ĀRYAVAIDYAN

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Of all the gifts,
the most precious is health

Vaidyaratnam P.S. Varier's
Arya Vaidya Sala, Kottakkal, Kerala

Aryavaidyan, the quarterly journal of Arya Vaidya Sala, Kottakkal, is intended to encourage scientific writing and intellectual interactions among scholars, academicians, practitioners and students of ayurveda and allied

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VAIDYARATNAM P.S.VARIER'S
ARYA VAIDYA SALA, KOTTAKKAL

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PLANT STUDY

Āryavaidyan, Vol. XXXII, No. 3, February - April 2019, Pages 05 - 10

Anatomical and antihyperglycemic activity of *Abutilon indicum* L. Sweet (Atibalā) fruits

Rajesh Bolleddu, Sama Venkatesh, Rao M. M. and Rachamalla Shyamsunder

ABSTRACT: The present study was aimed to establish the antihyperglycemic properties of *Abutilon indicum* fruit by screening various fractions of hydro alcoholic extract of fruits. Anatomical and morphological parameters were studied as per Ayurvedic Pharmacopoeia. Antihyperglycemic studies were carried out by Glucose tolerance test. Toxicity studies were performed as per OECD 425 guidelines and all extracts were observed to be safe. Ethyl acetate fraction has shown significantly ($p<0.01$) highest hypoglycemic activity (34.83%) at a dose of 400mg/kg against glucose load of 2gm/kg, followed by ethanolic extract. Transverse section of fresh fruit showed the presence of glandular trichomes, stellate hairs and group of lignified palisade cells. The current anatomical studies can be considered as reference standard for future studies on *Abutilon indicum* fruits. The observed significant antidiabetic activity of fruits is an evidence for further studies.

Key words: *Abutilon indicum*, Transverse section, Anatomy, Fractionation, Antihyperglycemic activity

Introduction

Abutilon indicum (L.) Sweet belongs to the family Malvaceae, is an important medicinal plant used in āyurveda and is commonly called as 'atibalā'. It bears characteristic schizocarpic fruits, golden yellow flowers and is found throughout India.[1] Its bark is recommended as febrifuge, anthelmintic and alexeteric. In āyurveda, it is known to remove the vitiated conditions of vāta and tridoṣa; allays thirst, vomiting and lessens perspiration.[2] Extracts from various parts of this plant has been reported to possess anticancer, anticonvulsant, antiasthmatic, anti-estrogenic, antimicrobial, hepato-protective, hypoglycemic, immunomodulatory, analgesic and wound healing properties.[3] Ethanolic extract of leaves have reported to possessing potent antibacterial[4], anti-diarrheal[5] and immunomodulatory activity[6]. Like all other parts, fruits and seeds also reported considerable medicinal uses. Traditionally, fruits were used to treat piles, gonorrhea, cough, haemorrhagic septicemia and seed powder was used as aphrodisiac and laxative.[7] Aqueous extract of seeds have been reported for its diuretic activity at 200 and 400mg/kg dose.[8] Seed oil has been reported for its antibacterial and antioxidant activities.[9] We have reported that the Ethyl acetate (86 mg GAE/g) and chloroform fraction (56 mg GAE/g) of fruits were found to contain high phenolic content and strong antioxidant potential.[10] Phenolic compounds are known for hypoglycemic properties and the leaves of *A. indicum* were reported to possess antihyperglycemic activity.[11] As the fruits are rich in phenolic content, an attempt was made to assess the antihyperglycemic property of ethanolic extract and its fractions. Anatomical studies of fruits were performed for identification.

Materials and methodes

Collection of plant material: The fresh fruits of *A. indicum* were collected from Suryapet, Telangana, India. The plant authentication was done in Botanical Survey of India, Hyderabad. The voucher specimen (GPRCP/AI/BR12/2015) was maintained in the Department of Pharmacognosy, G. Pulla Reddy College of Pharmacy, Hyderabad. Fresh fruits were used for anatomical studies, shade dried fruits were used for hypoglycemic studies.

Anatomical studies: The free hand transverse sections of *A. indicum* fruits were first treated with chloral hydrate reagent, mounted in glycerine and observed for histological characters using compound microscope. Presence of lignified tissues were determined by treating the section with phloroglucinol and concentrated hydrochloric acid (1:1). Presence of starch was determined by treating with iodine solution.[1a, 12]

Preparation of ethanolic extract: The shade dried fruits powder (#60) was extracted with 80% ethyl alcohol by maceration for eight days. The percentage yield of aqueous ethanolic extract was 6.5 % w/w .[13]

Fractionation of the mother extract: To the 100g of concentrated aqueous ethanolic extract, 500ml of distilled water was added and fractioned with petroleum ether (4X500ml), chloroform (4X500ml), ethyl acetate (4X500ml) and butanol (4X500ml). The percentage yield of petroleum ether, chloroform, ethyl acetate, butanol and left over fractions were 3.6, 1.4, 1.2, 3.7 and 74% respectively.[14]

Toxicity studies: Acute toxicity studies were done according to OECD 425 guidelines. The test used a maximum of 5 animals. A test dose of 2000 mg/kg was used. First animal was dosed and observed continuously for the initial period of 2hrs, intermittently for next 6 hrs and then 24 hrs for death and abnormality in behavioral changes. The animal survived. Then next four animals were dosed sequentially. All the animals survived- LD50 was>2000mg/kg.[15]

Test animals: Male wistar rats used in experiment were maintained under standard environmental conditions of temperature, relative humidity, dark/ light cycles and free access to feed and water *ad libitum* during the quarantine period. The animals were fasted for 16 hrs before experimentation but had been allowed free access to water. All the extracts of *A. indicum* fruit were tested for glucose tolerance test at doses of 200 and 400 mg/kg and were administered orally as fine aqueous suspension using 0.5% w/v carboxy methyl cellulose(CMC), as vehicle.

Effect of *A. indicum* fruit extracts on glucose tolerance in rats: Overnight fasted rats were divided in 12 groups of 6 rats each. Group 1 was served as control, received vehicle. Group 2 to 11 received various CMC suspensions of *A. indicum* fruit extracts at an oral dose of 200 and 400 mg/kg. Group 12 received glibenclamide as standard at an oral dose of 10 mg/kg. After 30 min of extract administration, the rats of all groups were orally loaded with 2g/kg of glucose. Blood samples were collected from the retro orbital plexus just prior to glucose administration and at 30, 60 and 120 min after glucose loading. Plasma was separated and blood glucose levels were measured immediately by glucose-oxidase method.[16, 17, 18]

The percentage variation of glycaemia was calculated for each group using the formula:

Percent variation in glycaemia = Gi-Gt/Gi X 100

Where, Gi- value of initial glycemia (0 hour) and Gt- glycemia at 30, 60 and 120min respectively.

Statistical analysis: All the values were expressed as mean ± SEM. Results were analyzed statistically by using analysis of variance (ANOVA) followed by Dunnett's test. Values of $P<0.05$ were considered significant.

Results and discussion

Morphological characters: Fresh fruits of *A. indicum* are green in colour, shizocarpic, circular in shape, consisting of 11-16 radiating densely pubescent mericarp per fruit, each mericarp flattened, somewhat boat shaped, black when ripe 1-2 cm in diameter consist of 2-3 seeds. Seeds were reniform, hairy, dotted, minutely scrobiculate, brownish black in colour and 3-5mm diameter. Odour characteristic; taste bitter. Figure 1.

Figure 1

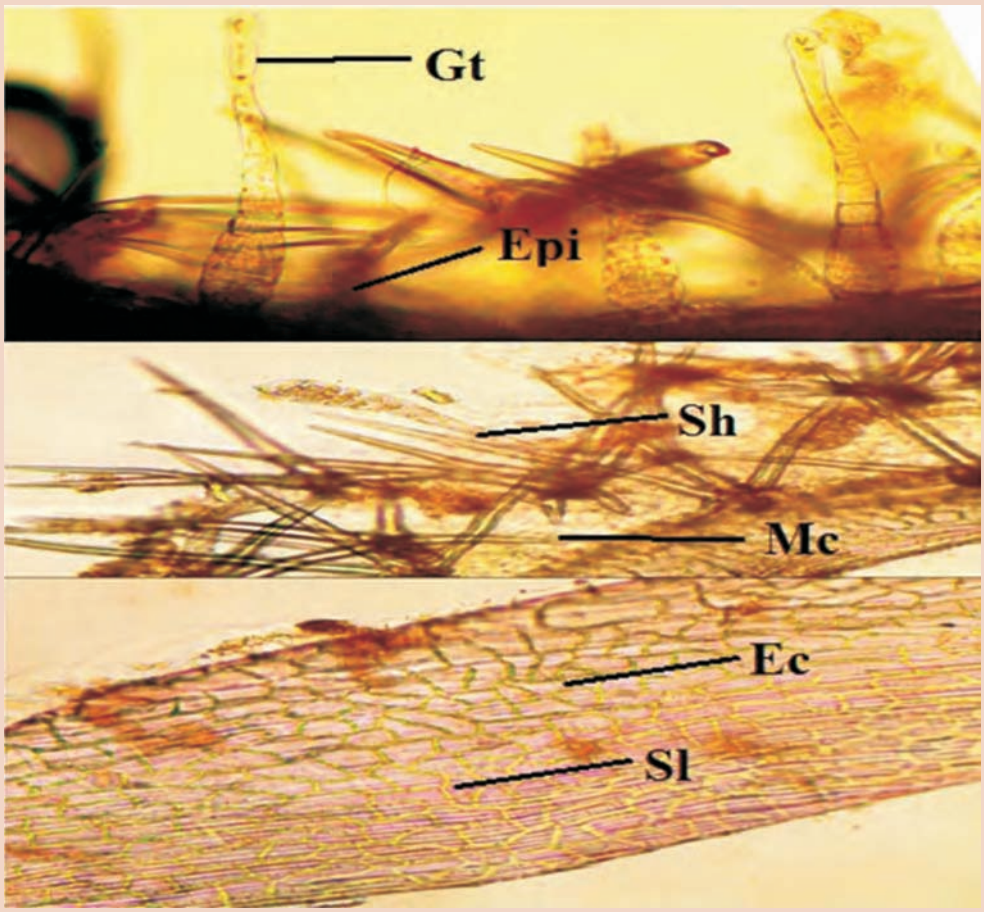
Abutilon indicum fruits (Fr) and seeds (Sd)

Anatomical characters: Fruit mericarp transverse section showed group of stellate hairs, glandular covering trichomes with multicellular uniseriate stalk with collapsed cells and unicellular globular head. Lignified irregular shaped endocarp cells, groups of fusiform lignified sclerenchymatous fibers running wavy crossing each other, presence of xylem vessels was also observed. Figure 2.

Transverse section of seed showed the presence of oval shaped cells of testa with lignified stroma and warty epidermis covered with cuticle. A narrow parenchymatous cell layer of hypodermis was located underneath this, followed by a layer of lignified palisade cells. Centrally located endosperm embedded with 'U' shaped embryo with their free

Figure 2

Transverse section of *Abutilon indicum* fruit mericarp



Gt- Glandular trichome; Epi- Epicarp; Sh- Stellate hair; Mc-Mesocarp; Ec- Endocarp; Sl-Sclerenchymatous layer

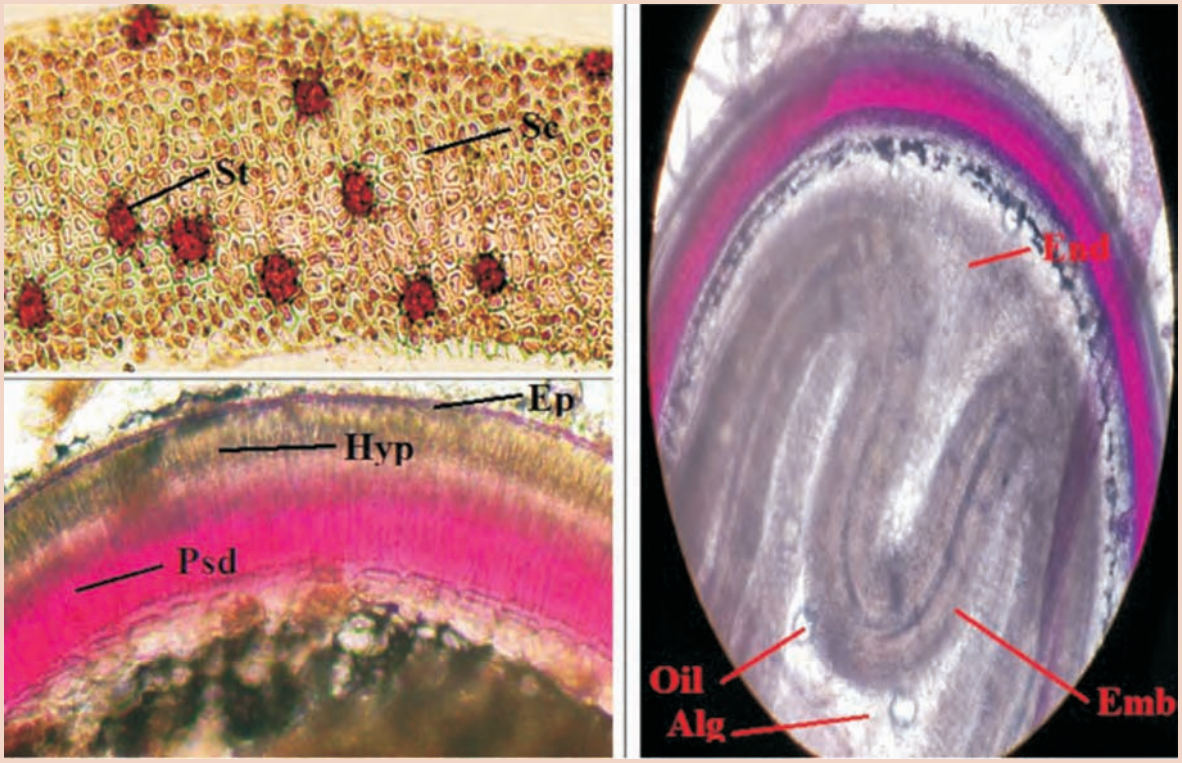
terminals pointing towards the hilar edge was noticed. Inside lumen was loaded with aleurone grains and few oil globules. Figure 3.

Toxicity studies

In oral acute toxicity studies, no mortality and abnormal behavioral changes observed in mice up to a dose of 2 g/kg body weight. All extracts of *A. indicum* was considered to be safe and further antihyperglycemic activity was tested at an oral dose of 200 and 400 mg/kg body weight.

Figure 3

Transverse section of *Abutilon indicum* seed



Sc-Seed coat (Testa); St- Stroma; Ep- Epidermis; Hyp- Hypodermis; Psd-Palisade cells; End-Endosperm; Emb-Embryo; Oil-Oil globule; Alg- Aleurone grains

Effect of *A. indicum* fruit extracts on glucose tolerance in rats: The effect of *A. indicum* extracts in glucose tolerance test is given in Table 1. All extracts produced antihyperglycemic properties. However, the ethyl acetate and aqueous ethanolic extracts produced a significant ($p<0.01$) dose dependent activity when tested at 200 and 400 mg/kg dose. The animals that received 400 mg/kg showed maximum activity at 60 min after glucose administration. Ethyl acetate extract at a dose of 400 mg/kg produced maximum protection (34.83%) followed by aqueous ethanolic extract and butanolic extract at 60 min after glucose administration. The activity produced by the extracts are not comparable with standard glibenclamide activity at the time of experiment. The standard glibenclamide (10 mg/kg) produced a significant maximum protection at 60 min (60.29%). Left over aqueous extracts did not produce any considerable antihyperglycemic properties. The percentage protection of ethanolic, petroleum ether, chloroform, ethyl acetate and butanol extracts at 60 minutes was 26.98, 7.74, 22.75, 34.83, and 24.16 respectively, when tested at 400mg/kg dose.

Table 1
Effect of *A. indicum* fruit extracts on glucose tolerance in rat

Group	Treatment	Dose mg/kg	Fasting	30min	60min	120min
1.	Control	--	73.58±1.39	114.79±1.04	167.37±1.21	113.14±1.36
2.	Aq. Et. Ext.	200	65.70±1.4**	109.43±1.33* (4.66)	140.78±1.09** (15.88)	104.16±1.14** (7.93)
3.	Aq. Et. Ext.	400	71.55±1.25**	102.81±1.39** (10.43)	122.2±1.3** (26.98)	102.16±1.4** (9.7)
4.	Pet. Eth. Ext.	200	69.67±0.88**	110.95±1.36** (3.34)	158.34±1.02** (5.39)	109.47±0.99** (3.24)
5.	Pet. Eth. Ext.	400	67.56±1.37**	107.26±1.38 (6.55)	154.41±1.22** (7.74)	106.86±1.23** (5.55)
6.	Chloroform Ext.	200	72.08±0.81**	98.81±1.25** (13.92)	138.2±1.3** (17.42)	95.66±1.38** (15.44)
7.	Chloroform Ext.	400	72.68±1.36**	96.88±1.3** (15.6)	129.29±1.27** (22.75)	92.96±1.08** (17.83)
8.	Ethyl Ac. Ext.	200	70.11±0.95**	92.09±1.04** (19.78)	113.41±1.43** (31.44)	86.8±1.41** (23.28)
9.	Ethyl Ac. Ext.	400	72.38±0.87**	85.16±1.41** (25.81)	109.07±1.21** (34.83)	81.2±1.26** (28.23)
10.	Butanolic Ext.	200	69.11±1.37*	99.56±1.23** (13.26)	131.41±1.43** (21.48)	107.02±1.15** (5.4)
11.	Butanolic Ext.	400	68.83±1.43**	97.69±1.26** (14.89)	126.92±1.23** (24.16)	99.68±1.17** (11.89)
12.	Glibenclamide	10	70.15±0.21**	62.92±0.46** (45.18)	66.46±0.94** (60.29)	53.37±0.46** (52.82)

Aq. Et. Ext. - Acquous ethanolic extract; Pet. Eth. Ext. Petroleum ether extract; Ethyl Ac. Ext.- Ethyl acetate extract;

Conclusion

For the first time, antihyperglycemic properties of *Abutilon indicum* fruits were reported. Among all fractions ethyl acetate fraction (34.83%) and ethanolic extract (26.98%) showed the highest therapeutic activity at 400 mg/kg dose. This scientific evidence will help the researchers to explore further at higher animal models to develop a clinical candidate for the treatment of diabetes. The presence of stellate hairs and glandular covering trichomes are the diagnostic characters for identification of fruits. Whereas, the presence of lignified palisade cells and 'U' shaped embryo are important characters in anatomy of seeds. These anatomical studies can be considered as reference standards for future studies.

