubal blockage, this effect seems to be more local than systemic. The Tila Taila [9,10,11,12] is Vranashodhaka and Vranapachaka. It is Krimighna too. Other than that its specific role on uterus and reproductive tract is also mentioned as Garbhashayashodhana and Yonishula-prashamana. All these properties indicate towards its antiseptic as well as anti-inflammatory effects. Its Vyavayi and Vikasi Guna show its potency to enter in minute channels and to get spread easily. Thus, it should be the best medium for any drug to reach in tubal cavity and remove the blockage. In some other study, result of only Tila Taila Uttar Basti on tubal blockage can also be studied to find out a cost-effective and easily available alternative. In both the groups, the selected drugs were also having the same Dosaghgnata. It also has Gulmanashana and Kaphanissraka Karma.

Any of the Kshara is said to be the best for not allowing recurrence. Hence, the Yava-Kshara works with its Tikshna and Vata-Kapha Shamaka properties in removal of blockage. It helps in scraping of obstructing substance and also removes the endometrial lining of tubes and uterus. And it is supported by the 64.29% patients, who complained of dark colour bleeding after Uttar Basti. It removes the fibrosed and damaged endometrium and promotes its rejuvenation. Thus, this management not only removes the blockage, but also creates a favourable environment inside the uterus for implantation. The statistical comparison of the results obtained from the haematological and urinary analysis of before and after

treatment show non-significant results (Table 5), which are evidence of no complication by the Uttar Basti.

The mode of action of Uttar Basti on tubal blockage can be described in following points:

- i) it removes the blockage of tubal lumen by directly acting on obstruction mechanically and restores the normal endometrium, as endometrial covering is there inner side of tubes too, scraping and regenerating of it also leads to normalization of tubal functions
- ii) it restores the normal functions of cilia by stimulating it
- iii) it may break the tuboperitoneal adhesions after getting spilled from fimbria.

## Conclusions

Intra uterine Uttar Basti with Yava-Kshara Taila is a highly significantly effective therapeutic measure against the fallopian tubal blockage. Possibility of any serious complication is not evident from the study.

Thus, with some further research studies, Yava-Kshara Taila Uttar Basti can be established as a data base, reliable, safe and easily accessible Ayurvedic treatment regimen of tubal infertility.

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## References

1. Howkins and Bourne, Shaw's Textbook of Gynaecology, eds. Padubidri, V.G. and Daftary S.N. Reed Elsevier India Private Limited, New Delhi, 2004, p.223.
2. Bharta Bhaishajya Ratanakara, 1937, 5th part, Shri Nagindas Chhaganlal Shah, Unjha Ayurveda Pharmacy, Ahemadabad, p.533-4.
3. Acharya Shalinatha. Rasamanjari, 'Siddhiprada' hindi commentary by Siddhinanadana Mishra, Chaukhaba Orientalia, Varanasi, 2003, 9/52-54.
4. Pharmacopeal standards for Ayurvedic formulations, C. C. R. A. S., New Delhi, revised edition, 1987.
5. Sushruta Samhita, Dalhana commentary, Ed. By P. V. Sharma, Chaukhamba Vishvabharati, Varanasi, 2003: 38/25.
6. Harish Doshi, Companion for obstetrics and gynec practical examination, Arihant Publishers, Ahamedabad, India, 2007, p.1-2.
7. Harish Doshi. Companion for obstetrics and gynec practical examination, Arihant Publishers, Ahamedabad, India, 2007, p.5.
8. Harish Doshi. Companion for obstetrics and gynec practical examination, Arihant Publishers, Ahamedabad, India, 2007, p.22-23.
9. Bhavamisra, Bhavaprakasha of Bhavamisra, eds. K. R. Srikantha Murthy, Krishnadas Academy, Varanasi, B. P. Taila Varga, 2002, p.2-7.
10. Bhavamisra, Bhava Prakasha, edt. Srikantha Murthy, Krishnadas Accademy, Varanasi. *Dhanya varga* 1998; 1: 652.
11. Anonymous. The Ayurvedic Pharmacopoeia of India, Ministry of Health and Family Welfare, dept. of ISM & H, govt. of India, New Delhi, 1999.
12. Sushruta, Sushruta Samhita, 'Ayurveda Tatva Samdipika' vyakhya, eds. Kaviraja Dr. Ambnika Dutta Shastri, Chaukhamba Samskrita Samsthana, Varanasi, 2003; 1: 45/ 112.

# Effects of *Indravati Rasa* on lipid peroxidation of diabetes induced rats

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## Abstract

Diabetes mellitus is a group of metabolic disorders. Ayurveda physicians have proved that diabetes mellitus may be controlled by some herbo-mineral preparations. Among there, *Indravati Rasa* has strongly been recommended for *Madhumeha* in various *Rasashastra* texts. Hyperglycaemia increases free radicals generation and free radical mediated injury leads to an increase production of malondialdehyde, a marker of lipid peroxidation. Circulating levels of malondialdehyde is higher in diabetic subjects. In this research, *Indravati Rasa* was evaluated for its possible effects on experimental diabetes mellitus and on lipid peroxidation in albino rats. Inbred Charles Foster albino rats 100-140.0 g were selected for this study. Streptozotocin in the dose of 70.0 mg / intra peritoneal produced significant increase in blood glucose and serum malondialdehyde at 48 h. *Indravati Rasa* in the dose of 133.0 mg/kg and 266.0 mg/kg was administered orally in two groups of rats. Analysis of data showed that *Indravati Rasa* attenuated the hypoglycaemic state, but it was statistically insignificant ( $p>0.05$ ). *Indravati Rasa* (133.0 mg/kg) produced significant lowering of streptozotocin induced blood malondialdehyde level ( $p<0.05$ ). The present findings suggest that the *Indravati rasa* at low doses (133.0mg /kg) possesses significant effect on lipid peroxidation.

## Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia, glycosuria, hyperlipidaemia, negative nitrogen balance and sometimes ketonaemia [1]. Diabetes mellitus represents a syndrome with disordered metabolism glucose due to either absolute deficiency of insulin secretion or reduction of its biologic effectiveness or both [2]. Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of langerhans in the pancreas leading to insulin deficiency. This type of diabetes can be further classified as immune-mediated or

idiopathic [3]. The majority of type 1 diabetes is of the immune-mediated nature, where beta cell loss is a T-cell mediated autoimmune attack. There is no known preventive measure against type 1 diabetes, which causes approximately 10% of diabetes mellitus cases. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages [4]. Type 1 diabetes can affect children or adults but was traditionally termed “juvenile diabetes” because it represents a majority of the diabetes cases in children.

Treatment of diabetes mellitus still remains one of the great challenges in the medical profession [5]. Ayurveda has elaborately mentioned that *prameha* is one of the *Maha roga* and *Madhumeha* is one sub type of *prameha* which is said to be incurable and it resembles closely to diabetes mellitus [6]. Ayurveda is a holistic and divine science. *Rasa shastra* is a branch of learning in Ayurveda pharmaceutics specially deals the medicine formulated with minerals, metals, precious stones, marine products and certain poisonous herbs. After a long time experience, ancient Ayurvedic physicians have proved that *Madhumeha* may be controlled by such herbo mineral preparations, among which *Indravati Rasa* is strongly recommended for *Madumeha* in various *Rasashastra* texts [7]. For the present study, *Indravati Rasa* was selected from *prameharoga Adhikara* of Bhaishajya Ratnawali [8] and it contains *Rasa sindura* (red-sulphide of mercury), *Vanga bhasma* (incinerated tin) and *Arjuna twak churna* (*Termenalia arjuna* Wight and Arn) in equal amount, this mixture is subjected to *bhavana* (triturating) [9] with *Shalmali mula swarasa* (juice of root bark of *Salmalia malabarica*) for seven days and finally it prepared in *vati* (pill) form. Dosage of *Indravati Rasa* mentioned in this text is one *masha* (1.0g) per day.