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ANALYTICAL STUDY

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A comparative pharmaceutico-analytical and *in vitro* anti-inflammatory study of aqueous and hydro-alcohol extracts of Rāsnāpañcaka kvāthaḥ

Praveen M. and Harshitha M.

ABSTRACT: Scarcity of raw materials is a major problem faced in āyurveda. Maximum extraction (herb-to-extract ratio) from the limited available raw materials without compromising the potency and minimize the extractive value in the residue is one of the solutions to surmount the problem. The extraction using 50% aqueous alcohol as solvent is being employed in this view. The fact that constituents that are insoluble in water may be soluble in alcohol and vice versa, may contribute to the enhanced potency, favors the industry for hydro-alcohol extraction. This has to be tested for each formulation. Potency and efficacy being important parameters, pre-clinical analysis of formulations includes pharmaceutical and *in vitro* tests besides the biological studies. HPTLC (High performance thin layer chromatography) is one of the methods to assess the potency of a drug pharmaceutically. Protein denaturation inhibition using bovine serum albumin is an *in vitro* method to assess the efficacy of a drug through its anti-inflammatory activity. Taken as the investigational product, Rāsnāpañcaka kvāthaḥ, the aqueous and the hydro-alcohol extracts of this formulation were tested and compared for its potency and anti-inflammatory efficacy by HPTLC and *in vitro* protein denaturation inhibition assay respectively. The results showed improved performance of hydro-alcohol extract of this formulation pharmaceutically as well as efficaciously.

Key words: Rasnāpañcaka kvāthaḥ, HPTLC, Protein denaturation inhibition assay

Introduction

Āyurveda, as a medical science, is evolved from the health needs of the society in the course of time. The evolution is visible in every field of āyurveda like preparation of medicines, development of new branches, approaches to diseases, etc. The very existence of āyurveda in the modern world as an independent and complete medical science is mainly due to its strong foundation in theories and principles and its adaptability to accept knowledge and ideas from contemporary sciences. Every science needs to be updated and revalidated frequently to keep pace with changing times for its existence. Āyurveda is no exception.

Perhaps, bhaiṣajyakalpana, pharmaceutics is one of the areas āyurveda has undergone tremendous change or development since its inception. The knowledge of conversion of raw materials into the desirable dosage forms is crucial in any medical science. Newer dosage forms evolved are brought into the practice to meet the health needs of the society. Preparations like avaleha, vaṭi, sneha and sandhānakalpana, etc. stands testimony to the efforts to make a drug more acceptable and make them available all seasons. Different dosage forms are introduced in the view of better extractive value, potency and shelf life besides better therapeutic efficacy and user friendliness.

Āyurveda is crucially dependant on the uninterrupted availability of raw materials of good quality in the right quantity. About 80% of the raw materials come from the vegetable kingdom both in fresh and dried forms. The demands for the herbal medicines are on the increase globally. This leads to severe scarcity of raw materials, especially of herbal origin. The need for their manufacture in bulk under industrialized and commercialized scenario has necessitated proper methodology for their scientific standardization.

Maximum extraction (herb-to-extract ratio) and thus minimize the extractive value in the residue is one of the solutions for the major problem of scarcity of raw materials in āyurveda. Increasing the yield of medicinal formulations to maximum from the available limited raw materials without compromising the potency is the need of the hour. Many newer techniques of extractions, such as solvent extraction, super critical extractions, etc. are being employed in the āyurveda medicine manufacturing industry now a days.

The Government of India has legalized the usage of 50% aqueous alcohol in the āyurveda medicine manufacturing industry for the extraction.[1] The fact that many constituents that are insoluble in water may be soluble in alcohol and vice versa, may enhance the potency of such extract. The advantages of hydro-alcoholic extracts compared to aqueous extract are many, namely, enhanced potency, more yield, minimum wastage, reduction in the usage of raw materials, reduction in bulk of dosage form and convenience in administering doses. But this advantage has to be ensured for each formulation by testing it phyto-chemically as the yield of extraction and potency varies from drug to drug and formulation to formulation.

Pre-clinical analysis of formulations includes pharmaceutical and *in vitro* studies besides biological studies. High performance thin layer chromatography (HPTLC) is one of the methods to assess a drug pharmaceutically. Protein denaturation inhibition using bovine serum albumin is an *in vitro* method to assess the efficacy of a drug through its anti-inflammatory activity.

Rāsnāpañcaka kvāthaḥ, mentioned in Cakradatta[2] is indicated in sāmavāta affecting sandhi, asthi and majja. Most of its ingredients possess an anti-inflammatory activity.

This study is an attempt to compare and analyze the potency and efficacy of both the aqueous and hydro-alcohol extracts of Rāsnāpañcaka kvāthaḥ yoga pharmaceutically and by *in vitro* method by protein denaturation inhibition for its anti-inflammatory activity.

Objectives

- To prepare aqueous and hydro-alcohol extracts of Rāsnāpañcaka kvāthaḥ.
- To carry out comparative pharmaceutico-analytical study of both the samples.
- To carry out comparative *in vitro* anti-inflammatory study by protein denaturation inhibition method.

Review of literature

Rāsnāpañcaka kvāthaḥ: The present study was based on Rāsnāpañcaka kvāthaḥ yoga. This formulation is mentioned in Cakradata in āmavātacikitsā. The classic treatises like Bhāvaprakāśaḥ[3], Yogaratnākaraḥ[4], Bhaiṣajya-ratnāvalī[5], Śāraṅgadharasamhitā[6] and the classical compendium of formulations in Malayalam, Sahasrayogam[7] also mention the formulation. The ingredients have the anti-inflammatory action. Tables 1 and 2 gives the basic information about the formulation.

Table 1
Rāsnāpañcaka kvāthaḥ ingredients, botanical identity and their ratio

Drug	Botanical name	Family	Part used	Quantity
Rāsnā	*Alpinia galanga* (L.)Willd.	Zingiberaceae	Rhizome	1 part
Guḍūcī	*Tinospora sinensis* (Lour.) Merr.	Menispermaceae	Stem	1 part
Eraṇḍa	*Ricinus communis* L.	Euphorbaceae	Root	1 part
Devadāru	*Cedrus deodara* (Roxb. ex. D. Don) G.Don	Pinaceae	Heart wood	1 part
Śuṇṭhī	*Zingiber officinale* Roscoe	Zingeberaceae	Rhizome	1 part

Table 2
Rasapañcaka of Rāsnāpañcaka kvāthaḥ as per Bhāvaprakāśa

Ingredients	Rasa	Guṇa	Vīrya	Vipāka	Karma
Rāsnā	Tikta	Guru	Uṣṇa	Kaṭu	Āmapācana
Guḍūcī	Tikta, kaṣāya	Guru, snigdha	Uṣṇa	Madhura	Āmapācana
Eraṅḍa	Madhura	Snigdha,tīkṣṇa	Uṣṇa	Madhura	Āmapācana
Devadāru	Tikta	Laghu, snigdha	Uṣṇa	Kaṭu	Āmapācana
Śuṇṭhī	Kaṭu	Laghu, snigdha	Uṣṇa	Madhura	Āmapācana

Protein denaturation inhibition assay: Denaturation of proteins is a well documented cause of inflammation. The bonding of a protein can easily be disrupted by a variety of physical and chemical agents as it is dependent on weak valence forces. The process is known as denaturation. The organized structure will lose and the protein assumes a highly disordered form. The protein will mislay its biological activity. Many globular proteins render less soluble on denaturation. Moist heat is a powerful denaturing agent. Extremes of acidity and alkalinity at moderate temperatures can disrupt the structure of proteins.

The compounds that inhibit the denaturation of proteins *in vitro* may be used as anti-inflammatory agents.[8] The anti-inflammatory effect of a sample can be performed by using bovine serum albumin (BSA). BSA assay seeks to eliminate the use of live specimens as far as possible in the drug development process. When BSA is heated, it undergoes denaturation (heat induced protein denaturation) and express antigens associated with type 3 hyper-sensitivity reactions, which can be related to the inflammation.

The anti-inflammatory efficacy of a drug can be expressed in terms of half maximal inhibition concentration (IC_{50}). It is a measure of the potency of a substance in inhibiting a specific biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganisms) by half. The values are typically expressed as molar concentration. The lower the IC_{50} value, the stronger the inhibitor is.

Materials and methods

For ease of understanding, the present work is categorized in three steps namely:

1. Pharmaceutical study
2. Analytical study
3. *In vitro* anti-inflammatory study

1. Pharmaceutical study: This involves the following steps;

a) Identification, collection and authentication of raw materials of the investigational product.

b) Preparation of aqueous and hydro-alcohol extracts of Rāsnāpañcaka kvāthaḥ yoga.

a) Collection and authentication of raw materials: Raw materials of Rāsnāpañcaka kvāthaḥ yoga were collected locally from Kottakkal, Kerala. The authentication of all the raw drugs was done at Center for Medicinal Plants Research Center, Arya Vaidya Sala, Kottakkal, Malappuram District, Kerala (CMPR).

b) Preparation of aqueous extract of Rāsnāpañcaka kvāthaḥ: Rāsnāpañcaka kvāthaḥ was prepared by conventional method. The washed and coarse powdered ingredients (200 grams each) were boiled under a controlled temperature in 16 liters of water to reduce to 1/8th (2 liters). The strained kvāthaḥ was then concentrated using mild heat till the liquid turns to paste or syrupy consistency. This was made into dry solid mass by dry heating it in a vacuum drier. The resultant dry powder, the aqueous

extract- was stored air tight for further analytical tests and *in vitro* study.

c) Preparation of hydro-alcohol extract of Rāsnāpañcaka kvāthaḥ: Hydro-alcohol extract of Rāsnāpañcaka kvāthaḥ yoga was prepared as per the guidelines of The Ayurveda Pharmacopoeia of India (API).[9] The washed and coarse powdered ingredients (200 grams each) were extracted with 3000 ml of 50% aqueous alcohol under reflux at a temperature between 80-85ºC for 3-4 hours using water bath. The extract was then strained. The marc was extracted three times more, filtering the extract each time into the same vessel. The combined filtrate was then concentrated using water bath to syrupy constituency. This was made into a dry mass by heating in a vacuum drier to eliminate the moisture content fully. The resultant dry powder, the hydro-alcohol extract- was stored air tight for further analytical tests and *in vitro* study.

2. Analytical study: The analytical evaluation of the samples was carried out at AYUSH accredited R. and D. Laboratory of Arya Vaidya Sala, Kottakkal, Malappuram, Kerala. The samples prepared were analyzed as per the guidelines of CCRAS.[10] The following analytical parameters were tested and tabulated.

- Organoleptic characters
- pH
- Loss on drying (LOD)
- Water soluble extractive
- Alcohol soluble extractive
- Ash value
- Acid insoluble ash
- HPTLC

3. *In vitro* anti-inflammatory study: The samples prepared were studied for their anti-inflammatory activities by protein denaturation inhibition capability, at Biogenix Research Center, using bovine serum albumin. Heat was used to denature the protein.

Diclofenac sodium was used as standard solution. Different concentrations of samples such as 62.5µg/ml, 125µg/ml, 250µg/ml and 500µg/ml prepared from a stock solution of 10 mg/ml were used for the study. The details of the solutions prepared are summarized in Table 3.

Table 3
Technical details of test solutions

Test Solution (0.5 ml): 0.05 ml of test solution + 0.45 ml of BSA (5% aqueous solution) • Test solution 1: 0.05 ml of aqueous extract (AE) + 0.45 ml of BSA • Test solution 2: 0.05 ml of hydro-alcoholic extract (HAE) + 0.45 ml of BSA
Test Control Solution (0.5 ml): 0.05 ml of distilled water + 0.45 ml of BSA
Product Control Solution : 0.05 ml of test solution + 0.45 ml distilled water. • Product control solution 1: 0.05 ml of test solution 1 + 0.45 ml of distilled water. • Product control solution 2: 0.05 ml of test solution 2 + 0.45 ml of distilled water.
Standard Solution (0.5 ml): 0.05 ml of diclofenac sodium + 0.45 ml of BSA.

All the solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37ºC for 20 minutes and the temperature was increased to 57ºC for 3 minutes. After cooling, 2.5 ml of phosphate buffer was added to the solutions. The absorbance was measured using UV-Visible Spectrophotometer at 416 nm.

The percentage inhibition of protein denaturation of each of the trial drug was calculated using the following formula.

Percentage of inhibition = 100 - [(OD_{TS} - OD_{PC}) ÷ (OD_{TC})] X 100.
OD, the optical density is measured by spectrophotometry.

The test was repeated thrice for the confirmation and the results were tabulated. The IC_{50} values of the investigational products were calculated using ED50 Plus V1.0 software and were tabulated.

Observations and results

The samples are represented by AE and HAE for aqueous and hydro-alcohol extracts of Rāsnāpañcaka kvāthaḥ yoga respectively.

The observed results of the physico-chemical tests are given in Table 4 and 5. The results of HPTLC are tabulated in Table 6 and 7 and in Figure 1 and 2. The observed values of *in vitro* anti-inflammatory activity of the samples by protein denaturation inhibition method are given in Table 8 and 9. The calculated IC_{50} values are enumerated in Table10.

Table 4
Organoleptic characters of AE and HAE

Characters/ parameters	AE	HAE
Physical appearance powder	Coarse powder	Coarse
Colour	Dark brown	Dark brown
Odour	Smell of ginger	Smell of ginger
Taste	Kaṣāya, kaṭu	Kaṣāya, kaṭu
Touch	Rigid	Rigid, sticky

Table 5
Observed values of physico-chemical analysis

No.	Parameters	Unit	AE	HAE
1.	LOD	% w/w	2.96	1.26
2.	pH of 10% solution of content	-	6.8	5.5
3.	Water soluble extractive	% w/w	67.51	47.99
4.	Alcohol soluble extractive	% w/w	16.28	43.58
5.	Total ash	% w/w	29.07	12.44
6.	Acid insoluble ash	% w/w	BDL	BDL

Table 6
Area and Peaks of AE and HAE at 254 nm

Peak	AE			HAE		
	Rf value	Area (AU)	% Area (AU)	Rf value	Area (AU)	% Area (AU)
1.	0.06	204.4	0.71	0.05	302.3	0.55
2.	0.15	4787.5	16.63	0.14	5072.0	9.18
3.	0.25	1435.7	4.99	0.24	768.9	1.39
4.	0.27	259.4	0.90	0.27	865.4	1.57
5.	0.32	4826.5	16.76	0.32	3246.8	5.88
6.	0.36	785.0	2.73	0.37	1135.9	2.06
7.	0.43	320.6	1.11	0.43	835.2	1.51
8.	0.48	3097.8	10.76	0.49	1484.3	2.68
9.	0.61	12148.7	42.20	0.54	2463.6	4.46
10.	0.77	925.1	3.21	0.65	33711.9	61.01
11				0.76	5366.9	9.71

Table 7
Summary of peaks and area under the curve

	AE	HAE
Total peak no.	10	11
Total area in AU	28790.7	55253.2

Table 8
Observed values of OD of Diclofenac sodium standard in spectrometry

Concentrations (µg/mL)	OD of TS	OD of PC	% of inhibition
Triplicate I: Absorbance of test control: 0.0243			
62.5	0.0257	0.0064	20.58
125	0.0468	0.0370	59.67
250	0.0878	0.0811	72.43
500	0.1266	0.1245	91.36
Triplicate II: Absorbance of test control: 0.0252			
62.5	0.0263	0.0070	23.41
125	0.0488	0.0384	58.73
250	0.0872	0.0821	79.76
500	0.1253	0.1237	93.65
Triplicate III: Absorbance of test control: 0.0268			
62.5	0.0307	0.0101	23.13
125	0.0498	0.0376	54.48
250	0.0911	0.0832	70.52
500	0.128	0.1262	93.28

Table 10
IC_{50} values calculated

Investigational product	IC50
AE	312.229µg/mL
HAE	285.209µg/mL
Diclofenac sodium	109 µg/mL

Table 9
Observed values of OD of test drugs AE and HAE in spectrometry

Concentrations (µg/mL)	OD of TS	OD of PC	% of inhibition
Triplicate I: Absorbance of test control: 0.0267			
Sample code: AE			
62.5	0.0281	0.0072	21.72
125	0.0472	0.0299	35.21
250	0.0829	0.0687	46.82
500	0.1017	0.0925	65.54
Sample code: HAE			
62.5	0.0468	0.0234	12.36
125	0.0642	0.0443	25.47
250	0.0921	0.0800	54.68
500	0.1121	0.1052	74.16
Triplicate II: Absorbance of test control: 0.0289			
Sample code: AE			
62.5	0.0301	0.0085	25.26
125	0.0520	0.0329	33.91
250	0.0792	0.0632	44.64
500	0.0993	0.0889	64.01
Sample code: HAE			
62.5	0.0521	0.0274	14.53
125	0.0699	0.0493	28.72
250	0.0984	0.0880	64.01
500	0.1218	0.1146	75.09
Triplicate III: Absorbance of test control: 0.0282			
Sample code: AE			
62.5	0.0296	0.0118	29.92
125	0.0486	0.0321	35.04
250	0.0818	0.0687	48.43
500	0.0973	0.0889	66.93
Sample code: HAE			
62.5	0.0558	0.0329	9.84
125	0.0846	0.0662	27.56
250	0.0987	0.0871	54.33
500	0.1255	0.1190	74.41

Figure 1

Overview graph of samples at various wave lengths

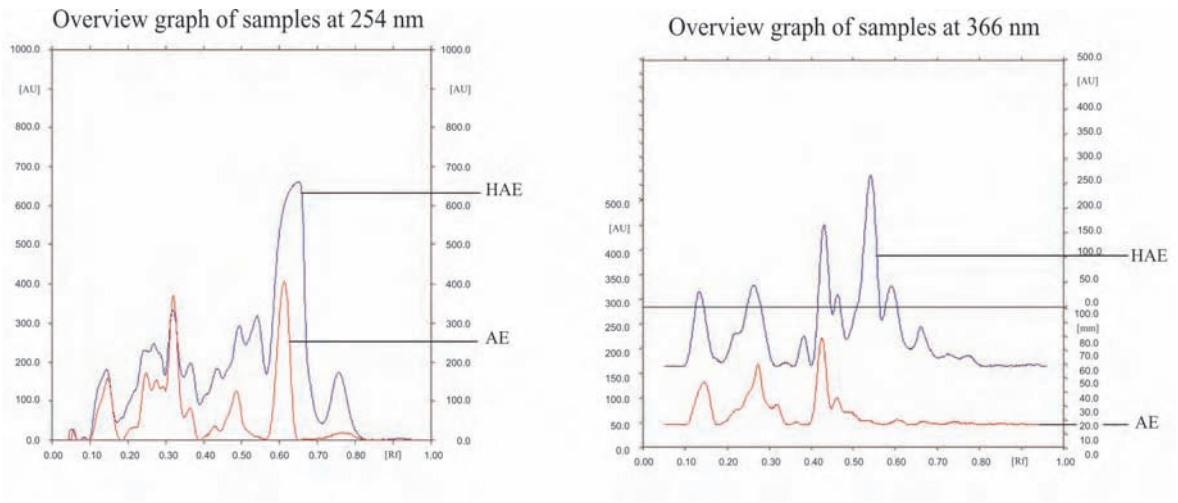
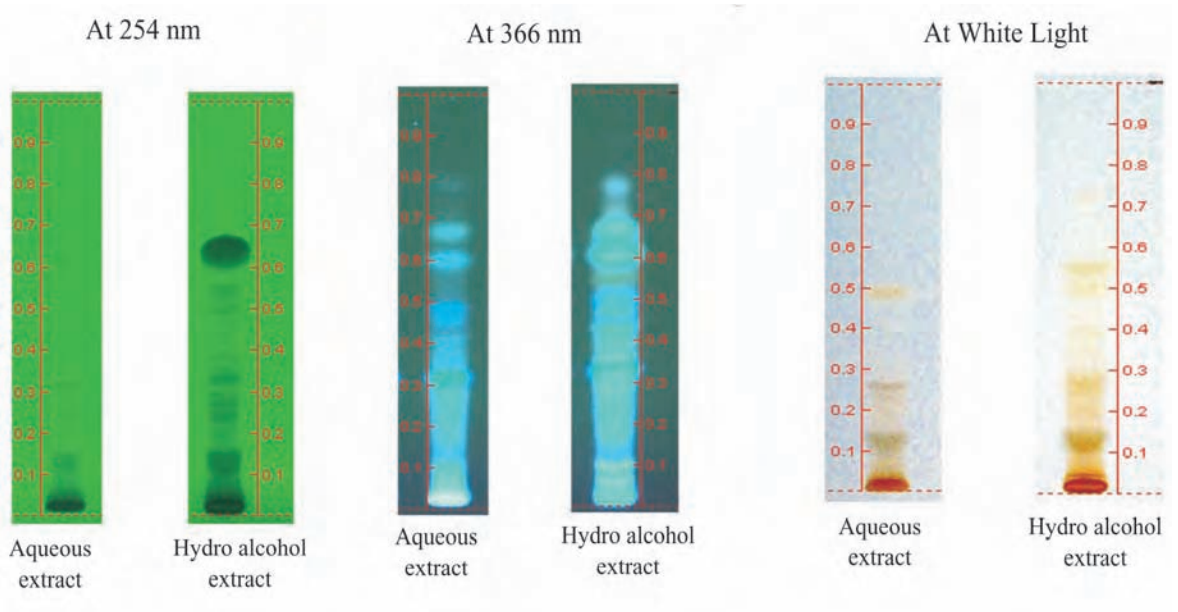


Figure 2

TLC Plate views of AE and HAE



Discussion

Scarcity of raw materials is a major problem faced in āyurveda. Maximum extraction (herb-to-extract ratio) from the limited available raw materials and thus minimize the extractive value in the residue is one of the solutions to the problem. Increasing the yield of medicinal formulations to maximum from the available limited raw materials without compromising the potency is the need of the hour. Newer extraction methods are experimented in the industry with this purpose. The extraction using 50% aqueous alcohol as solvent is one of them. The assumed better potency of the hydro-alcohol extract has to be tested for each formulation.

Taken as the investigational product, Rāsnāpañcaka kvāthaḥ, the aqueous and the hydro-alcohol extracts

of this formulation were tested and compared for its pharmaceutical potency and anti-inflammatory efficacy by HPTLC and *in vitro* protein denaturation inhibition assay respectively.

The extracts prepared show no visible difference. The yield of the extracts from 1 kg of raw materials is 95 grams for aqueous and 85 grams for hydro-alcohol extract.

All the physico-chemical values are compatible for both extracts. Hydro-alcohol extract is slightly acidic for its significance is not explored in the present study. Acid insoluble ash value of both extracts is below the detection level which indicates the absence of any foreign particles.

The HPTLC test in the present study was intended in view to analyze and compare the pharmaceutical potency of the samples. Profiles of both extracts were compatible. Both the samples have similar peaks, though hydro-alcohol extract has a small additional peak at Rf 0.54 (2463.6 AU of area). HAE has an area under the curve of 55253.2 AU whereas AE has only an area under the curve of 28790.7 AU.

The anti-inflammatory efficacy of a drug can be expressed in terms of half maximal concentration (IC_{50}) by calculating the percentage of inhibition. The lower the IC_{50} value, the stronger the inhibitor is. IC_{50} of AE is 312.229 ñg/mL and that for HAE, it is 285.209ñg/mL.

Conclusion

Based on the findings and the discussion, it is concluded that:

- There are no notable organoleptic differences between these extracts. All the physico-chemical indices are compatible for both extracts.
- The hydro-alcohol extract of Rāsnāpañcaka kvāthaḥ has more pharmaceutical potency and anti-inflammatory activity compared to its aqueous extract evidenced by chromatography and *in vitro* protein denaturation inhibition assay.

Scope for further study

- Safety profiling of the products by toxicological study
- Suitable *in vivo* animal study to substantiate the efficacy of the product.
- Clinical trial to substantiate the efficacy o the product on humans.

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EXPERIMENTAL STUDY

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Study on Miśreya arka (Hydro distillate of *Anethum graveolens* L.) prepared by three different methods

Shweta Paul, Karunanidhi Sharma, Sanjay Kumar and Parimi Suresh

ABSTRACT: Miśreya is an important drug used in Āyurveda, Unani and Modern system of medicine. In scientific world it is known as *Anethum graveolens* L., belongs to the family Apiaceae, commonly known as dill, sowa and soya. It is also an ingredient of dill water, which is used in children for abdominal discomfort. Arkakalpana, is an important dosage form in Āyurveda and Unani system of medicine, where water soaked miśreya along with water is placed in distillation apparatus and the distillate is taken as final product. There are different opinions on the ratio of water, drug and distillate in different texts. It is a conflict of interest that which one among these method is the best, present study has been designed to make a conclusion over this. Here three samples has been prepared by following the references of Arkaprakāśaḥ, The Ayurveda Formulary of India and National Formulary of Unani medicine and evaluated on the basis of method, yield and characters of prepared products. It was found that the methods of AFI and NFUM were more convenient, with enough yield and end product of acceptable quality, while the sample by Arkaprakāśaḥ was concentrated, very strong in taste and odour. It can be said that methods of AFI and NFUM can be used for preparing the arka generally.

Key words: Sowa arka, Distillate of soya, Arkakalpana, *Arq-e-Soya, Araq-e-Shibbt*.

Introduction

Anethum graveolens L. is an important herb that belongs to Apiaceae (Umbelliferae) family. It is also known as miśreya, śatapuṣpa (in Sanskrit), Shibt, Soya (in Urdu), Sowa, Sava and Soa (Common names) etc.[1] It is used much in Āyurveda and Unani system of medicine. In āyurveda it is indicated in colic pain, indigestion, stomach ache in children and uterine pain.[2] In Unani medicinal system it is also indicated in abdominal diseases.[3] Dill water is used as an ingredient in gripe water, given to relieve colic pain in babies and flatulence in young children.[4] It is used in various dosage forms like powder, phāṇṭa (infusion), taila (oil) and arka (hydro distillate).[2a] Though main constituent of miśreya is aromatic volatile oil, it can be said that arkakalpana is one of the best option to obtain maximum therapeutic value from it. Arkakalpana can be defined as a liquid preparation obtained by distillation of certain liquids or of drugs soaked in water using arka yantra.[5] It is also mentioned in fundamental dosage forms by Rāvaṇa in Arkaprakāśaḥ.[6] Arka is distilled liquid dosage form and arkayantra which is a classical distillation apparatus. In āyurveda, Arkaprakāśaḥ is a dedicated book to Arkakalpana has mentioned the use of miśreya arka in agnimāndya (impaired digestive fire), yonīśūla (uterine or vaginal pain) and kṛmiroga (worm diseases/ helminthiasis).[6a] While in Unani, *Su-e-Hazam* (dyspepsia), *Qillat-ul-Baul* (oliguria), *Nafkh-e-Shikam* (flatulance).[7] Both the systems has described their own method of preparation. Ayurveda Formulary has also described their method for preparation of miśreya arka. So now there are three main method of miśreya arka preparation. So it is the need of the time to evaluate the best method among these, so that the best quality of arka can be obtained to get maximum therapeutic properties. Considering this point in mind, the present study was planned, where miśreya arka was prepared by all these methods and analyzed in laboratory.

Materials and methods

Raw drug: Dried miśreya fruits were procured from Pharmacy, National Institute of Ayurveda, Jaipur. Distilled water was taken from Drug testing laboratory, Department of Rasashastra and Bhaishajyakalpana (RSBK), National Institute of Ayurveda (NIA), Jaipur.

Instruments: Round bottom flask, heating mantle, condenser, receiver flask, rubber tube, stands, clamps, etc.

- Samples of miśreya were analyzed in the Drug testing laboratory, Department of RSBK, NIA, Jaipur.
- The drug was tested for macroscopic, microscopic and physico-chemical parameters and were compared with standards mentioned in The Ayurvedic Pharmacopoeia of India.[1a] Table 1.
- After confirmation of authenticity, the miśreya was crushed and made into coarse powder.

Table 1
Showing the physico chemical characters of miśreya

Sl.No.	Physico-chemical parameters	API standards	Sample (analyzed)
1.	Foreign matter (Total %)	Not more than 5 %	0.92
2.	pH (10% aqueous solution)	Not mentioned	6.5
3.	Total Ash (% w/w)	Not more than 14 %	13.5
4.	Acid insoluble ash (% w/w)	Not more than 1.5 %	3
5.	Water soluble extractive (% w/w)	Not less than 15%	18.8
6.	Alcohol soluble extractive (% w/w)	Not less than 4%	12
7.	Volatile oil (% v/w)	Not less than 3 %	3%

- Preparation of arka was done in standard laboratory conditions.
- Three samples of arka were prepared with three different references i.e. Arkaprakāśaḥ, The Ayurveda formulary of India and National Formulary of Unani medicine where in all these references ratio and quantity of miśreya, water (taken) and arka (distilled) are different as mentioned in Table 2 and 3.

Table 2
Showing the ratio of water and miśreya in different texts

Reference	Ratio of		
	Miśreya	Water taken	Arka (distilled)
Arkaprakāśaḥ[6b,6c]	1	2	Not mentioned. (Till praśasta arka obtained)[6d]
Ayurveda Formulary of India[8]	1	7	5
Unani system of Medicine[7a,7b]	1	20	10

Table 3
Showing the quantity of water and miśreya according to three texts

Reference	Quantity of		
	Miśreya	Water taken	Arka (distilled upto)
Arkaprakāśaḥ	32 gm	64 ml	25 ml
Ayurveda Formulary of India	50 gm	350 ml	250 ml
Unani system of medicine	25 gm	500 ml	250 ml

• Same method of preparation was followed for preparation of samples as per all (three) above mentioned references.

• Coarse powder of miśreya were kept for overnight soaking and half amount of mentioned water in each case was added. On next morning remaining water was added to it and transferred into a round bottom flask. It was placed over heating mantle and distillation apparatus was assembled. Receiver flask was marked upto the amount of arka which was to be extracted. Then heating was started, temperature was gradually increased upto 100°C and kept for 30 minutes and then it was reduced to 50°C and was maintained throughout the procedure till appropriate quantity of arka was obtained. Then the arka was preserved in air tight container and marked as Sample 1 (Arkaprakāśaḥ), Sample 2 (AFI) and Sample 3 (National formulary of Unani medicine).

• After the preparation of arka all the three samples were analyzed for organoleptic and physico-chemical characters (i.e. pH, specific gravity and refractive index) in the Drug testing laboratory, Department of RSBK, NIA, Jaipur and the results were recorded.

• All the analytical tests i.e. foreign matter,[1b] total ash,[1c] acid insoluble ash,[1d] water soluble extractive,[1e] alcohol soluble extractive,[1f] volatile oil,[1g] specific gravity,[1h] and refractive index[1i] was carried out by following The Ayurvedic Pharmacopoeial references.

Results

Arka obtained:

Sample 1 (Arkaprakāśaḥ) - 25 ml; Duration: 5 hrs.

Sample 2 (AFI) - 250 ml; Duration: 7 hrs.

Sample 3 (NFUM) - 250 ml; Duration: 7 hrs. 45 min.

Results of analytical tests: See Table 4.

Table No. 4
Results of three samples of Miśreya arka

Character	Sample 1 (A.P.)	Sample 2 (AFI)	Sample 3 (NFUM)
Color	Transparent	Transparent	Transparent
Odour	Typical miśreya like (Strong)	Typical miśreya like	Typical miśreya like
Taste	Kaṭu, tikta (Strong)	Kaṭu, tikta	Kaṭu, tikta
Appearance	Watery (slight turbid)	Clear watery	Clear watery
Clarity	Floating oil drops	Floating oil drops	Floating oil drops
pH	6	6.3	6
Specific gravity	1.002	1.003	1.001
Refractive index	1.32	1.33	1.35

Discussion

Arka or *Araq* is an important dosage form in āyurveda, Unani system of medicine and also in Modern system of medicine,[3a,9] where it is used individually or in combination to improve digestion in children, to cure colic pain, indigestion (mainly) and other disease conditions. It is an aromatic plant and volatile oil is the main constituent. Hima, phāṇṭa, arka or *araq*, *saiyyalat*, *qutur* and *shrabat* are the dosage forms which were developed by the scholars of Āyurveda and Unani system of medicine for this type of drugs. Among all these arka kalpana is the best due to some reasons like it contains maximum volatile oils, aroma and its transparent color, having almost watery appearance and its taste not very strong. All these things make it much easier to take as a

medicine than all the other options. This high palatability and higher shelf life (6-12 months)[10,11] or till the presence of praśasta arka properties.[6e]

Instead of the arkayantra described in classics for the preparation of arka, simple distillation apparatus is used in today's practice and in present study also for preparation, as it is tedious job to prepare with the arkayantra. Simple distillation apparatus is easily available, very easy to assemble, easily controlled (temperature, quality of distillation etc.) and provides sophistication also. Coarse powder was preferred over powder or raw drug as it provides sufficient surface area for extraction of active constituents and also do not get chopped as fine powder. Coarse powder of the drug was soaked in water for overnight, hence, drug becomes soft and arka can be easily extracted out of it. Soaking time of yavāni and miśreya drugs in the water has given aṣṭaprahara in sunlight and aṣṭaprahara in moonlight[11] i.e. 48 hrs. of long duration which may cause over soaking, giving a bad smell to the mixture due to growth of microbes in it. So soaking time was also reduced to 12 hrs. and the mixture was subjected to simple distillation. In Arkaprakāśaḥ, miśreya is mentioned under kaṭhina dravya and so for the preparation of arka of kaṭhina dravya amount of water told is twice of the drug. While preparing on a small scale i.e. in the laboratory level for experimental study, it was found to be quite difficult to distill such small amount of arka as it gets charred and some turbidity is also seen. Initially it was heated for 30 minutes at 100°C and later the temperature was reduced to 50°C. It was maintained throughout the procedure. After boiling, the temperature was maintained at 50°C for evaporation of volatile substance and 35-50 minutes later arka started distilling out. After collection upto a marked amount (as per followed reference), heater was switched off. Odour and taste of the arka was same as miśreya, transparent in color and oil droplets were seen on the surface of the arka. In Arkaprakāśaḥ, agni pramāṇa (amount of heat) and time duration has been said as parameter for extraction of arka, but the definite amount is not clearly mentioned. Today, it is quite difficult to decide and carry out the distillation according to the agni pramāṇa. Beyond this, various instruments and methods are available which are more convenient and easy to control the heat during the process. Among all the references, method of Unani system of medicine seems better, where water quantity and distilled arka was found sufficient that it can drain maximum volatile oil from the drugs.