Most of herbo mineral preparations mentioned in *Rasashastra* are said to be potent in eliminating dreadful diseases and also rejuvenative, therefore the herbo mineral preparation *Indravati Rasa* has been selected for the study and effort has been made to evaluate for its possible

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hypoglycaemic effect and lipid peroxidation status in Type-1 Diabetes (Insulin Dependent Diabetes Mellitus - IDDM) experimentally. Increasing appreciation of the causative role of oxidative /injury in many disease states places great importance on the reliable assessment of lipid peroxidation. Malondialdehyde (MDA) is one of several low-molecular-weight end products formed via the decomposition of certain primary and secondary lipid peroxidation products [10]. Lipid peroxidation refers to the oxidative degradation of lipids in cell membranes. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lies methylene-CH<sub>2</sub>-groups that possess especially reactive hydrogen [11].

## Materials and Methods

### Animals

Inbred Charles Foster albino rats of either sex weighing between 100-140.0 g were procured from central animal house, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. All the animals were kept in colony cages at an ambient temperature of 25±2°C, with 45-55% relative humidity and 10:14 light and dark conditions. The animals were kept on standard rodent feed and water *ad libitum*. All the experimental animals acclimatized in the department for 3 days before experiment. All the experiments were conducted after Institute Ethical Committee approval and the principles of laboratory animal care and use (NIH Publication No 86-23, revised 1985) guidelines were adhered throughout the study.

### Drugs and Chemicals

#### Drugs

- (1). *Indravati Rasa* (containing red-sulphide of mercury, Vanga Bhasma (Incinerated Tin), Arjuna Twak Churna (*Termenalia arjuna* Wight and Arn) in equal amounts and triturated with Shalmali moola swarasa (juice of root bark of *Salmalia malabarica*) for 7 days and prepared 250 mg sized pills were prepared).
- (2). Insulin (Human Insulin – Regular) (Zydus Pharmaceuticals).

#### Chemicals

1. Streptozotocin (Sigma Aldrich U.S.A).
2. Citrate buffer (21.2g of citric acid monohydrate and 29.2 g of citrate were dissolved in one liter of carbon dioxide free water which served as stock solution) 280 ml of citric acid solution and 220 ml of trisodium citrate solution were mixed together.
3. Potassium chloride (KCl – 1.15%) was prepared by dissolving 1.15 g of KCl of 50 ml of distilled water was added to make the final volume of 100 ml.
4. Sodium lauryl sulphate (SLS 8.1%) was prepared by dissolving 8.1g of SLS in distilled water to a final

volume of 100ml. Complete dissolution was achieved by gently heating in the water bath.

5. Acetic acid (20% pH 3.5): 20 ml acetic acid was mixed with 80 ml of distilled water and pH was adjusted to 3.5 by slowly adding 0.1 sodium hydroxide.
6. Thiobarbituric acid (TBA-O.8) (Merks comp): 800 mg of TBA was dissolved in 100 ml distilled water. Complete dissolution was achieved by slightly heating in the water bath.
7. n-Butanol/pyridine (15:1v/v) Solution of n-Butanol and pyridine in the ratio of 15:1 was prepared by mixing 75ml of n-Butanol with 5ml of pyridine.
8. Tetraethoxypropane (TEP) was used as an external standard for obtaining the standard curve.
9. Potassium chloride, sodium lauryl sulphate, acetic acid, n-Butanol, pyridine and 1, 1', 3, 3'-Tetraethoxypropane used in the experiment were of analytical grade.

## Methods

**Table 1: Grouping of animals and dose schedule**

S. No	Group	Drug dose
1	Control vehicle (C)	2% gum acacia solution
2	Control streptozotocin (STZ)	70.0mg/kg i.p, single dose
3	<i>Indravati Rasa</i> (IVR- X dose)	133.0mg/kg/day,p.o
4	<i>Indravati Rasa</i> (IVR- 2X dose)	266.0mg/kg/day,p.o
5	Insulin (Human insulin regular)	4.0IU/kg,s.c/day

#### Drug administration

*Indravati Rasa* was suspended in 2% gum acacia and was given orally through aero gastric tube. The dose of *Indravati Rasa* was selected on the basis of the human therapeutic dose (dose for 60.0 kg adult is 1g orally per day). The dose of *Indravati Rasa* in the experimental study was 10 times of human adult dose.

#### Induction of diabetes mellitus (IDDM) [12]

Diabetes was produced by intraperitoneal injection of 70.0mg/kg streptozotocin in citrate buffer. Control animals received equal volume of buffer solution. Blood glucose was measured after 48 hours of streptozotocin injection to ascertain induction of IDDM. Blood was collected by puncturing the para orbital venous plexus with capillary tube. Blood glucose and serum

malondialdehyde levels were measured after 48hrs of streptozotocin injection and on day 21and day 28.

#### Estimation of blood glucose

The blood drop directly dropped on glucometer (Jhonson and Jhonson U.S.A) strip to measure the blood glucose level. Blood glucose level was expressed as mg/dl.

#### Estimation of serum malondialdehyde

Malondialdehyde estimation in the serum was carried out by using thiobarbituric acid (TBA).

#### Procedure

1.0 ml of serum was added to 2.0ml of the trisodium citrate acid - thiobarbituric acid - hydrochloric acid (TCA-

TBA-HCL) reagent and mixed thoroughly. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 g for 10 min. The absorbance of the sample was determined spectrophotometrically at 535.0nm, against a blank that contains all the reagents except serum.

#### Calculation

Malondialdehyde concentration of the sample was calculated using a TEEP standard curve and expressed as nmol/ml.

#### Statistical analysis

The data generated during the study was analyzed by employing paired and unpaired student 't' test.

## Results

**Table 2: Effect of *Indravati Rasa* on blood glucose level (mg/dl) in streptozotocin (STZ) induced albino rats (n=6)**

Group	Blood sugar level (mg/dl) mean, SEM		
	Initial (48 hours after STZ injection)	After Treatment	
		After 21 days	After 28 days
Control (vehicle)	69.90± 2.83	69.32±3.38	70.70± 2.76
Control (STZ)	4.24.56 ± 11.15	322.16± 17.44 (n=5)	322.15 ± 14.0 (n=4)
<i>Indravati Rasa</i> (single dose)	417.42 ± 9.79 <sup>(a)</sup>	315.04± 10.50 <sup>(a)</sup> (n=5)	292.14 ± 7.73 <sup>(a)</sup> (n=4)
<i>Indravati Rasa</i> (double dose)	401.08± 4.51 <sup>(b)</sup>	289.95± 6.01 <sup>(a)</sup>	289.86 ± 7.17 <sup>(b)</sup> (n=5)
Insulin	404.62± 9.51 <sup>(b)</sup>	254.18± 9.23 <sup>(c)</sup>	198.13 ± 16.35 <sup>(c)</sup>