Conclusion

After completion of the study it was concluded that maximum yield was obtained by following the method of Unani system. Arka obtained by the method of Arkaprakāśaḥ was concentrated, with strong taste and smell. Methods of AFI and Unani system were found more convenient for the preparation of arka generally, but for concentrated and highest potency, method of Arkaprakāśaḥ is the best. Evaluation of the same through preclinical and clinical studies are suggested on the basis of the present study.

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CASE STUDY

Āryavaidyan, Vol. XXXII, No. 3, February - April 2019, Pages 25 - 30

A case study on healing effect of Apāmārga kṣāra (caustic therapy) in śalyajanya nāḍī vraṇa after chedana karma

Rajasree G. and Anita Patel K.

ABSTRACT: A pilonidal sinus or śalyajanya nāḍī vraṇa is a cavity filled with hairs and granulation tissue. There are several methods to treat pilonidal sinus, but the recurrence rate is more due to unhealthy healing of excised wound in post-operative period. According to Suśruta, treatment of śalyajanya nāḍī vraṇa is nirharaṇa of śalya followed by mārgaśodhana and ropaṇa. Here, single case is reported with pre and post study of apāmārga (*Achyranthes aspera* L.) kṣāra application in śalyajanya nāḍī vraṇa after chedana karma, which is assessed based on colour, pain, discharge, granulation tissue and size of wound on every week, till the wound gets completely healed. By this treatment pilonidal sinus got completely healed by 42 days. Study was concluded as single application of apāmārga kṣāra after chedana karma is having good healing effect, as it makes the surgical wound healthy and clean.

Key words: Pilonidal sinus, Śalyajanya nāḍī vraṇa, Pratisāraṇīya kṣāra, Chedana karma

Introduction

A pilonidal sinus (śalyajanya nāḍī vraṇa) is a cavity filled with hairs and granulation tissue. Common site of pilonidal sinus is posteriorly in the midline over sacro-coccygeal area and natal cleft. It is an acquired disease that often takes a chronic course. The incidence of the disease is calculated to be 26 per 100,000 people.[1] Pilonidal disease has a male predominance.[2] The disease occurs 2.2 times more often in men than women.

Hair broken off by friction due to continuous sitting collect in the cleft and enter into the sweat glands. After the initial entry dermatitis and inflammation start around the loose hair and once the sinus is formed, intermittent negative pressure of the area may suck other loose hair into the pit. It affects inter gluteal furrow. The sinus passes upwards and forwards towards the sacrum. It may possess branching side channels. It occurs in young adults or teenagers.

There are several methods to treat pilonidal sinus like Karydakis procedure, Bascom's technique, Z-plasty, Radical excision, etc. But the recurrence rate is more due to unhealthy healing of excised wound in post-operative period.

In āyurveda there are eight types of nāḍī vraṇa. Among that śalyajanya nāḍī vraṇa or āgantuja nāḍī vraṇa is taken as pilonidal sinus as it is caused by hair. Kṣārasūtra application is widely practised treatment modality in śalyajanya nāḍī vraṇa (pilonidal sinus), but it needs weekly thread changing and it is a painful procedure through out the course of treatment (till the sinus track gets completely cut open). Ācārya Suśruta has explained the treatment of śalyajanya nāḍī vraṇa as nirharaṇa of śalya followed by mārgaśodhana and ropaṇa.[3] In the indication of pratisāraṇīya kṣāra, Suśruta has included nāḍī vraṇa. In the indication of chedya vyādhi, asthi-māmsaka śalya is also included.

Hence, an attempt was made to treat pilonidal sinus by nirharaṇa of roma (hair) śalya with the help of chedana karma (excision) which was followed by mārga-śodhana and ropaṇa by application of apāmārga kṣāra.

Method of preparation of apāmārga kṣāra

10 kg of apāmārga (whole plant) was collected and formed into a heap. The whole plant was burnt into ashes, and it was allowed to cool by itself. The whole

ash was collected (1 kg) and mixed with six litres of water and stirred well, allowed to settle overnight. Next day it was filtered through a double folded cloth for 21 times and the residue was thrown out. Obtained amber coloured filtrate was subjected to mandāgni (mild fire). When the content was reduced to half; about1/3rd of kṣārajala (caustic water) was taken out of the vessel. 100 gm of śukti (conch shell) was heated red hot and was then mixed with 1/3rd of kṣārajala to dissolve it completely. Thus dissolved śukti was added to the boiling kṣārajala and continued to boil. Meanwhile 10 gm of citrakamūla (*Plumbago indica* L.) kalka was added to the boiling kṣārajala and allowed to boil for a few more minutes. When the content attained consistency as described by Suśruta[4] (not too liquid nor too solid) it was removed from fire and transferred into a separate container with lid. Figure 1.

Figure 1
Apāmārgakṣāra

Case Report

A 21 year old female patient came to surgery OPD on March 2016 with complaints of pus discharge and pain around the natal cleft since one week. She had a past history of pilonidal abscess and did incision and drainage in 2012 and 2013 from an allopathic hospital. In 2015 December pilonidal sinus appeared and it was treated with incision and drainage. It was healed and they were advised to do excision for the same from that modern hospital. Due to recurrence the patient was not willing to go for modern treatment.

On examination opening was present at natal cleft (sacral region) with pus discharge. On palpation induration was present with tenderness. The direction of sinus was downwards i.e., towards skin pit. Figure 2. On probing the length of the track was almost 5cm. Figure 3. Blood routine, liver function test, renal function test, lipid profile and blood sugar was in normal limits. HIV, HBsAg and VDRL were negative. ECG was normal.

Figure 2
Pilonidal sinus

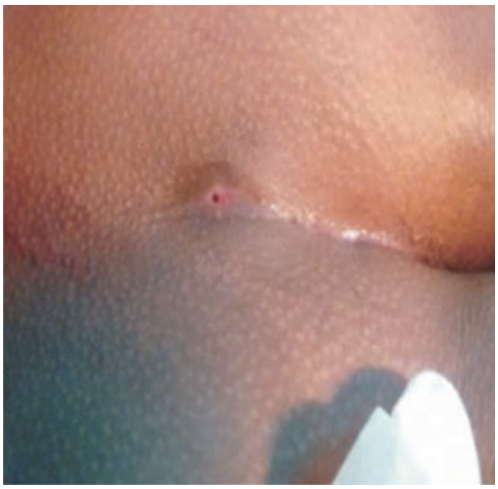
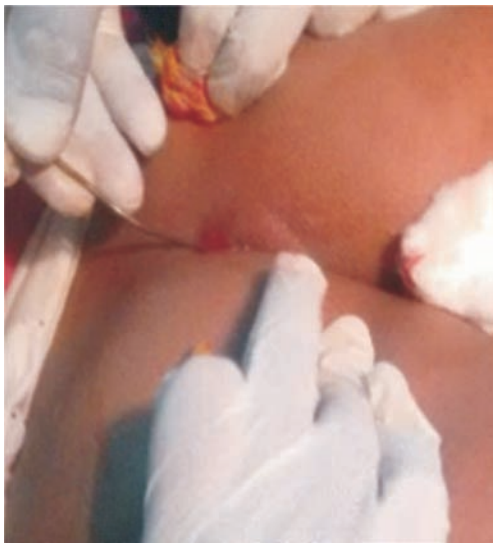


Figure 3
Probing the Pilonidal sinus



Procedure planned was chedana karma (excision) followed by single application of apāmārga kṣāra.

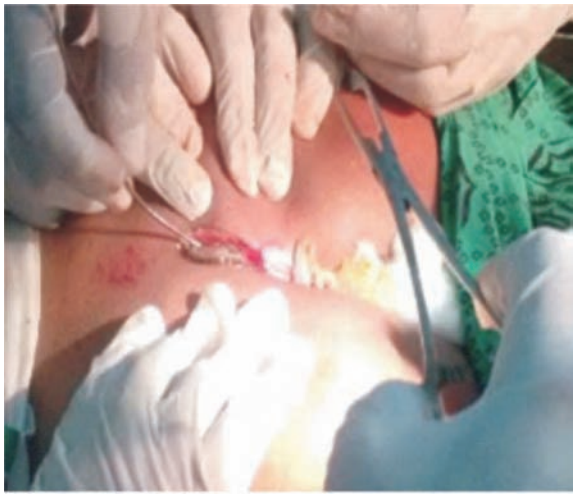
Pūrva karma (Pre-operative preparation)

Sodium phosphate enema (proctoclysis) was given early in the morning on the day of operation. Injection T.T. (Tetanus toxoid) 0.5 ml IM was given and plain Xylocaine 2% was given intradermal for sensitivity test. Gorocanādi guḷika was given as a premedication.

Pradhāna karma (Operative procedure)

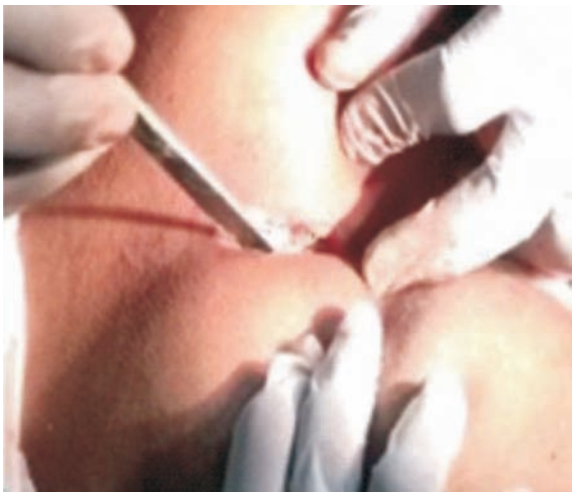
Patient was made to lie down in prone position. Painting to the operative site was done with triphalā kaṣāya. Followed with draping. Local anaesthesia was given to the site of pilonidal sinus with Xylocaine 2%. Probing was done to see the length of the tract. With the help of scalpel (blade no.11 and B.P handle no. 3) an elliptical incision was made around the pilonidal sinus. The whole sinus tract was excised deep up to the presacral fascia. Figure 4.

Figure 4
Doing excision around the Pilonidal sinus track



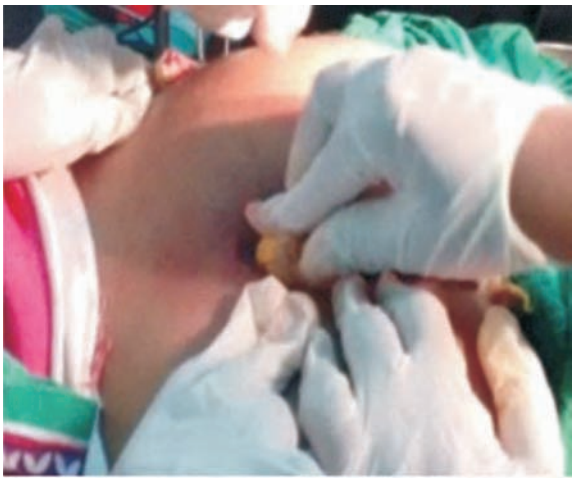
During the whole surgical process hemostasis was maintained by giving pressure with surgical mop. After the above chedana karma pratisāraṇīya kṣāra prepared from the apāmārga was applied. Figure 5. After application of the kṣāra, it was kept for 100 mātrā kāla (1 minute) and it was washed with jambīra

Figure 5
Application of Apāmārga kṣāra



[*Citrus limon* (L.) Osbeck] svarasa to operative wound. Figure 6. Hence, neutralization occured by alkaline and acid reaction.

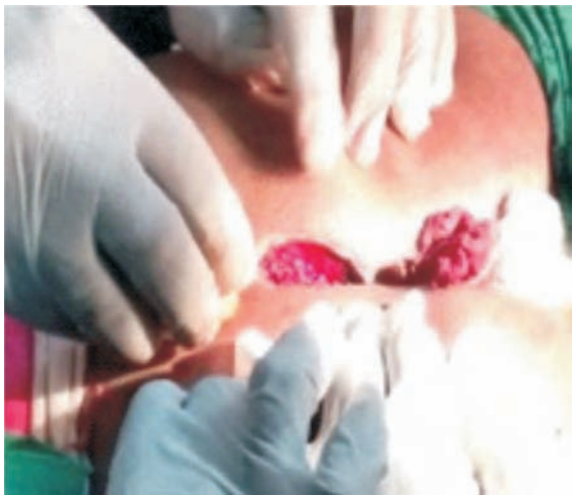
Figure 6
Washing with Jambīra svarasa



Pascāt karma (Post-operative procedure)

In the post-operative wound, chemical cauterization occurred and bleeding was arrested. Figure 7. Daily cleaning and dressing was done with normal saline till the wound gets completely healed. Weekly shaving was done around the operative wound. The wound was assessed weekly till it got completely healed.

Figure 7
Post operative wound on surgical day



Results and Discussion

Results are given in Figures 8, 9, 10, 11 and 12 and Table 1.

Figure 8
Post-operative wound on 7th day

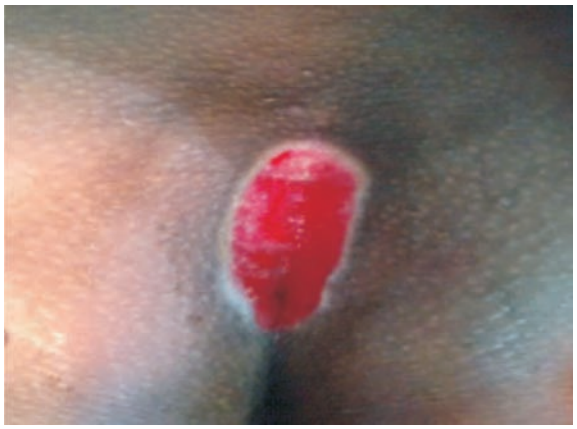


Figure 9
Post-operative wound on 14th day

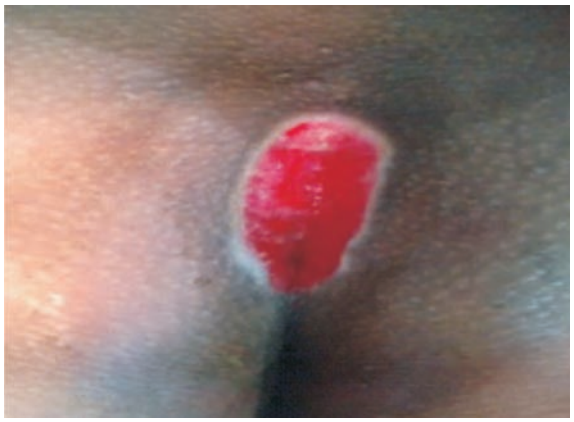


Figure 10
Post-operative wound on 21st day

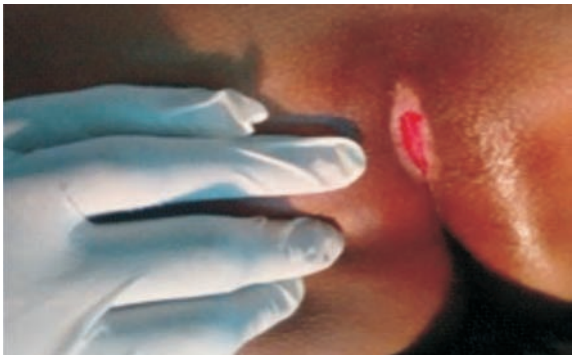


Figure 11
Post-operative wound on 28th day

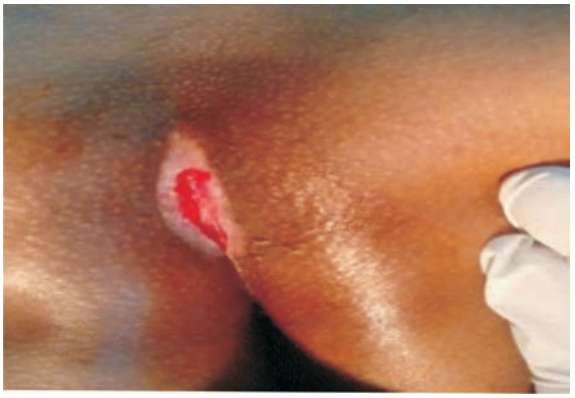


Figure 12
Post-operative wound on 35th day

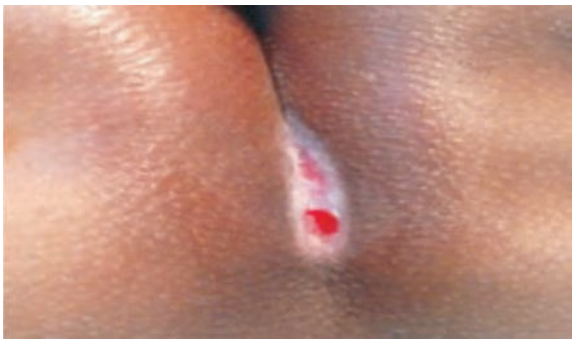


Figure 10
Post-operative wound on 42nd day

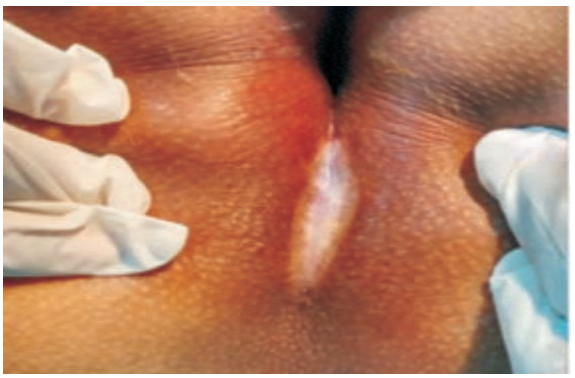


Table 1 Changes observed during the course of treatment						
Days	7	14	21	28	35	42
Colour of wound	Red	Red	Red	Red	Red	Healed
Pain	Absent	Absent	Absent	Absent	Absent	Absent
Discharge	Pale	Absent	Absent	Absent	Absent	Absent
Size(cm^2)	11.5	11.5	8	4	1.5	0
Granulation tissue	Healthy	Healthy	Healthy	Healthy	Healthy	Healed

- Pain was present only for 1st two days after surgery and it was relieved by use of analgesics.
- The excised wound was completely healed within 42 days.
- Application of apāmārga kṣāra after excision of pilonidal sinus helped in scraping of pits in the surrounding tissue of the sinus.
- Apāmārga kṣāra acted as chemical cauterization. Immediately after chedana karma the bleeding was arrested and it had rakta-sthambhana property.
- pH value of apāmārga kṣāra was 11.1
- According to Arrhenius theory, the acids are substances which produce hydrogen ions and base produces hydroxide ions. Hence, neutralization occurs by hydrogen ions and hydroxide ions reaction and produce water. Hence, pratisāraṇīya kṣāra was washed with jambīra svarasa neutralises it.

$$H^+ + OH^- = H_20$$

- It has anti-inflammatory and antibacterial property.
- It avoided the formation of unhealthy granulation tissue and the wound got healed from the base.
- Healing was more seen in breadth wise rather than length wise healing.
- It performs chedana, bhedana and lekhana properties among the aṣṭavidha śastra karma.[5]
- Since three years there is no reccurence of the disease.

Conclusion

- Application of apāmārga kṣāra in pilonidal sinus after excision helped to heal the wound within 42days.
- Post-operative pain was very less compared to other treatment modalities.
- Minimum hospitalisation is required.
- Bleeding was absent.
- There is no scope for reccurence since all pits adjecent to sinus track are scrapped.

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CLINICAL STUDY
Āryavaidyan, Vol. XXXII, No. 3, February - April 2019, Pages 31 - 39

Management of jalodara *vis-a-vis* ascites with special reference to Jalodarāri rasa as virecana yoga

Sanjay Kumar Giri and Sanghamitra Patnaik

ABSTRACT: Jalodara is a clinical condition described in āyurveda that closely resembles with the clinical features of ascites. Elimination of both fluids and morbid factors through virecana (therapeutic purgation) is the first line of treatment in the management of jalodara in āyurveda, whereas, standard diuretic therapy is the first line therapy for ascitis as per modern medicine. A single blind clinical study was conducted at Department of Post graduate studies in Kayachikitsa, Gopabandhu Ayurveda Mahavidyalaya, Puri, Orissa, to evaluate the efficacy of Jalodarāri rasa, a virecana yoga mentioned in āyurveda classics, in patients suffering from jalodara and to compare the āyurveda line of treatment with that of modern medicine. Thirty patients aged between 29-63years were divided into 20 patients in trial group and 10 patients in control group. The patients in the trial group were prescribed with Jalodarāri rasa, cow's milk and lājāmaṇḍa (a preparation made from puffed rice) and those in the control group were prescribed a diuretic Lasilactone (20mg Furosemide and 50mg Spironolactone). The assessment was based on the changes in the clinical signs and symptoms. In the trial group 5%, 50%, 30% and 15% patients experienced maximum, moderate, mild and no improvement whereas, in the control group it was 0%, 30%, 40% and 30% respectively. It was observed that patients in both groups experienced comparable changes after treatment but the patients in the trial group experienced additional benefits in terms of general debility ($p<0.001$) and fluctuant abdomen ($p<0.001$). Thus it was found that Jalodarāri rasa is a very potent virecana yoga that can pursue out the morbidity associated with Jalodara. This study also proved that āyurveda treatment is equally effective as modern diuretic therapy in the treatment of jalodara or ascitis.

Key words: Ascitis, Diuretics, Jalodara, Pañcakarma, Virecana

Introduction

Jalodara, a condition described in āyurveda, characterized in udara guha is accompanied by symptoms such as anorexia, heavy distended abdomen, tastelessness of mouth, scanty urination and hard faeces.[1, 2] It is mainly caused by the intake of cold water after samśodhana, loss of digestive fire, etc.[2a,3] It is analogous to ascites in the modern parlance where accumulation of several litres of serous fluids in peritoneal cavity is found.[4] Ascites is secondary to hepatic diseases, renal diseases, malignancies, cardiac failures, tuberculosis, malnutrition and so on. Jalodara has been described as a terminal condition of udararoga in āyurveda. In malnutrition the collection of intra-peritonial fluid occurs due to hypoproteinemia.[5] In view of hypoproteinemia elimination by purgation of both fluid and morbid factors is the foremost aim in the management of ascites. Virecana, one among the pañcakarma, is considered as the prime line of treatment in jalodara.[6] Considering this fact Jalodarāri rasa, a virecana yoga referred in Bhaiṣajyaratnāvali was selected for the clinical trial under the Department of Post graduate studies in Kayachikitsa, Gopabandhu Ayurveda Mahavidyalaya, Puri, Orissa.[7]

Jalodarāri rasa, commonly used in practice in the treatment of jalodara especially in this part of the country gives satisfactory result. Hence, this study was designed to establish the efficacy of this classical virecana yoga by using scientific parameters with statistical analysis.

Further, there is no much documentation on the comparative evaluation of virecana in āyurveda to

that of diuretic therapy in modern science. Hence, the present study was conducted.

Materials and methods

Source of study: OPD and IPD of Gopabandhu Ayurveda Mahavidyalaya, Puri, Orissa.

Design of the study

Number of patients (sample size): Trial group (T_1) - 20 and Control group (T_2) -10.

Duration of study: 30 consecutive days for both trial and control group patients.

Name of the trial drug with ingredients: Jalodarāri rasa contains haridrā, pippalī, maricaḥ, tāmra bhasma and purified jepāḷaḥ.

Bhāvana dravya: Snuhīkṣīra: Quantity sufficient

Statistical design

T_1: B.T. Vs A.T. - effectiveness of Jalodarāri rasa was assessed.

T_2: B.T. Vs A.T. - effectiveness of Lass lactone was assessed.

Name of the control drug with composition: Lassilactone- Frusemide - 20mg, Spironolactone - 50mg

Dose of trial drug with vehicle and control drug: Dose of trial drug - 250 mg twicw daily with cow milk,

Dose of control drug- 2 tab twicw daily.

Type of study: Single blind study

Objectives of the study: To assess the clinical efficacy of both trial and control drug and to compare the efficacy of both.

Diet and regimen: Patients were advised to remain on a diet of cow milk alone with lājāmaṇḍa. They were not allowed for normal bath except sponging.

Procurement of the drug: The raw materials were procured from authentic sources. Medicine was prepared in the rasaśāla of Gopabandhu Ayurveda Mahavidyalaya, Puri, Orissa.

Follow up: After the completion of trial, follow up was made after one month.

Criteria for the selection of cases

Inclusion criteria: On the basis of cardinal features of hypo-protienemia due to malnutrition i.e., enlarged and fluctuated abdomen, increased body weight, general debility and retention of urine and stool, the patients were screened before including for trial.

Patients of age group 29-63years and both sex were included.

In multiple phases of the study subjects were allocated by random sampling.

Exclusion criteria: Nephro-pathology, tubercular peritonitis, malignancy, cardiac failure, liver cirrhosis, diabetes mellitus and haemorrhagic diseases were excluded.

Clinical parameters for assessment: Patients were screened based on the proforma accepted by the Department of Post graduate studies in Kayachikitsa, Gopabandhu Ayurveada Mahavidyalaya, Puri, Orissa.

Assessment criteria

Subjective

i. Fluctuated abdomen. ii. General debility

Objective

i. Enlargement of abdomen (abdominal girth)
ii. Suppression of urine (frequency/24 hours)
iii. Retention of bowel (frequency/24 hours)
iv. Urine output (volume/24hrs)
v. Passage of stool (quantity/24hrs)
vi. Body weight (to evaluate swelling)

Method of preparation of Jalodarāri rasa: [7]

Ingredients: Haridrā: 125 mg, pippalī: 125 mg, maricaḥ: 125 mg, tāmra bhasma: 31.25 mg, śodhita jepāḷa: 500 mg, bhāvana dravya (snuhīkṣīra) - Quantity sufficient. Table 1.

Table 1
Rasapañcaka and chemical composition of the ingredients of Jalodarāri rasa

Drug name	Rasa	Guṇa	Vīrya	Vipāka	Indication	Therapeutic action	Chemical composition
Haridrā (*Curcuma longa* L.)	Tikta, kaṭu	Rūkṣa laghu	Uṣṇa	Kaṭu	Kapha vāta śāmaka Pitta recaka Pitta śāmaka	Śotha, yakṛt-plīha roga	Curcumin, volatile oils
Pippalī (*Piper longum* L.)	Kaṭu, madhura	Laghu snigda tīkṣṇa	Anuṣṇa-śīta	Madhura	Kapha vāta śāmaka	Agni māndya, vibandha, śūla	Volatile oils, piperin, piperidine piplartine
Maricaḥ (*Piper nigrum* L.)	Kaṭu	Laghu rūkṣa tīkṣṇa	Uṣṇa	Kaṭu	Kapha vātahara	Aruci agnimāndya, śūla	Volatile oils, piperin, piperidine piplartine
Tāmra bhasma (*Cuprum*)	Madhura amla, kaṭu tikta, kaṣāya	Snigda sara laghu	Uṣṇa-śīta	Madhura	Kapha pitta śāmaka	Pāṇḍu, yakṛt-plīha roga, grahaṇi	CuSO4, CuS, Cu2O, CuO and Cu metal
Jepāḷaḥ (*Croton tiglium* L.)	Kaṭu	Guru, tīkṣṇa	Uṣṇa	Kaṭu	Vāta kaphahara	Śopha, jalodara	Steamic, palmitic, tiglic, oleum crotonis
Snuhī kṣīra (*Euphorbia neriifolia* L.)	Kaṭu	Snigda laghu	Uṣṇa	Kaṭu	Vātahara	Gulma, udara ādhmāna	Euphorbon, Starch

All the kāṣṭauṣadhī (herbal ingredients) were made into fine powder. Tāmara bhasma was added in khalva yantra, then soaked in snuhī kṣīra (qs) and was given mardana (trituration). This process was continued for one day and snuhī kṣīra was added accordingly to the need. Then vaṭi (tablet) weighing 2 ratti (250mg) mātra was prepared and allowed to dry. After drying it was preserved in a tight container.

Assessment scale

Enlargement of abdomen (abdominal girth)

Grade 0: (No improvement) proportionate with body parts.

Grade 1: (Mild improvement) 2-4 cms.

Grade 2: (Moderate improvement) 5-8 cms.

Grade 3: (Maximum improvement) above 8 cms.

Fluctuated abdomen

Grade 0: Soft, not fluctuant

Grade 1: (Mild), soft, fluctuation changes with change of posture

Grade 2: (Moderate), soft, fluctuation not changes with change of posture.

Grade 3: (Severe), dull, fluctuation not change with change of posture.

Suppression of urine (in frequency/24hrs)

Grade 0: 6 times/day with proper quantity

Grade 1: (Mild) >3 times/day, Scanty

Grade 2: (Moderate) 2-3 times/day, Scanty

Grade 3: (Severe), 1-2 times/day, Scanty

Retention of bowel and flatus (frequency/24hrs)

Grade 0: Defecation 2 times/day with satisfaction and flatus passed regularly.

Grade 1: (Mild), defecation < 2times/day with satisfaction having flatus passed.

Grade 2: (Moderate), defecation < 1-2times/day, having flatus passed occasionally.

Grade 3: (Severe), defecation restricted to once per day or less having flatus not passed.

Urine output (in ml/24hrs)

Grade 0: (Normal) around 1500ml/day
Grade 1: (Mild), >551ml/day - <1500ml/day with turbidity
Grade 2: (Moderate), >451ml/day - <550ml/day with turbidity
Grade 3: (Severe), <450ml/day with turbidity

Passage of stool (in gms/24hrs)

Grade 0: > 200 gm/day
Grade 1: (Mild), 101-200gm/day
Grade 2: (Moderate), 51-100 gm/day
Grade 3: (Severe), 0-50 gm/day

Body weight reduction in kg

Grade 1: 1-2 kg (Mild improvement)
Grade 2: 2-3 kg (Moderate improvement)
Grade 3: above 3 kg (Maximum improvement)

General debility

Grade 0: (Normal)
Grade 1: Debilitated, having normal decubitus
Grade 2: Debilitated upto the extent of inability to perform the normal decubitus with help.
Grade 3: Debilitated up to the extent of inability to perform the normal decubitus not even with assistance.

Assessment scale and score

The grade points were seen to assess the severity of different signs and symptoms. Table 2.

Table 2
Assessment scale and score

Sl.No.	Sign	Severity	Grade	Grade points (score)
1.	+++	Severe	G 3	3
2.	++	Moderate	G 2	2
3.	+	Mild	G 1	1
4.	-	Normal	G 0	0

Clinical assessment of results

1. Maximum improvement: Improvement in all signs and symptoms more than 50% to 75%.
2. Moderate improvement: Improvement upto 25% to 50%.
3. Mild improvement: Improvement upto 25%.
4. No Improvement: Not observed or < 25%.

Statistical analysis

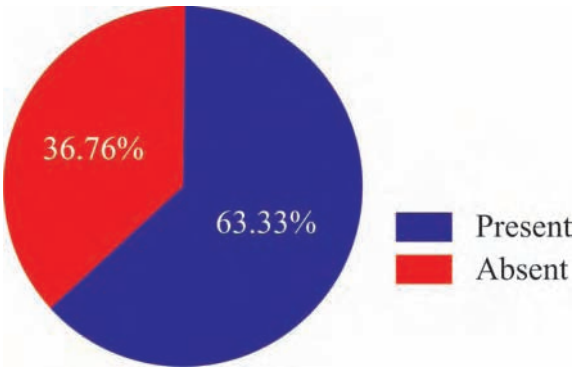
For statistical analysis the mean SD value before treatment of each sign and symptom was compared with mean SD value after 15 to 30 days of treatment. Paired 't' test was used to attain the test of significance. The $p < 0.05$ was set for significance.

Observations and results

In this study it was observed that in both trial and control group all the patients were having the objective and subjective signs and symptoms.

This study showed that out of 30 patients, 19 (63.33%) patients had history of udararoga where as 11 (36.675%) patients did not have the history of udararoga. Figure1.

Figure 1
Past history of Udararoga



It was observed that out of 30 patients, 11 (37%) patients were having addiction to tea and coffee, followed by 8 (27%) patients using betel leaf and smoking, 5 (17%) patients using tobacco, 3 (10%) patients each having addiction towards alcohol and bhanga. Figure 2.

Statistical analysis showing the effectiveness of the trial drug and control drug to different signs/ symptoms after 15 and 30 days are given in Table 3 and 4 respectively.

Figure 2
Incidence of addictions

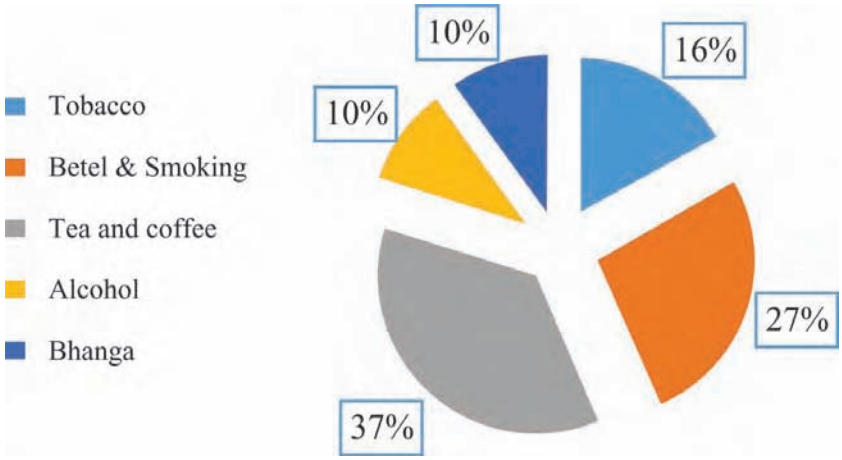


Table 3
Statistical analysis showing the effectiveness of the trial drug to different sign/symptoms after 15 and 30 days[2]

Sl. No.	Signs/ symptoms	Before treatment	After (15 days) treatment	After (30 days) treatment	t-value	p-value
1.	Enlargement of abdomen (Abdominal girth)	78.65±7.9	74.1±8.08	70.8±9.15	8.73 8.79	<0.001 <0.001
2.	Fluctuated abdomen	2.15±0.72	1.95±0.74	1.45±0.74	2.23 5.43	<0.05 <0.001
3.	Suppression of urine (Frequency in number/24hours)	2.1±0.9	2.0±0.8	2.0±0.8	0.58 0.58	>0.05 >0.05
4.	Retention of bowel and flatus (Frequency in number/24hours)	1.1±0.8	2.75±1.08	2.05±0.86	8.48 5.68	<0.001 <0.001
5.	Urine output	445±126.4	448.0±125.0	445.5±125.5	0.89 0.78	>0.05 >0.05
6.	Passage of stool	50.9±27.3	350.5±30	298.6±32	9.8 8.7	<0.001 <0.001
7.	Body weight	50.9±2.47	49.7±2.55	49.0±2.5	8.9 8.0	<0.001 <0.001
8.	General debility	2.05±0.7	1.75±0.6	1.45±0.7	2.85 5.36	<0.05 <0.001

<0.05: Significant; <0.001: Highly significant

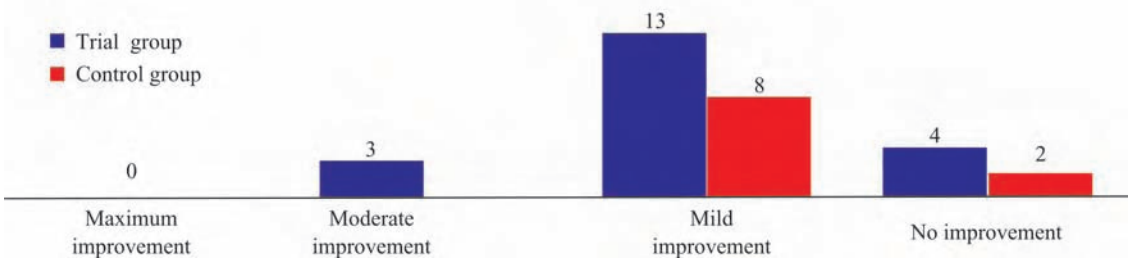
Trial drug is significantly effective to reduce enlargement of abdomen and fluctuated abdomen, to increase bowel and also to increase the passage of bowels, to decrease the body weight and to reduce the general debility. But trial drug (T .D) showed non- significant result in urine output and suppression of urine.