a=p>0.05 b=p<0.01 c=<0.001

**Table-3: Effect of *Indravati Rasa* on serum MDA level (n mol/dl) in streptozotocin (STZ) induced albino rats**

Group	Serum MDA level (n mol/dl) mean SD		
	Initial (48 hours after STZ injection)	After treatment	
		After 21 days	After 28 days
Control (vehicle)	0.03±0.02	0.03±0.20	0.33± 0.20
Control (STZ)	6.90±0.42	7.51±0.28 (n=5)	8.12±0.72 (n=4)
<i>Indravati Rasa</i> (single dose)	7.77± .304 <sup>(a)</sup>	7.50± 0.67 <sup>(a)</sup> (n=5)	6.88± 0.28 <sup>(b)</sup> (n=5)
<i>Indravati Rasa</i> (double dose)	7.16± .458 <sup>(a)</sup>	7.17± .245 <sup>(a)</sup>	6.89± .388 <sup>(a)</sup> (n=5)

a=p>0.05 b=p<0.01 c=<0.001

## Discussion

Analysis of the data showed that streptozotocin induced hyperglycaemia could be antagonized by insulin. *Indravati Rasa* in the dose of 133.0mg/kg and 266.0mg/kg per day were administered per orally in two groups of rats. 133.0mg/kg of *Indravati Rasa* could attenuate the streptozotocin induced hyperglycaemic status of rats only after 28 days. However, the reduction in blood glucose level was not statistically significant. But, *Indravati Rasa* 266.0 mg/kg dose also reduced hyperglycaemic status only after 28 days of treatment. The reduction in blood glucose level was statistically significant in this dose. The present findings in the light of conventional Ayurvedic use of individual components of *Indravati Rasa* justify the use of this novel product in experimental IDDM. Free radicals are known to be associated with diverse pathological conditions including diabetes mellitus, many degenerative diseases and ageing. An attempt was made to ascertain the status of lipid peroxidation in IDDM and possible role of *Indravati Rasa* in inhibition of lipid peroxidation. The present findings showed that *Indravati Rasa* at low doses (133.0 mg/kg per day) attenuated streptozotocin enhanced serum Malondialdehyde (MDA) level. Inability at higher dose of *Indravati Rasa* to produce similar effect is unknown to explain in the present experimental set up.

Analysis of IDDM and MDA data is suggestive of free radical involvement in the causation of IDDM. The ability of *Indravati Rasa* to antagonize streptozotocin induced hyperglycaemia and enhanced lipid peroxidation opens up a new window for *Indravati Rasa* in the armamentarium of pharmacotherapy of IDDM.

## Conclusions

*Indravati Rasa* is a herbomineral preparation is strongly recommended for the management of *Madhumeha* in rasa shastra texts. *Madhumeha* resembles closely to diabetes mellitus. Many of the reactions associated with hyperglycaemia may acutely and chronically increase the production of free radicals resulting in an oxidant antioxidant imbalance.

As a consequence of this imbalance free radical mediated injury ensures and this leads to an increased production of malondialdehyde a marker of lipid peroxidation. In this study experimental results reveals that *Indravati Rasa* is effective in streptozotocin. Insulin dependent diabetes mellitus (IDDM). The mechanism of anti hypoglycaemic effect might also be due to its influence on lipid peroxidation.

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## References

1. Michael S, Michael G. Hutchson's Clinical Methods, 22nd edition, Saunders Elsevier, London, 2007, p.280.
2. Amos AF, McCarty DJ, Zimmel P. The rising global burden of Diabetes and its complications estimates and projections to the year 2010, *Diabet Med* 1997; **14**: (Suppl) S7-S85.
3. Bonnefont Rousselot D, Bastard JP, Jaudon MC, Delatré J. Consequences of the diabetic status on the oxidant /anti oxidant balance. *Diabetes Metab* 2000; **26** (3): 163-76.
4. Southon PA. Free radicals in medicine, Chemical nature and biological reactions. *Mayo Clin* 1998; **63**: 381-9.
5. Andrew J Krenz, Clifford J Belly. Type 2 Diabetes in practice. Royal Society of Medicine Press Ltd, London 2001.
6. Yadavji Trikamji Acharya, Charaka Samhita, Chaukambha Prakasha, Varanasi, (Ch.Ch.6) 2009; p.445-50.
7. Bhatt Sri Gopal Krishna. Rasendra Sara Samgraha, edited by Kaviraj Radha Vallabh Pant, 1949, Delhi.
8. Siddhinandana Mishra, Bhaisajjya Rathnawali, Chaukambha Surabharati Academy, Varanasi, 2005, (BR.37.120-121), p. 708.
9. Govinda Das, Bhaishajya Ratnavali, Pandit Lalchandraji Vaidya, Hindi, Motilal Banarasidas Publishers, Delhi, 1924.
10. David R Janero. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury, Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, 556 Morris Ave., Summit, NJ 07901, USA, 2003.
11. Cross E, Halliwell B, Borish ET, Pryor WA, Ames BN, Saul RL, McCord JM, Harman D. Oxygen radicals and human disease. *Ann Intern Med* 1987; **107**: 526-45.

# Therapeutic potentials of Ayurvedic Rasayana in the management of Asthi Kshaya vis-à-vis osteopenia/osteoporosis

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## Abstract

Ayurveda attributes prime importance to safeguard the health of healthy individuals and to mitigate diseases of the ailing. The equilibrium of Dhatus is health. Among the Dhatus, Asthi (bone) does sharira dharana. Any commotion in equilibrium of Dhatus leads to abnormalities in the body. Asthi kshaya is a condition in which there will be kshaya of Asthi dhatus. In contemporary science Asthi kshaya may be compared to osteopenia/osteoporosis where there is a decrease in the bone tissue. Osteoporosis is a global problem that will increase consequently with the ageing population. Females have the higher risk compared to males. Treatment available in the contemporary science is not devoid of adverse effects. Hence, the present study was carried out to find natural, safe and effective therapy for the management of osteopenia/osteoporosis. The patients were divided into two groups – A and B. In group A, Ayurvedic Rasayana compound consisting of Ashwagandha, Shatavari, Shukti bhasma and Lakshadi guggulu was given for 4 months with milk as vehicle and in group B, modern control drug, tablet Shelcal 500 mg was given with water for 4 months. The Ayurvedic Rasayana regimen provided significant results in subjective as well as objective parameters including bone mineral density (BMD) compared to control drug.

## Introduction

Ayurveda defines human body as a hospitable homeostasis of Dosha, Dhatus and Mala [1]. The equilibrium of Dhatus is health [2]. Any commotion in their equilibrium leads to diseases. This commotion may either be increase, decrease or movement away from their natural abode [3]. Asthi kshaya is a condition in which there will be kshaya of Asthi dhatus. Asthi kshaya may be compared to osteopenia/osteoporosis where there is a decrease in the bone tissue. Osteopenia means decrease in the bone tissue. Osteoporosis is defined as “a progressive systemic skeletal disease characterised by

low bone mass and micro architectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture” [4].

Osteoporosis is a global problem. Females are at higher risk. This risk even increases at menopause, which is the period of hormonal imbalance. One in three women and one in five men over the age of 50 will experience an osteoporotic fracture in their lifetime [5,6].