Table 4
Statistical analysis showing the effectiveness of the control drug to different sign/symptoms after 15 and 30 days[2]

Sl.No.	Signs/ symptoms	Before treatment	After (15 days) treatment	After (30 days) treatment	t-value	p-value
1.	Enlargement of abdomen (Abdominal girth)	77.65 8.36	74.1 8.08	71.8 9.73	5.12 4.6	<0.001 <0.01
2.	Fluctuated abdomen	2.00 0.77	1.9 0.80	1.60 0.91	0.98 1.98	>0.05 >0.05
3.	Suppression of urine (Frequency in number/24hours)	1.2 0.4	2.0 0.8	2.0 0.8	9.03` 0.58	<0.001 <0.001
4.	Retention of bowel and flatus (Frequency in number/24hours)	1.5 0.5	1.5 0.5	2.05 0.86	-----	(the control drug has no action)
5.	Urine output	315.0 100.12	860.0 73.5	690.0 152.9	11.59 9.8	<0.001 <0.001
6.	Passage of stool	58 10.5	58.5 11.0	57.9 10.9	0.98 0.78	<0.001 <0.001
7.	Body weight	50.3 1.95	48.8 2.99	48.9 1.97	6.72 6.41	<0.001 <0.001
8.	General debility	1.9 0.83	1.9 0.83	1.6 0.9	-- 1.79	(drug do not effect insignificant) <0.05

Control drug is significantly effective to reduce the enlargement of abdomen and decreased fluctuated abdomen. It is significantly effective to increase the frequency of urine as well as urine output and passage of stool and to reduce the body weight. But in case of general debility the control drug is not significantly effective.

Figure 3
Clinical assessment of results after 15 days of treatment in trial and control group



Clinical assessment of results at 15 days and 30 days of follow up: The clinical assessment of the result showed that among trial group, after 15 days of treatment, out of 20 patients 3 (15%) patients got moderate improvement, 13 (65%) patients had mild improvement and the rest 4 (20%) patients could not get any improvement. Among the control group, out of 10 patients 8 (80%) patients got mild improvement but another 2 (20%) patients did not have any such improvement. After 30 days of treatment, in the trial group, out of 20 patients, 1 (5%) patient got maximum improvement, 10 (50%) patients got moderate improvement, 6 (30%) patients got mild improvement, but the rest 3 (15%) patients could not get any improvement. In the control group, out of 10 patients, 3 (30%) patients got moderate improvement, 5 (50%) patients got mild improvement but the rest 2 (20%) patients could not get any improvement. Figure 3 and 4.

The percentage of improvement in different signs and symptoms are given in Table 5 and Figure 5.

Figure 4
Clinical assessment of results after 30 days of treatment in trial and control group

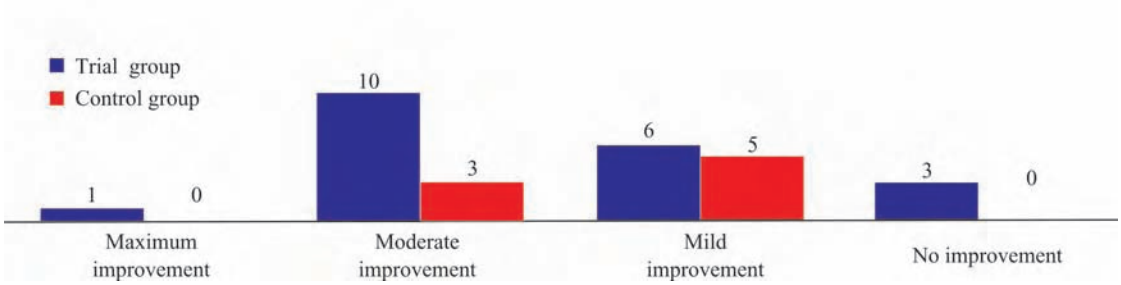
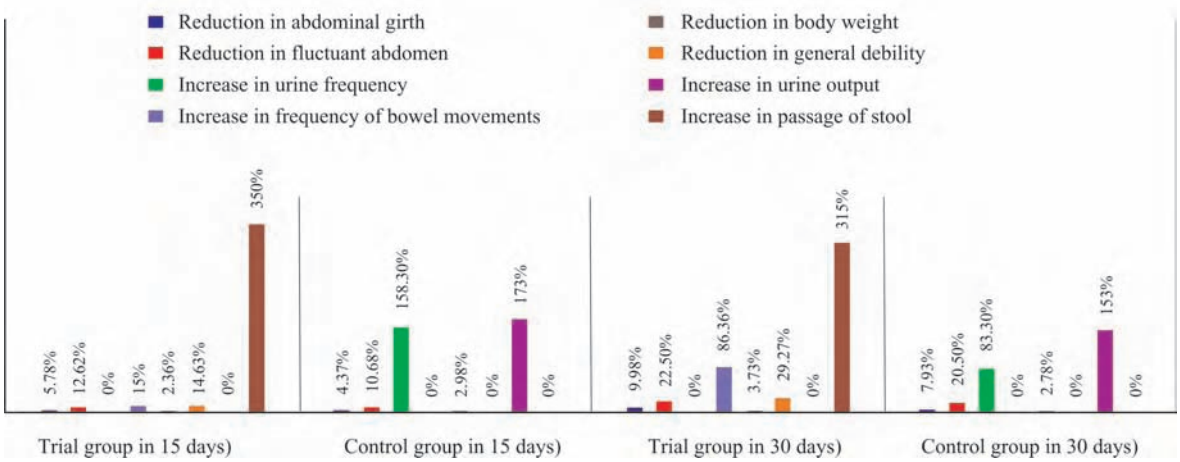


Table 5
The percentage improvement of different signs and symptoms are given below

Sl. No.	Groups and duration of treatment	Signs and symptoms							
		Reduction in abdominal girth	Reduction in fluctuant abdomen	Increase in urine frequency	Increase in frequency of bowel movements	Reduction in body weight	Reduction in general debility	Increase in urine output	Increase in passage of stool
1.	Trial group (in 15 days)	5.78%	12.62%	0%	15%	2.36%	14.63%	0%	350%
2.	Control group (in 15 days)	4.37%	10.68%	158.3%	0%	2.98%	0%	173%	0%
3.	Trial group (in 30 days)	9.98%	22.5%	0%	86.36%	3.73%	29.27%	0%	315%
4.	Control group (in 30 days)	7.93%	20.5%	83.3%	0%	2.78%	0%	153%	0%

Figure 5
Percentage of improvement of different signs and symptoms



Discussion

Jalodara is a clinical entity that demonstrate by increase in abdominal girth and dullness of abdomen caused due to accumulation of fluid in the abdomen.[8] Svedavaha and udakavaha srotas as well as prāṇa and apāna vāta are predominantly affected in jalodara. Pathological changes occur due to the obstruction in the transport of the fluid in the body were analogous

to ascites based on the patient presentation. Several authorities in āyurveda have accepted śodhana cikitsa particularly virecana, to eliminate the obstruction in jalodara.

Discussion on trial drug formulation[9,10]: Jalodarāri rasa, the trial drug, was formulated with the following ingredients and the mode of action of each drug is explained henceforth. Pippalī and maricaḥ enhance the agni. Jepāḷaḥ is an irritant purgative. Tāmra bhasma is having kaphanāśaka and śothahara properties. Haridrā is anti-infective as well as anti-inflammatory in nature. Thus, the depletion in symptoms with jalodarāri rasa as the virecana yoga, is justified.

Discussion on observation: Highest incidence being observed within the age group 29-35 with lower socioeconomic status, which indicates the impact of poverty contributing to malnutrition. The people of ānūpadeśa (habitat), laborers and housewives (occupation) were more reflecting irregular diet and regimen. Most of the patients were having agnimāndya and koṣṭhakāṭhinya. According to the intensity of symptoms maximum dominating signs and symptoms were protuberant abdomen, everted umbilicus, enlarged blood vessels, shifting dullness as well as fluid thrill and general debility. According to the overall percentage of result in trial group, 5% showed maximum improvement whereas in control group it was 0%, moderate improvement of 50% was found in trial group and it was 30% in control group, mild improvement was 30% in trial group and 40% in control group and no improvement observed was 15% in trial group and it was 30% in control group. In view of this comparison in response, the trial drug is more complimentary than the control drug specifically in general debility and fluctuated abdomen.

So the principle of treatment mentioned against jalodara may also be comprehended by Jalodarari rasa and it can be considered as a highly potent formulation. It is fit to purge out the morbidity associated to jalodara. The response derived by the drug is complimentary to declare and accept the drug as a suitable formulation for the treatment of jalodara.

Below flow chart is showing the mode of action of virecana in jalodara.

Uṣṇa, tīkṣṇa, sūkṣma, vyavāyi ⟹ Vīrya of drug reaches hṛdaya ⟹ Circulate in dhamani, sthūla and aṇu srotas ⟹ Uṣṇa guṇa (viṣyandana), Tīkṣṇa guṇa (chedana) ⟹ Doṣa pracalana and bahirgamana from koṣṭha ⟹ Pṛthvī and jala mahābhūta bhuyiṣṭha ⟹ Adhobhāga prabhāva ⟹ Gudamārga doṣa haraṇa

Conclusion

The results were carefully assessed considering the cardinal signs and symptoms along with statistical adjudication. Assessment of patients treated with Jalodarāri rasa for 15 days and 30 days revealed that there was 50% (moderate improvement), 30% (mild improvement) 5% showed maximum improvement and 15% showed no improvement. Whereas, patients treated with furosemide demonstrated that there was 40% (mild improvement), 30% (moderate improvement) maximum improvement was not observed and 30% not showed any improvement. Overall comparison showed that Jalodarāri rasa has brought additional benefits when compared to the patients who received only furosemide therapy. In jalodara, udaka-svedavaha srotas as well as apāna-prāṇa vāta are mainly affected which can be well rectified using the aforementioned Jalodarāri rasa. This shows its effectiveness in the management of jalodara or ascites.

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ANALYTICAL STUDY

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Comparative X-ray fluorescence analysis of Kāsīsa purified by different methods

Manali Anil Visaria and Sheela Pargunde

ABSTRACT: In Rasaśāstra, śodhana is a procedure by which a dhātu or a substance is purified to remove mala or impurities from it. Only after śodhana, dhātu can be subjected to māraṇa (incineration) thereby making it efficient for consumption by human beings. Kāsīsa is classified under the uparasa in Rasaśāstra. Various grantha (ancient texts) have mentioned kāsīsa śodhana by different methods with different dravya like bhṛṅgarāja svarasa, kāsamarda rasa, pañca pitta, rājakośātaki, etc. This study was aimed to compare XRF analysis of kāsīsa purified by different methods. Aśuddha kāsīsa was purified as per the reference mentioned in Rasataraṅgiṇi, Rasaratnasamuccaya and Rasārṇava. XRF analysis of aśuddha kāsīsa and śuddha kāsīsa was done. The results of XRF analysis showed that aśuddha kāsīsa (Iron-71.8%, Sulphur-25%), śuddha kāsīsa done by svedana in ḍoḷāyantra with bhṛṅgarāja svarasa (Iron-74.58%, Sulphur-16.57%), śuddha kāsīsa done by svedana in ḍoḷāyantra with bhṛṅgarāja kvāthaḥ (Iron-69.2%, Sulphur-17.9), śuddha kāsīsa done by dissolving in bhṛṅgarāja svarasa (Iron-76.46%, Sulphur-20.75%), śuddha kāsīsa done by bhāvana with kāsamarda kvāthaḥ (Iron-70.7%, Sulphur-18.3%). Among all the methods of śodhana, percentage of iron and sulphur were found more in śuddha kāsīsa done by the method of dissolving in bhṛṅgarāja svarasa.

Key words: Kasis, XRF analysis, Iron, Sulphur

Introduction

Śodhana is defined as a procedure in which a dhātu or a substance is purified by peṣaṇa, svedana, etc. to remove mala from it.[1] Śodhana is an important concept explained in Rasaśāstra for preparing any medication especially the oral medications. Hence, śodhana is considered as an initial procedure of preparation of any medication. Kāsīsa is one such metal described in Rasaśāstra under uparasa varga. Śodhana of kāsīsa is mentioned in various grantha of āyurveda. It can be purified by various dravya such as bhṛṅgarāja [*Eclipta alba* (L.) Hassk.] svarasa as mentioned in Rasataraṅgiṇi and Rasaratna-samuccaya, kāsamarda rasa, pañca pitta and rājakośātaki as mentioned in Rasārṇava, etc. After the śodhana of kāsīsa, its elemental composition can be derived from XRF (X-ray fluorescence) analysis. XRF can be defined as an emission of characteristic X-ray from a material that has been excited by bombarding with high-energy X-rays or gamma rays.[2]

Aim of the study

To compare XRF analysis of kāsīsa purified by different methods.

Objective of the study

Determination of elemental composition of śuddha kāsīsa by XRF analysis.

Materials and methods

Śodhana of aśuddha kāsīsa was done as per the reference mentioned in Rasataraṅgiṇi, Rasaratna-samuccaya and Rasārṇava.

a) First and second methods of purification was done as per the references mentioned in Rasataraṅgiṇi. First method of purification was done by subjecting aśuddha kāsīsa to svedana in ḍoḷāyantra with bhṛṅgarāja svarasa for three ghaṭika. Figure 1.

Second method of purification was done by svedana in ḍoḷāyantra with bhṛṅgarāja kvāthaḥ for three ghaṭika.[3] Figure 2.

Figure 1
Svedana in Ḍoḷāyantra with Bhṛṅgarāja svarasa

Figure 2
Svedana in Ḍoḷāyantra with Bhṛṅgarāja kvāthaḥ

b) Third method of purification was done as per the reference mentioned in Rasaratnasamuccaya. It was done by dissolving aśuddha kāsīsa in bhṛṅgarāja svarasa and it was kept to dry in sunlight.[4] Figure 3.

c) Fourth method of purification was done as per the reference mentioned in Rasārṇava. It was done by giving three bhāvana(trituration) of kāsamarda (*Cassia occidentalis* L.) svarasa.[5] Figure 4.

Figure 3
Dissolving Aśuddha Kāsīsa in Bhṛṅgarāja svarasa

Figure 4
Bhāvana in Kāsamarda svarasa

XRF analysis of aśuddha kāsīsa and śuddha kāsīsa were done.

Result

The following tables (Table 1, 2 and 3) are showing the quantity of kāsīsa before and after śodhana; XRF analysis: Composition of oxide- (Unit- mass %) and XRF analysis: Composition of element- (Unit- mass %) respectively.

Table 1
Quantity of Kāsīsa before and after śodhana

Kāsīsa	Svedana in ḍoḷāyantra with bhṛṅgarāja svarasa	Svedana in ḍoḷāyantra with bhṛṅgarāja kvātha	Dissolving in bhṛṅgarāja svarasa	Bhāvana with kāsamarda svarasa
Before śodhana (weight)	500 gm	500 gm	500 gm	500 gm
After śodhana (weight)	350 gm	315 gm	300 gm	410 gm

Table 2
XRF analysis - Composition of Oxide (Unit - mass %)

Element	Aśuddha kāsīsa	Śuddha kāsīsa (ḍoḷāyantra with bhṛṅgarāja svarasa)	Śuddha kāsīsa (ḍoḷāyantra with bhṛṅgarāja kvāthaḥ)	Śuddha kāsīsa (dissolving in bhṛṅgarāja svarasa)	Śuddha kāsīsa (bhāvana with kāsamarda svarasa)
Ferric oxide (Fe2O3)	60.8%	65.171%	62.0%	65.183%	63.2%
Sulphur trioxide (SO3)	36.9%	31.609%	28.0%	34.555%	28.5%
Chromium oxide (Cr2O3)	0.444%	0.439%	0.682%	0.66%	0.849%
Silicon dioxide (SiO2)	0.326%	0.111%	0.955%	-------	0.923%
Manganese oxide (MnO)	0.106%	0.024%	0.226%	0.264%	0.502%
Calcium oxide (CaO)	0.0125%	0.3385%	0.521%	0.1385%	0.264%
Potassium oxide (K2O)	-------	3.246%	2.86%	0.525%	0.127%
Cupric oxide (CuO)	-------	0.37%	0.0220%	-------	0.0257%

Table 3
XRF analysis - Composition of Element (Unit - mass %)

Element	Aśuddha kāsīsa	Śuddha kāsīsa (ḍoḷāyantra with bhṛṅgarāja svarasa)	Śuddha kāsīsa (ḍoḷāyantra with bhṛṅgarāja kvāthaḥ)	Śuddha kāsīsa (dissolving in bhṛṅgarāja svarasa)	Śuddha kāsīsa (bhāvana with kāsamarda svarasa)
Iron	71.8%	74.58%	69.2%	76.46%	70.7%
Sulphur	25.0%	16.57%	17.9%	20.75%	18.3%
Chromium	0.513%	0.503%	0.745%	0.873%	0.930%
Silicon	0.257%	0.657%	0.713%	-------	0.691%
Manganese	0.139%	0.299%	0.279%	0.459%	0.622%
Calcium	0.0151%	0.5351%	0.595%	0.2151%	0.302%
Potassium	-------	5.23%	3.79%	0.87%	0.168%
Copper	-------	0.07%	0.0281%	--------	0.0328%

Discussion

After purification by four different methods, weight of kāsīsa before and after śodhana was compared. Loss of kāsīsa by svedana in ḍoḷāyantra might be due to heating procedure after complete dissolving of kāsīsa in bhṛṅgarāja svarasa or bhṛṅgarāja kvāthaḥ. Maximum loss was seen in śodhana done by dissolving śuddha kāsīsa in bhṛṅgarāja svarasa as during its exposure to direct sunlight for drying, loss was observed due to its spillage. Minimum loss was seen in śodhana done by bhāvana with kāsamarda svarasa in khalvayantra as no spillage occurred during śodhana. Their XRF analysis was done. It showed that composition of iron, sulphur and their oxide were maximum in kāsīsa purified by dissolving it in bhṛṅgarāja svarasa. Composition of chromium, manganese and their oxide were maximum in kāsīsa purified by bhāvana with kāsamarda svarasa. Composition of silicon, calcium and their oxide were maximum in kāsīsa purified by svedana in ḍoḷāyantra with bhṛṅgarāja kvāthaḥ. Composition of potassium, copper and their oxide were maximum in kāsīsa purified by svedana in ḍoḷāyantra with bhṛṅgarāja svarasa.

Conclusion

It can be concluded that through XRF analysis, the main composition of kāsīsa which are iron and sulphur were maximum in kāsīsa purified by dissolving it in bhṛṅgarāja svarasa.

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REVIEW ARTICLE
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An approach to lifestyle disorders and its management in children

Chethan Kumar V. K., Shubhangi Rathore and Harshitha M. S.

ABSTRACT: Lifestyle disorders are also called as the disease of civilization. As it takes a long time for a civilization to establish, similarly, these lifestyle disorders takes long time to occur in a healthy being and it becomes difficult to reverse the condition from diseased to healthy. The faulty daily habits like gorging on junk food, addiction to television and aversion to outdoor activities are the three major reasons. Also high fat and sugar rich diet, late night sleep and an inappropriate relationship with their environment lead to lifestyle disorders. The prevalence of childhood obesity, diabetes, hypertension and other life style disorders are increasing alarmingly in India, and are affecting much younger populations than in the West.[1] Even the maternal lifestyle during pregnancy can affect the offspring to have such disorders. An association between such faulty lifestyle and lifestyle disorders were reviewed in different āyurveda and modern classics. Āyurveda offers several measures related to lifestyle which includes dinacarya (daily regimen), ṛtucarya (seasonal regimen), daivavyapāśraya cikitsa (divine/spiritual therapy), satvāvajaya cikitsa (psycho-behavioral therapy), regular pañcakarma (regular internal bio-purification), rasāyana (rejuvenative measures), sadvṛtta (ideal routines) and ācāra rasāyana (code of good conducts) and āhāra and vihāra (dietary patterns and discipline of eating). These measures help to modify lifestyle, promote psychosomatic health and thus help in prevention and management of a wide range of lifestyle disorders and hence serve as measures for maintaining the well-being of children and a healthy nation. This article highlights the different lifestyle disorders in children and their management according āyurveda and modern classics.

Key words: Lifestyle, Lifestyle disorders, Eating habits, Childhood obesity, Prevention

Introduction

Lifestyle disorders are the disorders based on daily habits and an inappropriate relationship with the environment. They are also called as disease of civilization or non-communicable disease.[2,3] A child is dependent for his nourishment when he is in mother's womb and therefore, maternal lifestyle also has an influence on the growth and development of a child.[4] For this reason, āyurveda explains the regimen, a pregnant woman should follow to have a healthy offspring. Ācārya have explained in detail regarding māsānumāsika paricarya (month wise regimen of a pregnant woman) by which the overall growth and development of the fetus can be taken care to have a healthy progeny.[5,5a] If this monthly regimen is not followed by the pregnant mother, it may lead to fetal abnormalities in the antenatal period itself. With the same concept, Barker hypothesis developed by the modern science explains fetal programming which is due to the effect of maternal under nutrition or maternal insult during critical period of the pregnancy.[1] Dietary and living standards of a mother during pregnancy has a very significant effect on the fetus to develop disorders like obesity, insulin resistance and fetal alcohol syndrome which leads to conditions like diabetes mellitus and coronary heart disease in childhood.[6] Childhood is the period of appropriate growth and development of the child. The three main reasons for children to get lifestyle disorder are gorging on junk food, addiction to television and aversion to outdoor activities.[7] The excessive intake of high fat diet like pizza, burgers, etc. along with physical inactivity is the major cause for the development of childhood obesity which in turn leads to many other types of disorders like

diabetes, hypertension, hyperlipidemia, etc. Physical inactivity is the real killer, associated with a 'Sedentary death syndrome' of which obesity and type 2 diabetes mellitus are the most prevalent.[8,9]

There are increased prevalence of obesity, because of decline in physical activity and awareness, together with irresponsible food marketing practices and the widespread use of cheap energy dense foods.[10] Hence, it is the need of the present era of technology and rapid urbanization with faulty lifestyle in children to prevent these disorders through holistic approach.

Literary review

Childhood obesity is a major public health problem of the modern world which has shown increasing trends globally in the recent years.[11] WHO has mentioned it as one of the most serious public health challenges in the 21st century due to its rapidly increasing prevalence and tracking seen till adulthood. Out of the predicted 1.5 billion overweight people in 2015, it is estimated that children will constitute around 10% of which 75% are from developing countries.[12] Parallel rise of conditions like dyslipidemia, hypertension, abnormal glucose tolerance and reduced health related quality of life is seen amongst pediatric age group.

Authors Booth and Lees have reported that based on declining activity levels with children and adolescents now spending as much as 45 hours each week in sedentary screen based pastimes, every child in the United States will be obese by 2044.[9] Obesity is characterized by increased storage of fatty acids in an expanded adipose tissue mass and in peripheral tissues such as the skeletal muscle and liver which is explained as medoroga in āyurveda where the causes are explained as physical inactivity, intake of sweet and high fat diet leading to the development of insulin resistance, explained by the ācārya as prameha pūrvarūpa.[13] A study by Borg and colleagues states that a high fat diet could literally leave you with 'fat on brain'. Studies have shown in both animals and humans that consuming excessive amounts of fat in the diet leads to an increased accumulation of lipid in peripheral tissues which are not designed for fat storage, particularly the skeletal muscle and the liver. Ectopic fat storage which interferes with their ability to function normally leading to co-morbidities associated with diet induced obesity, in particular insulin resistance.[14]

Type 2 diabetes mellitus is due to the combination of insulin resistance with relative insulin deficiency and is becoming prevalent among children due to rise in childhood obesity.[6a] Excessive intake of heavy, unctuous, saline substances, use of new rice or fresh wine, excessive sleep, no mental or physical exercise with sedentary habits leads to augmentation of pitta, meda, kapha and māmsa which obstructs the vāta and drives it down to urinary system leading to madhumeha (diabetes mellitus).[15] Ācārya have described two types i.e.; Jāta pramehi and sthūla pramehi and by looking on to the etiological factors and the features explained they can be compared with type 1 and type 2 diabetes mellitus respectively which are now seen in children.[15a]

It is now considered that most of the chronic disorders like obesity, diabetes mellitus, hypertension bronchial asthma, coronary artery diseases, COPD (chronic obstructive pulmonary diseases), chronic liver diseases, psoriasis, arthritis, etc. are resulted due to faulty lifestyle.[16] Therefore, lifestyle modification is to be considered as major criteria for the prevention and management of such type of disorders. Āyurveda offers a wide variety of dietary supplementation and a range of non-pharmacological measures for prevention and management of lifestyle disorders. There is a great need of time to globalize dietary supplementation and non-pharmacological measures as described in āyurveda system of medicine to promote the health and to reduce the disease burden on the society.

Discussion

Āyurveda offers holistic approach towards prevention and management of diseases. Āyurveda

intervention targets towards complete physical, psychological and spiritual wellbeing, which makes it a wonderful option in treating lifestyle disorders. Āyurveda provides great options in the form of proper dietary management, lifestyle modifications, measures for internal bio-purification and rejuvenation.

Lifestyle medicine is defined as the application of environmental, behavioral, medical and motivational principles to the management of lifestyle-related health problems in a clinical setting.[17] A healthy life style includes a proper balanced diet and physical activity which must be adopted to combat these diseases.

Āyurveda described dinacarya (daily regimen) and ṛtucarya (seasonal regimen) which include dietary and lifestyle modification for an individual depending on his prakṛti (psychosomatic constitution) to maintain the health and hence, to prevent the diseases due to lifestyle modifications. Āyurveda explains various measures for the management of lifestyle disorders which plays a significant role to maintain health and for preventing diseases like; āhāra (diet/dietary supplementation), daivavyapāśrayacikitsa (divine/spiritual therapy), satvāvajayacikitsa (psycho-behavioral therapy), dinacaraya (daily regimen), ṛtucarya (seasonal regimen), pañcakarma (five technologies of internal bio-purification), rasāyana (rejuvenative measures), the sadvṛtta (ideal routines) and ācāra rasāyana (code of good conducts).[18]

WHO developed preventive Global strategy of diet, Physical activity and health (DPAS) in 2004 to overcome lifestyle disorders.[19] Āhāra and vihāra play an important role in the life according to āyurveda. In āyurveda, āhāra and vihāra have been given utmost importance for better living, health and wellness. Āyurveda emphasizes more on āhāra and is considered as prāṇa (basis of life).[20] Āhāra has been described as one of the trayopastambha (three subsidiary pillars) of life.[21] Similarly, diet is considered as vital for a human body as it provides the basic nutrients and promotes longevity. Āyurveda always emphasizes on consuming healthy and nutritious diet for maintaining good health.

As per the view point of āyurveda, both the living human body and the diseases afflicting it are the products of āhāra. Use of hitāhāra (wholesome diet) promotes health and longevity and ahitāhāra (unwholesome diet) promotes manifestation of different disorders.[22] Unfortunately in modern era, the concept of hitāhāra is continuously being ignored leading to the development of lifestyle disorders. Āyurveda offers different pathyāpathya (do's and don'ts) regarding diet/dietary supplementations which definitely help in the prevention and management of a wide range of lifestyle disorders. It has been explained by the ācārya to take guru (heavy to digest) and apatarpaṇa (non-nourishing) āhāra for the management of obesity as it is explained as santarpaṇottha-janya-vyādhi (disease due to over nourishment).[23]

Vyāyāma (exercise) is said to be a preventive management for the disorders due to sedentary lifestyle. Physical activity normalizes elevated leptin level in obesity.[24] In previous studies exercise has proved to be an effective approach for promoting fat oxidation, reducing triglyceride content in skeletal muscle and improving peripheral insulin sensitivity.[25]

In addition to food and diet, āyurveda has a separate concept of medicinal dietary supplements in the context of rasāyana (rejuvenative measures). Rasāyana can be used as nutritional supplement as well as medicine depending upon its various types. Most rasāyana act by promoting the agnibala, acting as direct nutrients and by way of sroto-prasādana (purification of body channels), resulting in an improved nutritional status which leads to an improved quality of dhātu or body tissues.[26] Various studies on rasāyana suggest their action as immunomodulator, adaptogenic, antioxidant, nootropic and antistress.[27]

Dinacarya (daily regimen) is very important in day to day life to maintain biological clock. Therefore, one has to stay aware about this daily regimen for day to day promotion of health, boost immunity and prevention from lifestyle disorders.

Each ṛtu (season) has different effects on the body as well as on the environment. Āyurveda has depicted various regimens (carya), regarding diet and lifestyle to adjust with the seasonal enforcement easily without altering body homeostasis.[18] Hence, ṛtucarya is a very important aspect of preventive measure for various illnesses including lifestyle disorders as mentioned in āyurveda.

Āyurveda describes code of good conducts under the heading of sadvṛtta and ācāra rasāyana. It is a protective factor for maintaining mental health. Furthermore, community participation and civic engagement are associated with better self-reported mental health.

Lifestyle disorders are very common in the present era due to unawareness and ignorance towards proper daily regimen, seasonal regimen and good code of conducts. The therapeutic application of āhāra and vihāra as described in āyurveda is very vast and more scientific. It needs further validation in the management of lifestyle disorders as per the need of present era.[28]

Conclusion

In present era, due to the rapid advancements in technology and rising influence of western lifestyle, parents are unaware about the lifestyle as mentioned in āyurveda. So they are not able to train their children to follow a proper lifestyle mentioned by the ācārya in āyurveda. Children develop the habit of improper lifestyle with excessive eating especially the junk food or fast food along with the addiction to television which makes them to be physically inactive. Due to these reasons, they start to develop lifestyle disorders like childhood obesity, diabetes mellitus, hypertension, hyperlipidemia etc. which affect their growth and development in a significant way. As children are the wealth of a nation, it is our responsibility to guide the parents as well as the children in a holistic way to follow dinacarya, ṛtucarya, sadvṛtta and ācārarasāyana, so as to lead a healthy life and a healthy nation.

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REVIEW ARTICLE

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Toxicity profile of Malla sindūra- a kūpīpakva rasāyana

Pallavi M., Pavan Kumar and Doddamani M. S.

ABSTRACT: Malla sindūra (MS) is a unique rasa yoga, with pārada, gandhaka and malla prepared by kūpī pāka method having indications in vātaroga, āmavāta, viṣūcikā, etc. In this study the pharmaceutical processing as per texts and various physico-chemical and instrumental analysis of malla sindūra was carried out. The objectives of the study included review of malla sindūra, malla, pārada, gandhaka and other associated drugs. Preparation of malla sindūra kajjaḷi (MSK) and malla sindūra as per classical reference, physico-chemical analysis of malla sindūra kajjaḷi and malla sindūra and evaluation of toxic effect of malla sindūra on albino rats by acute and sub acute oral toxicity method were done. Pharmaceutical study included (i) the extraction of pārada from hiṅguḷa by ūrdhva pātanayantra (ii) Gandhaka śodhana in godugdha by subjecting to kūrmapuṭa by bhūdharayantra method (iii) Malla śodhana in kāravellaka svarasa by subjecting to svedana in ḍoḷāyantra for two yāma and (iv) Preparation of MSK. MSK was prepared from pārada, gandhaka and malla in the ratio of 1:1:1/2 and subjected to kūpīpāka in vālukāyantra for 56 hrs. The kaṇṭhastha product was collected. In the analytical study physical, chemical and instrumental analysis was carried out for MSK and MS. MS is a unique kūpīpāka preparation, which is a sāgni, sagandha mūrcchana of pārada, gandhaka and malla in the ratio of 1:1:1/2 through kramāgni pāka for 36-48hours. Śodhana of each ingredient will modify the raw drug into its safe, bioactive, therapeutic form and is an essential preliminary step for all the pharmaceutical procedures of kūpīpakva rasāyana. In the preparation of MS, MSK was subjected to kramāgni pāka in vālukā yantra. The process completed in 56 hrs. Chemical tests denoted that the drug does not contain free mercury and sulphur which proves its safety. A major percentage of mercury was in mercuric form; sulphur in sulphide form and arsenic in arsenate form. Overall assessment was done by observing the analytical parameters and it was found that MS is safe at therapeutic dose.

Key words: Mallasindūra, Kūpīpāka, X-ray Diffraction, Fourier transmission infrared spectroscopy

Introduction

Rasacikitsa is an important stepping stone in the development of āyurveda. Rasaśāstra deals with the preparations of medicines mainly with the help of mercury, minerals, metals and herbs. There are four types of rasauṣadhi which are described in Rasaśāstra. Kharaḷīya rasāyana, parpaṭi rasāsyana, kūpīpakva rasāyana, poṭṭali rasāyana.

Indian alchemy developed a wide variety of chemical processes for the transmutation of metals and preparation of elixir of life, in which mercury occupied a prime position. The literature on rasaśāstra is perceptibly voluminous and methodical in the presentation of a variety of processes whose number is countless. Of these processes, kūpīpakva rasāyana deserves special mention because of its minimal dosage, augmenting effect and long lasting potency.

Malla sindūra[1] is one of the important classical kūpīpakva rasāyana containing hiṅguḷottha pārada, śuddha gandhaka and śuddha malla in 1:1:1/2 proportions. It is sagandha, sāgni, bahirdhūma, kaṇṭhastha kūpīpakva rasāyana potentiated with agni samskāra for 36-48 hours. The process converts the metal into a chemical compound with necessary medicinal benefits like in the treatment of vātaroga, āmavāta, viṣūcikā, etc.

Materials and methodes

Drug review

All together seven varieties of malla sindūra has been mentioned in classics. All these have different ingredients but the similarities being all are kūpī

pakva rasa. Pārada, gandhaka and malla are the common ingradients in all types of malla sindūra.

Ingredients: Śuddha malla : 5 tola, śuddha pārada : 10 tola, śuddha gandhaka : 10 tola and kumārī svarasa : Q.S.

Procedure: The ingredients were taken in the said quantity to prepare kajjaḷi. After attaining kajjaḷi siddhi lakṣaṇa, fine powder of śodhita malla was added and bhavana with kumārī [*Aloe vera* (L.) Burm.F.] svarasa was done and allowed to dry. It was filled in a kācakūpi and was kept in vālukāyantra by giving kramāgni.

Materials

Raw drugs: Major and associated raw drugs.
Equipments: Major and associated equipments.
Collection of raw materials: Raw drugs which were having similar grāhya lakṣaṇa as mentioned in the classics were collected from the market.