Europe and the Americas accounted for just over half of all these fractures while most of the remainder occurred in the Western Pacific region and in Southeast Asia [7]. In India, approximately 26 million were suffering from osteoporosis in 2003 and the number may reach to 36 million by 2013 [8].

The adult skeleton undergoes a continuous process of remodeling (formation and resorption). When bone resorption exceeds formation, then osteopenia/osteoporosis occurs. Modern treatment is mainly intended at preventing further bone loss and fractures, maintaining bone mass, calcium and vitamin D supplementation, hormone replacement therapy (HRT), and use of certain drugs like bisphosphonates, selective estrogen receptor modulators (SERMs), anabolic steroids and strontium etc. HRT, bisphosphonates, SERMs and anabolic steroids may produce side/adverse effects. Hence, it is need of the hour to carry researches for finding efficient, economic, natural and safer formulations to manage osteopenia/osteoporosis. The Ayurvedic rejuvenation therapy (Rasayana) may be useful in treating osteopenia/osteoporosis. Keeping these principles in mind the present study was carried out to clinically evaluate the disease Asthi Kshaya vis-à-vis osteopenia/osteoporosis and to assess the efficacy of Ayurvedic Rasayana in its management.

## Aims and Objectives

- To evaluate the efficacy of Ashwagandha, Shatavari, Shukti Bhasma and Lakshadi Guggulu in the management of Asthi Kshaya vis-à-vis osteopenia/osteoporosis.

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2. To evaluate the Rasayana effect of these drugs on degenerative changes of aging related to bone.
3. To compare the efficacy of the Ayurvedic Rasayana drugs with the modern standard control drug Shelcal 500 mg (containing calcium with vitamin D3).

## Materials and Methods

**Study design:** Present study was a randomized, open, standard controlled clinical research.

**Source of data:** The patients were selected by conducting free osteoporosis diagnosis camps in the Hospital of the Institute for Postgraduate Teaching and Research in Ayurveda (I.P.G.T & R.A), Gujarat Ayurved University, Jamnagar, India.

### Inclusion and exclusion criteria

Osteopenic/osteoporotic patients of either sex who's BMD (t-score) is equal to or less than – 1, patients presenting with the classical signs and symptoms of Asthi Kshaya vis-à-vis osteopenia/osteoporosis and patients between the age group of 40-80 years were included in the present study.

The patients aged below 40 and above 80 years, BMD (t-score) above – 1, suffering from neoplasms and tuberculosis of the bone, and systemic disorders like uncontrolled hypertension, thyrotoxicosis, hyper parathyroidism, rheumatoid/gouty arthritis, Paget's disease, Cushing's syndrome were excluded.

Patients fulfilling above criteria were subjected to physical, radiological, haematological and urine examinations (as and when required). A special clinical proforma was prepared incorporating both Ayurvedic and modern parameters.

**Group A** (study group): patients in this group were given;

1. Ashwagandha  
(*Withania somnifera*) powder – 3 gm b.i.d.
2. Shatavari  
(*Asparagus resimosa*) powder – 3 gm b.i.d.
3. Tablet Lakshadi guggulu – 500 mg b.i.d.
4. Capsule Shukti bhasma – 500 mg b.i.d.

All these drugs were given with cow's milk for 4 months.

**Group B** (control group): patients in this group were given Tab. Shelcal 500 mg (calcium 500 mg and vitamin D<sub>3</sub> 250 IU) one tablet b.i.d. with water for 4 months.

### Parameters of assessment

**Subjective parameters:** Shula (Pain), Kesha pata (Hair fall), Danta vikara/pata (Dental deformity/fall), Nakha vikara (Nail deformity) and Daurbalya (General debility) [9].

**Objective parameters:** Serum calcium, serum alkaline phosphatase, X-ray Singh's index, BMD (t-score). To measure the BMD the FDA approved McCue CUBA

Clinical Ultrasound Bone Sonometry system was used which was sponsored by the Elder Pharmaceutical, Mumbai, India.

**Follow up study:** The patients were followed up for 2 months. All the subjective parameters of assessment were assessed during the follow up.

**Assessment of total effect of therapy:** Total effect of therapy was assessed as follows;

1. Marked improvement: more than 75% but less than 100% improvement.
2. Moderate improvement: more than 50% but less than 75% improvement.
3. Mild improvement: more than 25% but less than 50% improvement.
4. No improvement: less than 25% improvement.

**Statistical analysis:** The results were analyzed statistically by applying students' t-test (paired and unpaired) and Chi square test.

## Observations

Total, 117 patients were registered; 64 in group A and 53 in group B. Among these 117 patients, 110 completed the treatment, 60 in group A and 50 in group B and 7 patients discontinued the treatment, 4 in group A & 3 in group B.

Maximum numbers of patients were between the age group of 40-50 years (37.61%), were females (64.1%). The main complaints observed were back pain and joint pain (100%) followed by skeletal pain (58.12%) and weakness in joints (53.85%), hair fall (100%), general debility (93.16%), dental deformity/fall (76.07%) and nail deformity (59.83%). Positive family history of fracture was found in 20.51% of patients. Out of 75 females, 4% were in their perimenopausal stage and 82.67% had attained menopause. Maximum number of patients, consumed Katu rasa (pungent taste) dominant food (71.79%), irregular diet (52.13%), were addicted to tobacco chewing (18.80%), smoking (9.40%) and alcohol (2.56%). Total, 65.81% performed less exercise, 70.08% were afflicted by chinta and 78.63% were of vata pitta predominant prakriti. Among the 117 patients, 72 patients (61.54%) were osteopenic and the remaining 45 (38.46%) were osteoporotic.

## Results

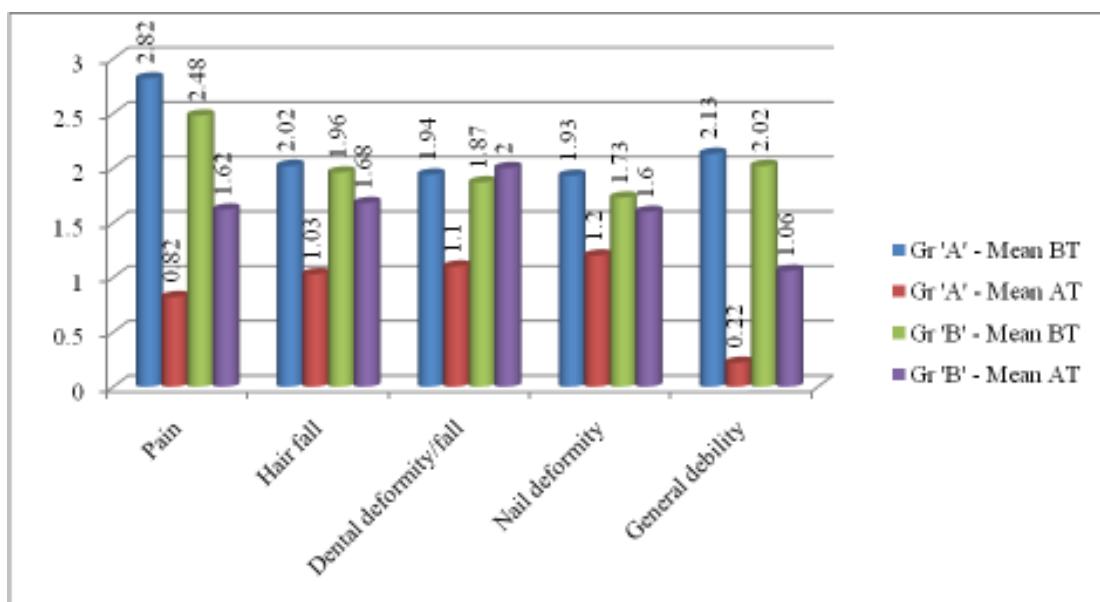
### Effect of therapy on subjective parameters

The percentage relief from pain in group A and B was 70.92% and 34.68% respectively. Effect of therapy in group A was statistically highly significant than group B at p<0.001(unpaired 't' test and Chi square test). Relief from kesha pata was 48.51% in group A and 14.28% in group B. It was highly significant in group A than group B at

$p<0.001$ . Percentage relief from danta vikara/pata was 43.29% in group A whereas it worsened by 6.95% in group B. Statistically group A was highly significant at  $p<0.001$ . Percentage relief from nakha vikara was in group A was 37.82% and 7.51% in group B. On comparison, group A was highly significant at  $p<0.001$ . There was 90.14% relief

in daurbalya group A and 47.52% in group B. Group A showed highly significant results at  $p<0.001$ . But, Chi square test was not significant ( $p>0.10$ ) indicating that the ratio between the number of patients significantly improved and non-significantly improved in both the groups was almost similar.

**Figure 1: Effect of therapy on subjective parameters in groups A and B**

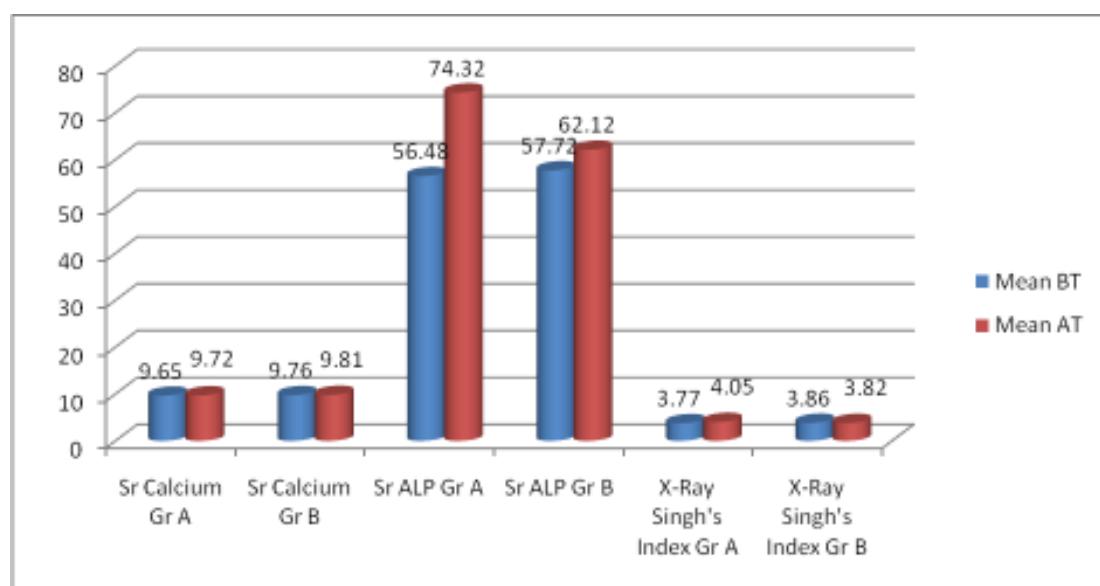


#### Effect of therapies on objective parameters

The effect of therapy on serum calcium was statistically not significant in both groups at  $p>0.10$ . But, serum alkaline phosphatase increased significantly in group A at  $p<0.001$  and the increase was not significant in group B at  $p>0.10$ .

The effect of therapy on X-ray - Singh's index was statistically highly significant in group A at  $p<0.001$  and not significant in group B at  $p>0.10$ . On comparison, unpaired t-test showed that effect of therapy in group A was highly significant than group B at  $p<0.001$ .

**Figure 2: Effect of therapies on objective parameters in groups A and B**



### Effects of therapies on BMD

Since the Ultrasound Bone Sonometer was not available at Jamnagar, the after treatment analysis of the BMD was not possible after exactly 4 months. Depending upon the duration analysis of BMD before and after treatment (BT and AT) the patients of both the groups were divided into 3 sub groups viz A1, A2, A3 and B1, B2, B3 respectively. BMD was assessed after 3½ months in A1 and B1, 4 months in A2 and B2 and 5½ months in A3 and B3.

Statistical analysis was also done separately in each sub group. The effect of therapy was statistically highly significant in groups A1 at  $p<0.001$  and not significant in group B1 at  $p>0.10$ . The effect of therapy was statistically not significant in both the sub groups A2 & B2 at  $p>0.10$ . The effect of therapy was statistically not significant in group A3 at  $p>0.10$  whereas it was highly significant in group B3 at  $p<0.01$ .

### Discussion

Pathogenesis of Asthi kshaya is a complex mechanism and hence no single drug can be used to reverse the pathogenesis. It requires a '*Holistic approach*' to manage Asthi kshaya effectively. Rasayana helps in the formation of quality body tissues. It increases the life span, memory, promotes intellect, youthfulness, luster, color. It gives pleasant voice, strong body and senses [10]. Hence, in this study Ashwagandha, Shatavari were used as systemic Rasayanas whereas, Lakshadi guggulu and Shukti bhasma were used as Asthi Dhatu specific Rasayanas in group A. In group B, tablet Shelcal-500mg was used as standard control.

Recent researches have suggested that Ashwagandha is anti-inflammatory, anti-rheumatic and anti-arthritis and hence useful in painful conditions [11]. It is also an analgesic which helps to relieve pain associated with osteodystrophic disorders and osteoarthritis [12,13]. Asthi shrinkhala (*Cissus quadrangularis*) which is one of the ingredients of Lakshadi guggulu has a proven analgesic effect comparable to aspirin or anti-inflammatory drugs like ibuprofen [14]. The anti-inflammatory features suggest that it acts by preventing the conversion of arachidonic acid to inflammatory prostaglandins [15]. Hence, Ayurvedic drugs were potent enough to overcome the pain compared to the control drug.

Ashwagandha and Shatavari are systemic Rasayanas and Lakshadi guggulu and Shukti bhasma may be considered as Asthi Dhatu specific Rasayana (Naimittika Rasayana). Hence, they nourish the Dhatu. When Asthi Dhatu is nourished and is brought back to normalcy, simultaneously its mala, i.e. kesha and nakha are nourished and hence the hair roots may become strong. This may reduce hair fall and brittleness of hair. Similarly the brittleness of the nails is reduced and they become strong.

Scientific studies have proved that Ashwagandha, Shatavari and Nagabala are having anabolic and tonic actions on the human body. Shatavari is adaptogenic and immune-stimulator [16] and is indicated in general debility [17].

Lakshadi guggulu is indicated in Asthi bhagna and is said to make the body as strong as vajra (diamond). Shukti is the samana guna bhuyishtha dravya of Asthi.

Recent researches have shown the anti-osteoporotic effect of Ashwagandha and Asthisrinkhala. A study conducted by Nagareddy *et al.* in 2006 showed potent anti-osteoporotic activity of Ashwagandha in ovariectomized rats [18].

Treatment with Ashwagandha root extract which is known to contain estrogen like withanolides, particularly withaferin-A significantly prevented net bone loss. It is possible that the presence of a large number of withanolides, particularly withaferin A, an estrogen-like compound, may have contributed to anti-resorptive activity (Mishra *et al* 2000). Treatment with Ashwagandha appeared to maintain normal integrity, structure and compactness of the bone.

The analysis of Shukti bhasma in our pharmaceutical chemistry laboratory revealed that it contains 36.73% of elemental calcium. *Terminalia arjuna* is also the richest source of natural calcium, the bio-availability of which may be more. Since the purification of guggulu was done with cow's milk, Lakshadi guggulu also contained 6.18% of elemental calcium. Hence by all these facts the drugs of group A by their holistic approach might have increased the BMD in the patients of Asthi Kshaya. However, the effect of therapy on BMD was not statistically significant in subgroups B1, A2, B2 and A3.

There were no significant changes in the serum calcium levels after treatment. But, in serum alkaline phosphatase levels there was a significant increase in group A. ALP is biomarker of bone resorption. Its levels are high during increased bone resorption. But, after treatment even when there was increase in BMD, the reason for its increase is unknown.

In group A, 2 patients complained of nausea and vomiting by taking Ashwagandha may be because of its bitter taste and typical odour. The other unwanted effect was weight gain in previously over weight /obese patients. This may be because of continuous use of Ashwagandha and Shatavari for 4 months. But, it was a positive effect for those patients who had low Body Mass Index (BMI  $< 19 \text{ kg/m}^2$ ). In group B, 43.67% of the patients complained of flatulence and epigastric distress. Majority of the patients felt gastritis, if the Shelcal tablets were taken on empty stomach. Constipation was another adverse effect noted in 36.68% patients of group B.

## Conclusions

The latest signs and symptoms of osteopenia/osteoporosis are almost similar to those of Asthi kshaya. Hence, in the present study osteopenia / osteoporosis was vis-à-vis correlated with Asthi kshaya. By the effect of therapy obtained in the control group B, it is clear that only calcium and vitamin D<sub>3</sub> supplementation is not the complete treatment for osteopenia/osteoporosis.

Management of Asthi kshaya requires a holistic approach, taking into account the nutritional, metabolic and hormonal aspects, which is fulfilled by Ayurvedic Rasayana therapy. The Ayurvedic herbo-mineral Rasayana drugs used in the present study provided significant relief from signs and symptoms like Kati, Asthi and Sandhi shula, Kesha pata, Danta vikara /pata, Nakha vikara and Daurbalya. The results on X-ray (Singh's index) and BMD eventhough were encouraging but were not significant statistically. The antique therapeutic potentials of Ayurvedic Rasayana holds good even today in the management degenerative disorders related to ageing.

## References

1. Sushruta, Sushruta Samhita, Chaukhamba Orientalia, Varanasi, 4th edition, India, Sutrasthana, 1980, 15/3.
2. Agnivesha, Charaka Samhita, Chaukhamba Sanskrit Sansthan, Varanasi, Fifth edition, India Sutrasthana, 2001, 9/4.
3. Agnivesha, Charaka Samhita, Chaukhamba Sanskrit Sansthan, Varanasi, Fifth edition, India, Cakrapani on Sutrasthana, 2001, 9/4.
4. WHO Scientific Group on the Prevention and Management of Osteoporosis (2000: Geneva, Switzerland) (2003). "Prevention and management of osteoporosis: report of a WHO scientific group" (pdf). [http://whqlibdoc.who.int/trs/WHO\\_TRS\\_921.pdf](http://whqlibdoc.who.int/trs/WHO_TRS_921.pdf). Retrieved 31-05-2007.
5. Melton LJ, Atkinson EJ, O'Connor MK, et al. Bone density and fracture risk in men. *J Bone Miner Res* 1998; **13**: 1915.
6. Melton LJ, Chrischilles EA, Cooper C, et al. Perspective – how many women have osteoporosis? *J Bone Miner Res* 1992; **7**: 1005.
7. Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. *Osteoporos Int* 2006; **17** (12): 1726-33.
8. Action Plan Osteoporosis. Consensus Statement of an Expert Group. Osteoporosis Society of India, New Delhi, 2003.
9. Kadlimatti Sanjay, Chandola HM, Maheshwari KS. Critical analysis of the concept of Asthi Kshaya vis-à-vis osteoporosis. *AYU* 2009; **30**: 447-58.
10. Agnivesha Charaka Samhita, Fifth edition, Chaukhamba Sanskrit Sansthan, Varanasi, India, 2001, Chikitsa sthana 1-1/7-8.
11. Mishra LC, Singh BB, Dagenais S. Scientific basis for the therapeutic use of *Withania somnifera* (Ashwagandha): a review. *Altern Med Rev* 2000; **5**: 334-46.
12. Nadakarni KM. Indian Materia Medica, 3rd edition, Popular Prakashan, Mumbai, India, 1996, p. 1198.
13. Kulkarni R et al. Treatment of osteoarthritis with a herbomineral formulation: a double-blind, placebo-controlled, cross-over study. *J. Ethnopharmacol* 1991; **33** (1-2): 91-5.
14. Panda. *J Res Ayurv Siddha* 1990; **11**: 7.
15. Hoffman Karl (2009), *Cissus quadrangularis* [http://www.cuttingedgemuscle.com/Forum/art\\_showarticle.15.\(01.02.2009\).](http://www.cuttingedgemuscle.com/Forum/art_showarticle.15.(01.02.2009).)
16. Rege NN, Thatte UM, Dahanukar SA. Adaptogenic properties of six rasayana herbs used in Ayurvedic Medicine. *Phytother Res* 1999; **13**: 275-91.
17. Michael Thompson, Shatavari – Asparagus recemosus monograph, 2009, <http://www.phytomedicine.com/> (22.04.2009).
18. Nagareddy PR, Lakshmana M. *Withania somnifera* improves bone calcification in calcium deficient ovariectomized rats. *J Pharm Pharmacol* 2006; **58** (4): 513-9.

# Scientific studies of a popular Sri Lanka indigenous therapeutic agent “Rathakalka” used in paediatric practice

A P G Amarasinghe<sup>1</sup>

## Abstract

The test drug Rathakalka, selected for these studies, is a popular Sri Lankan indigenous recipe specially used for children. A clinical study of Rathakalka recipe revealed significant changes in serum Immunoglobulins (IgG, IgM and IgA) and serum complements (C3 and C4) levels in infants and young children. Animal experiment with albino rats showed its highest anti-inflammatory activity 3 hours after induction of edema. In-vivo experiment demonstrated that Rathakalka reduced yeast induced elevation of the body temperature in rats. In-vitro experiment revealed that the recipe has anti-bacterial effect on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Listeria monocytogenes*. In-vivo experiment showed that the prolonged administration does not produce any toxicity changes in rabbits. Microbiological study indicated that the microbial colony counts observed in this study were within the limits acceptable by the World Health Assembly (WHA). These results scientifically evaluate that the drug samples are tested and deemed microbiologically safe and up to the microbial quality standards. These studies confirmed the presence of immune enhancing effect, anti-bacterial effect, anti-pyretic effect, anti-inflammatory effect, non toxicity, and microbiological safety in Rathakalka.

## Introduction

In Sri Lanka the indigenous system of medicine has been practiced successfully since several centuries. The test drug undertaken for this study is a popular compound of Sri Lankan indigenous medicine prescribed routinely for infants by traditional physicians. It is found in Watikaprakaranaya [1] a compilation of indigenous medicine in Sri Lankan in the name of *Desadunkalka*. Another indigenous medical text called Vaidyaka Tatvadarshana [2] also carries reference of the same drug by the name of *Dvichandna kalka*. It has been included in Ayurvedic Pharmacopeia [3] published by the government of Sri Lanka in the recent past known as *Desadunkalka or Rathakalka*.

The name Desadum kalka or Dvichandna kalka implies the presence of two kinds of chandana (sandal) viz. Swetha chandna (sandalwood) and raktha chandana (red sandalwood) in the ingredients. According to the indigenous medical texts, this particular drug has been

used for two main purposes, one to use it as a preventive medicament to protect infants from rakhaja roga, diseases caused by vitiated blood and the other to use it as a curative measure for fever, inflammation, respiratory diseases and some skin disease conditions.

Rakhja roga also have been named as Rathagaaya in indigenous medical system in Sri Lanka [4]. This very drug have been known as “Rathakalka” as it is used for Rathagaaya.

The ingredients of Rathakalka are:

1. *Dolichos biflorus* – Kollu (S), Horse gram (E)
2. *Glycyrrhiza glabra* – Welmee (S), Licorice (E)
3. *Acorus calamus* – Wadakaha (S), Calamus (E)
4. *Pterocarpus santalinus* – Rathandun (S), Red sandalwood (E)
5. *Santalum album* – Suduhandun (S), Sandalwood (E)
6. Rock salt – Sahind lunu (S)  
(S – Sinhala, E – English)

Fine powder of all above herbal ingredients in equal parts and rock salt five parts are taken. After mixing, these are ground with lime (*Citrus medica*) juice to make it into form of kalka (paste).

Although this recipe Rathakalka has been used in Sri Lankan traditional medicine for many centuries, claims regarding its properties were mainly anecdotal and had not been subjected to any type of scientific confirmation. This is not only the first controlled study carried out with Rathakalka, but also pioneering study in Sri Lanka, of indigenous recipe used in traditional medicine for treatment of various disease conditions in infants and young children.

## Effect of oral Rathakalka on immunoglobulin and complement levels in neonates

One of the main uses of this recipe is as a preventive medicament against various disease conditions. Therefore, it was hypothesized that the recipe may help in stimulating the immune system.

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Randomized samples of neonates were used. Test group (n=20) was given Rathakalka 250 mg for two months and control group was on placebo for two months. Serum immunoglobulins (IgG, IgA and IgM) and complements (C3 and C4) assessed in neonates from cord blood and later at the end of two months follow up by Single Radial Immunodiffusion technique. Pre-treatment levels of immunoglobulins and complements were compared with the post treatment levels.

**Table 1: Effect of Rathakalka on immunoglobulins and complements**

Immunoglobulin and complements	Control mg/dl	Treated mg/dl
Ig G	470	765**
Ig A	27.2	45.2**
Ig M	42.5	68.5**
C3	77.1	91.3*
C4	12.2	15.8*

\*\* p<0.001

\* p<0.01

The recipe of Rathakalka revealed significant changes in serum IgG levels in the treated group. Normally serum IgG levels decrease with the increase in age during infancy. These levels have decreased in both the groups but the physiological decrease of IgG was found less in the treated group.

Post-treatment IgA and IgM levels increased significantly (p<0.001) in the treated group. Serum C3 and C4 levels were also found enhanced significantly (p<0.01) in treated group[5].

#### Anti-inflammatory effect of Rathakalka

Although this recipe is being used as an anti inflammatory agent for ages, till now no scientific study has been reported. The objective of this study was to expose the recipe to animal experimentation to evaluate its anti-inflammatory effects.

This experiment was carried out using Sprague Dawley albino rats (250 - 275g). Three groups were used (n=6/group). The rats were orally administered with Rathakalka – 312.5 mg/animal in 200 ml of distilled water (treated), Ibuprofen – 21.4 mg/animal in 2 ml of distilled water (reference) and 2 ml of distilled water (control) 1 hr before induction of paw edema.

Paw edema was induced in rats using 0.05ml of 1%

carageenan (s/c). The volume of hind paw was determined by the water displacement technique.

The volume of displaced water was considered equivalent to the volume of the left hind paw. The paw volume measurements were taken before and after (at 1, 2, 3, 4, 5, 24 hours) carageenan injection.

The edema at each time was calculated in relation to the volume of hind paw before carageenan injection.

**Table 2: Anti inflammatory effect (edema inhibition ratio) of Rathakalka**

Interval after carageenan injection	Anti inflammatory effect /edema inhibition ratio	
	Rathakalka	Ibuprofen
01 hour	46.58	36.79
02 hours	42.18	57.53
03 hours	56.84*	59.84
04 hours	42.47	61.40**
05 hours	32.89	64.17
24 hours	33.51	15.02

\* p<0.05

\*\*p<0.01

Rathakalka showed its highest anti-inflammatory activity at 3 hrs after induction of edema (p<0.05, students' t-test) while Ibuprofen elicited its maximum activity at 5 hrs after carageenan treatment (p<0.01) [6].

#### Anti-pyretic effect of Rathakalka

The present study was undertaken to evaluate the antipyretic effects of Rakthakalka on yeast-induced fever in Wistar strain albino rats. Animals were randomly divided in to five groups of six each. After measuring the basal rectal temperature, animals were injected subcutaneously with 10 ml/kg body weight of 15% w/v yeast, suspended in 0.5% w/v methylcellulose solution.

Rathakalka was administrated orally 19 hours after yeast injection, at the dose of 100, 200 and 300 mg/kg body weight to 3 groups of animals respectively.

Distilled water (2 ml/animal) administrated orally to the control group. The fifth group of rats received standard drug, Paracetamol 150 mg/kg body weight orally.

The subcutaneous injection of yeast suspension markedly elevated the rectal temperature at the 19th hour after administration. Treatment with Rathakalka at the dose of 100, 200 and 300 mg/kg body weight decreased the rectal temperature of the rats in dose dependent manner.

**Table 3: Anti-pyretic effect of Rathakalka**

Treatment	Rectal temperature (°C) before and after yeast injection at				
	0 h	19 hrs	20 hrs	21 hrs	22 hrs
Control 2ml dw/animal	37.3	39.6	39.6	39.5	39.5
Paracetamol 150mg/kg <sup>-1</sup>	37.2	39.7	38.3*	38.3*	37.7*
Rathakalka 100 mg/kg <sup>-1</sup>	37.3	39.6	38.7*	38.3*	38.2*
Rathakalka 200 mg/kg <sup>-1</sup>	37.4	39.8	38.8*	38.3*	38.2*
Rathakalka 300 mg/kg <sup>-1</sup>	37.1	39.6	38.6*	38.2*	38.0*

\* p < 0.01, as compared to control values for the corresponding hour

The anti-pyretic effect started as early as one hour after drug administration, and effect was maintained for 4 hours. The standard drug Paracetamol at the dose of 150 mg/kg body weight reduced the yeast-induced elevation of body temperature.

The results obtained from both, standard drug Paracetamol and Rathakalka treated group were compared with the control group. A significant reduction (p<0.01) in the yeast-elevated rectal temperature was observed.

Rathakalka has the capacity to reduce yeast induced elevation of the body temperature in rats. Results of this study may justify more scientific testing of the use of Rathakalka as an anti-pyretic agent in humans [7].

#### Anti-bacterial effects of Rathakalka

This particular drug is being used as a curative measure for fever, some skin diseases and respiratory disorders. One of the main causes for above disease conditions is bacterial infection.

Although individual studies of some of the ingredients in Rathakalka have been reported to have anti bacterial activity, the whole compound itself has not yet been proved to have this effect. Based on this foundation it was hypothesized that Rathakalka may have antibacterial effect as whole.

Hence the objective of this study was to evaluate the antibacterial effect of Rathakalka.

0.1gm of Rathakalka was dissolved in 5 ml of sterile distilled water and filter sterilized using Hemmings filter. This sterile soluble extract of Rakthakalka was used in this study. Anti-bacterial assay was performed using cylinder plate method in nutrient agar and incubated for 48 hours at room temperature. Bacterial species used were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Listeria monoytogenes*.

**Table 4: Anti-bacterial effects of Rathakalka**

Bacterial species	Diameter of inhibition zones
<i>Staphylococcus aureus</i>	2.40 cm
<i>Pseudomonas aeruginosa</i>	1.75 cm
<i>Listeria monocytogenes</i>	1.40 cm
<i>Eschichia coli</i>	-

A clear inhibition zone of bacterial lawns were observed repeatedly in all plates except *E. coli* indicating that the water extract of Rathakalka has anti-bacterial effect on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Listeria monocytogenes* [8].

#### Toxicity study

In view of extensive use of Rathakalka in neonates over a long period it is argued that toxicity test in animals are superfluous. On the other hand there is growing awareness among scientists and general public of the ill effects of drugs. The objective of this study was to expose the recipe of Rathakalka to animal experimentation to determine the presence of toxic effects if any.

Thirty Belgium strain white rabbits (weight 900-1000 g) were randomized into three groups of ten, each group comprising an equal number of male and female rabbits. One group served as control and received 15ml/Kg body weight distilled water and the other two groups received Rathakalka at a dose of 1 gm and 3 gm/Kg body weight respectively for 60 days. The animals were observed for any signs of toxicity throughout the period of the experiment. On day 61 blood and urine samples collected and the animals were sacrificed and observed for any macroscopical changes and histo-pathological examination of target organs.

No mortality was observed during the course of experiment. Urinalysis and haematological parameters were within the normal range. No observable gross abnormalities that could be attributed to drug toxicity were noticed in the treated group. Histo-pathological examination of the target organs revealed total normalcy. The study concluded that the recipe of Rathakalka does not show any evidence of toxicity when tested on rabbits. It is probable that the same absence of toxicity would be observed when Rathakalka is used as a medication in infants and children [9].

#### **Total viable count and specific micro organisms in Rathakalka**

Basic ingredients of Rathakalka are mainly plant origin. All such materials contain a natural inherent microbial flora and also may contain added contaminant during processing, preparation, and storage.

Considering these facts the World Health Assembly in its resolutions WHA - 31:33, 40:33, and 42:43 has emphasized the need to ensure the microbial quality standard of medicinal plant products by using modern techniques and applying suitable standards [10].

The main objective of this study was to enumerate the total viable count of bacteria and the specific micro organisms such as *E-coli* and *Salmonella*.

Fifteen market samples of different manufactures were studied. Three different samples of each manufacturers with different manufacturing dates were selected for the study. Nutrient agar and potato dextrose agar were used as common culture media for bacteria and fungi.

Routine sterilization process was followed to sterile the culture media and glass ware. 1.0 gram of Rathakalka was dissolved in 10 ml of sterile distilled water and three dilutions of  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$  were made using this solution. 0.1 ml of this solution was used to study the microbial load. Pour plate technique and spread plate technique were used on nutrient agar and potato dextrose agar respectively. Microbial count on nutrient agar and potato dextrose agar were taken after 24 hours and 72 hours. It was assumed that each colony was formed by a single organism. Coliform test was done with single strength MacConkey broth by using probable number technique.

*Salmonella* was performed according to the international standard. The same procedures were repeated three times to confirm the colony count and the specific micro organisms.

The results obtained in this study indicate the presence of bacteria and fungi in this preparation. None of the drug samples was positive for Coliforms or *Salmonella*. According to the limits adapted from the provisional guide lines established by the World Health

Assembly, the microbial colony counts observed in this study were within the limits acceptable by the W.H.A. These results were statistically analyzed by using t-test.

Mean of the colony count is not significant at 0.05. There is no difference of standard mean of the colony count and sample colony count. These results scientifically evaluate that the drug samples tested microbiologically safe and up to the microbial quality standard [11].

#### **Comments**

These studies have been conclusively demonstrated for the first time in Sri Lanka, the presence of immune enhancing effect, anti-bacterial effect, anti-pyretic effect, anti-inflammatory effect, non toxicity, and microbiological safety in an indigenous recipe Rathakalka, and thus help to establish the value of traditional medicines in the therapy of infantile diseases.

Sri Lankan traditional physicians have used this recipe of Rathakalka on the basis of their own indigenous knowledge and clinical experience but not on the findings of any scientific research. Therefore, the results of these preliminary studies justify scientifically the use of indigenous recipe of Rathakalka in infants and young children by traditional Ayurvedic practitioners in Sri Lanka during the last few centuries.

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#### **References**

1. De Alwis ES Aylapperuma, Watika Prakaranaya. 1st Edition, Granthaprakasana Press, Colombo, 1877.
2. Kavitillaka BLS, Vaidyaka Tathva Dharshana, 1932.
3. Ayurveda Pharmacopeia, Vol. I, Part I, Department of Ayurveda, Sri Lanka, 1970.
4. Don Wilion. Ratha Veda Potha, 1902 (reprinted by the Department of Ayurveda, 1977).
5. Amarasinghe APG, Sharma RD, Singh Usha. Effect of oral Rathakalka on immunoglobulin and complement levels in neonate. Proceedings of the 55th Annual Session of the Sri Lanka Association for the Advancement of Science 1999. p.10.
6. Amarasinghe APG, Mendis MGU. Preliminary study of anti-inflammatory effect of Ayurvedic recipe Rathakalka. Proceedings of the 56th Annual Session of the Sri Lanka Association for the Advancement of Science, 2000, p. 42.

7. Amarasinghe APG, Nageeb BM. Antipyretic effect of an Ayurvedic recipe Rathakalka. Proceedings of the 59th Annual Session of the Sri Lanka Association for the Advancement of Science, 2003, p. 1.
8. Amarasinghe APG, Widanapathirana S. Preliminary study of anti-bacterial effect of an Ayurvedic recipe Rathakalka. *Indian Journal of Research in Ayurveda and Siddha*, 2005; **4**: 1-5.
9. Amarasinghe APG, Arya NC. Toxicity study of an Ayurvedic recipe Rathakalka. Proceedings of the 56th Annual Session of the Sri Lanka Association for the Advancement of Science, 2000, p. 35.
10. World Health Organization, Geneva, Quality Control Methods for Medicinal Plant Materials, WHO Library cataloguing in publication data, ISBN: 942, 1988.
11. Nageeb BM, Amarasinghe APG, Widanapathirana S. A study of total viable count of microorganisms and specific micro-organisms in the compound preparation of Rathakalka, *Ayurveda Sameekshava* 2010: **II/VI**, 205-8.

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