Major drugs

Malla: Malla is a crystalline or amorphous substance, white in colour. Its powder resembles the flour of wheat but is much heavier. The surface of malla has a peculiar shine and sometimes there occurs a yellowish tinge which meets with the qualities of grāhya malla.[2]

Hiṅguḷa: It is dark red in colour, heavy with silvery white shining lines on the surface.[3]

Gandhaka: It is yellow, crystalline with smooth surface and strong sulphur odour.[3a]

Place of procurement

Malla: Amrith Kesari, Bangalore.
Hiṅguḷa: Mamata Herbals, Thane, Maharastra
Gandhaka: Jogappa Shanbhaug, Udupi.

Associated drugs

Kāravellaka (*Momordia charantia* L.) svarasa for śodhana of malla, nimbū [*Citrus limon* (L.) Osbeck] svarasa for hiṅguḷottha pārada, haridrā (*Curcuma longa* L.) cūrṇa for hiṅguḷottha pārada, milk for gandhaka śodhana and kumārī svarasa for bhāvana of MSK.

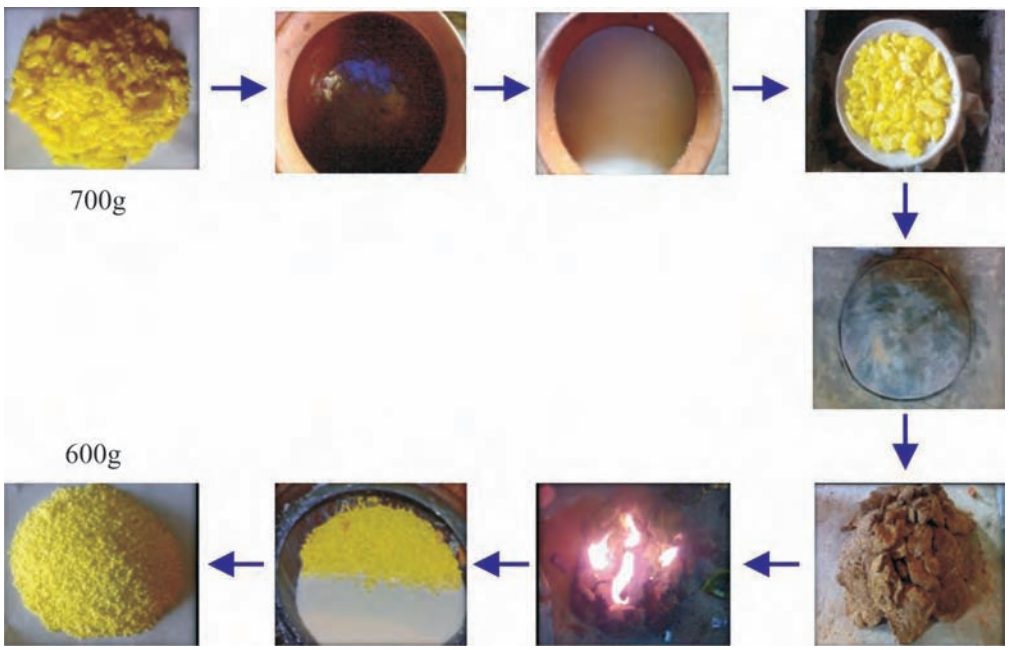
Main equipments: Khalva yantra, ūrdhvapātana yantra, kūrmapuṭa and vālukā yantra.

Methods

The whole method of preparation includes;

1. **Śodhana of raw materials**: Śodhana of malla was in kāravellaka svarasa by svedana method[4] and śodhana of gandhaka in godugdha by kūrma puṭa method.[5] Figure 1.

Figure 1
Gandhaka śodhana



2. **Extraction of pārada from hiṅguḷa**: It was done by ūrdhvapātana method.[6] Figure 2.

3. **Preparation of MSK**: Preparation contained 1part of hiṅguḷottha pārada and 1part of śuddha gandhaka. Trituration was done till kajjaḷī siddhi lakṣaṇa was obtained. Later, half part of śodhita malla (finely powdered) was added and triturated for about 6 hours for a homogeneous mixture of MSK. Later, bhāvana was given to this kajjaḷi with kumārī svarasa and was dried completely in shade. Figure 3 and 4.

4. **Preparation of MS**[1]

1. **Pūrvakarma** (Figure 5)

Figure 2
Hiṅguḷottha pārada nirmāṇa

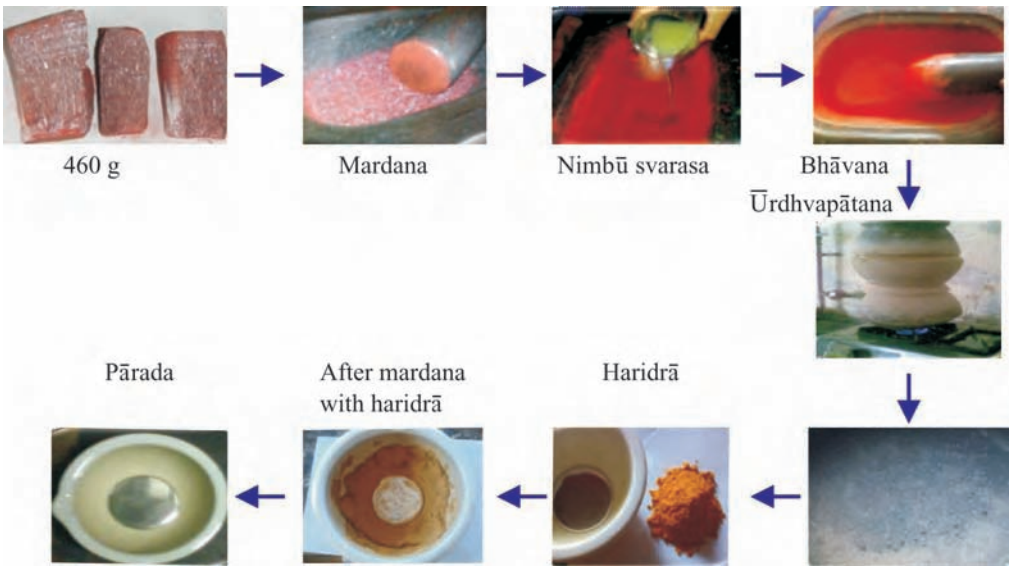


Figure 3
Malla śodhana

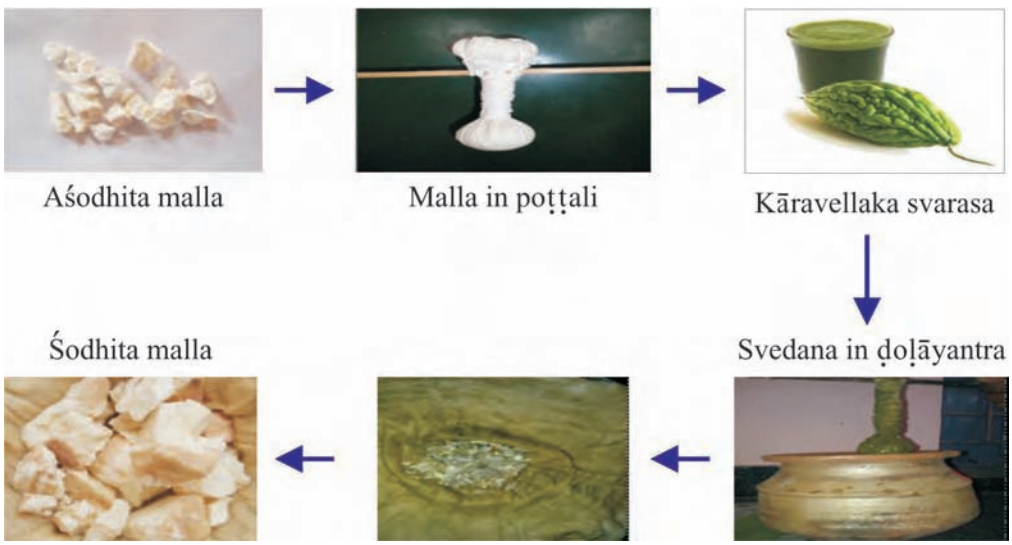
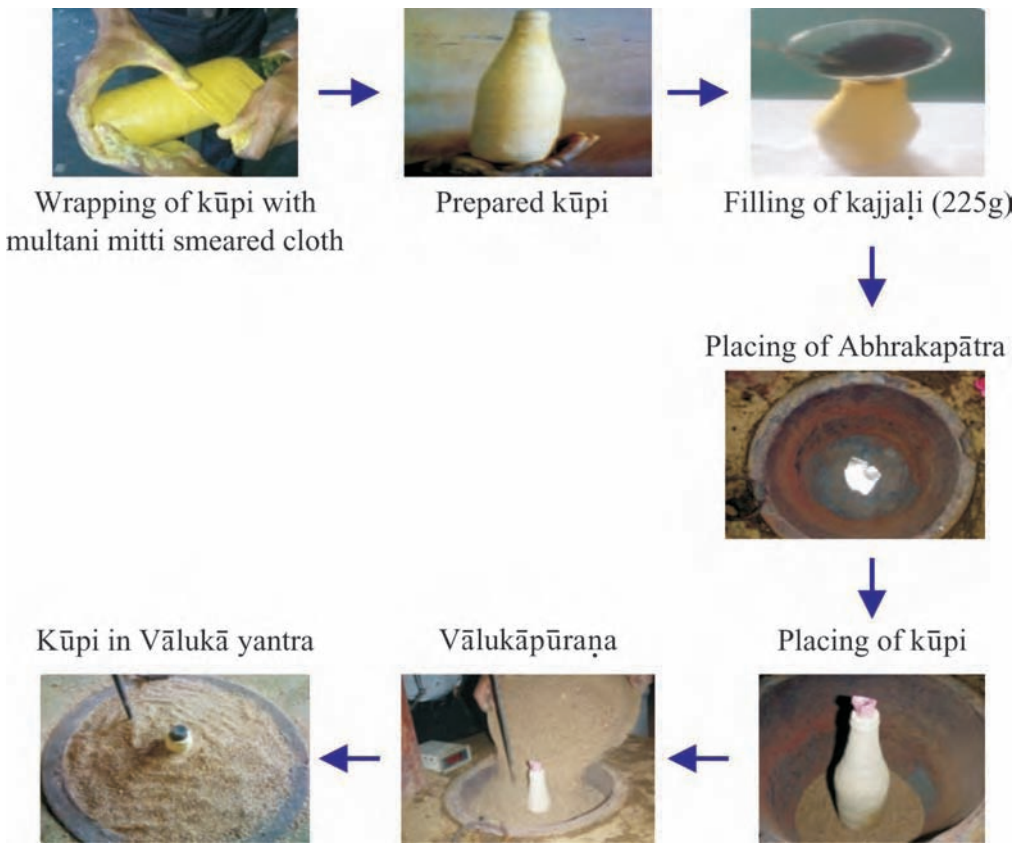


Figure 4
Prepartion of Malla sindūra kajjaḷi

Figure 5
Pūrvakarma in the preparation of Malla sindūra



a. **Preparation of kācakūpī**[7]: Amber coloured glass beer bottle of 650 ml was taken. The bottle was cleaned and completely dried. At the base of the bottle paste of gopīcandana (multani mitti) was applied. A cloth smeared with gopīcandana measuring 6cm x 6cm width and breadth was covered and dried completely. Another cloth smeared with gopīcandana measuring 116cms x 4cms length and breadth was covered in circles, starting from the bottom of the bottle upto mouth of the bottle and was allowed to dry completely.

After completion of 7 layers, one layer of red clay was smeared. Later, one more layer of gopīcandana smeared cora cloth was applied and kept for drying. In this way nine layers were covered over the surface of the bottle.

b. Filling of kajjaḷi in kācakūpī

c. Placing of kācakūpī in vālukā yantra

2. **Pradhānakarma** (Figure 6)

a. Heating schedule (Kramāgni tāpa). Table 1.
b. Observation and recording of temperature.
c. Corking kācakūpi and self-cooling of theapparatus.

3. **Pascātkarma** (Figure 7)

a. Removal of kācakūpi from vālukāyantra.
b. Breaking of kācakūpi.
c. Collection of final product.

Analytical study: Physical tests MSK and MS (Table 2 and 3), Chemical tests MSK and MS (Table 4), X-ray diffraction (Table 5), Particle size of MSK and MS (Table 6), EDX of MSK and MS (Table 7),

Figure 6
Pradhānakarma in the preparation of Malla sindūra

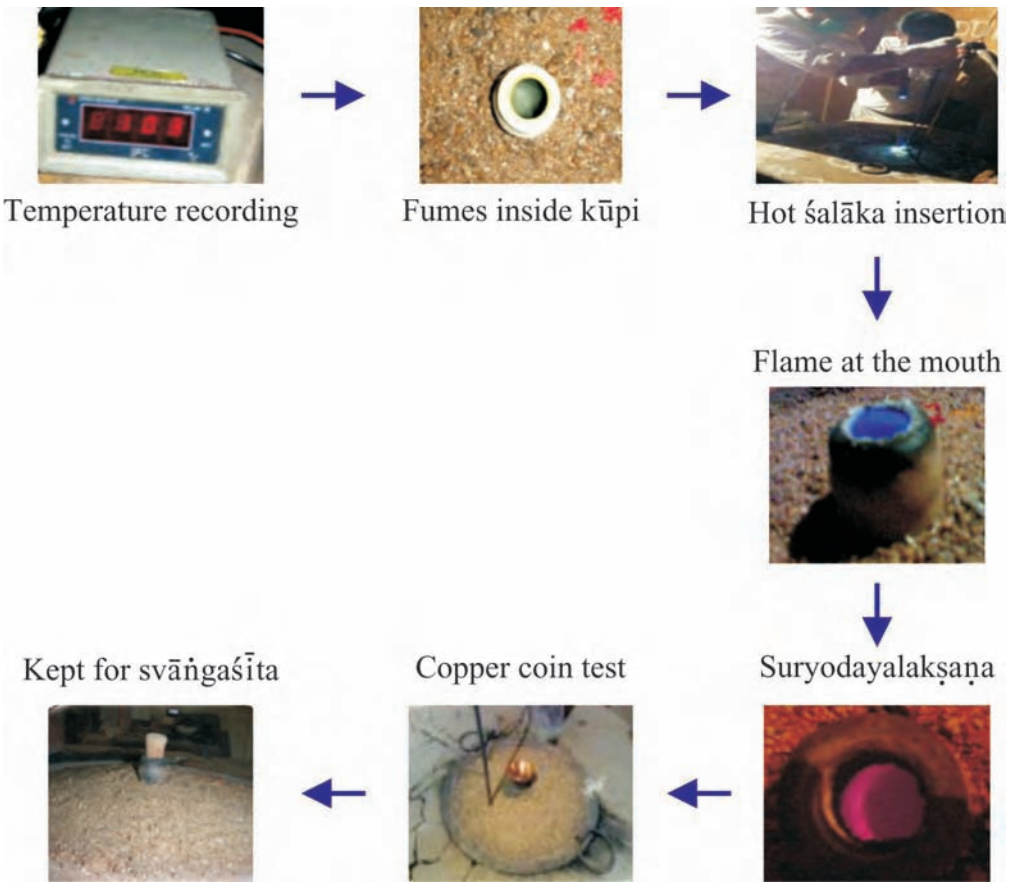
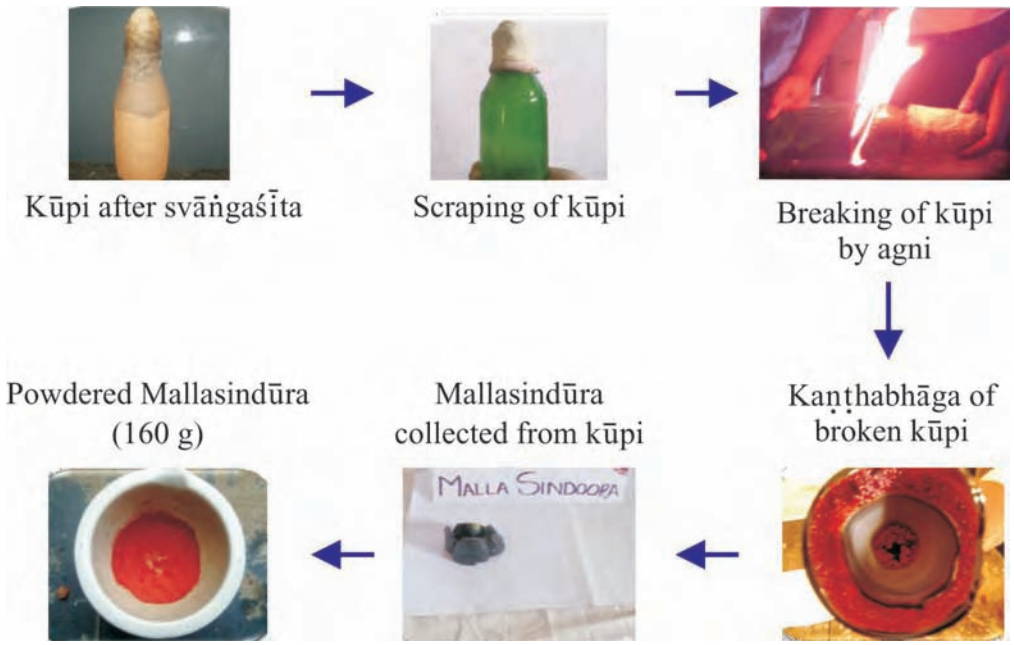


Figure 7
Pascātkarma in the preparation of Malla sindūra



and The Namburi Phased Spot Test (NPST) of MSK and MS (Table 8).

Toxicological study

Acute toxicity study[8]

- According to OECD 425 GUIDELINES, dosing was started with 175mg, as first rat did not showed any toxic symtoms.
- The next animals were dosed with 550mg, 2000mg, 2000mg and 2000mg, according to staircase method (AOT software).
- Further dosing was stopped following the guidelines
- No morbidity and mortality was seen
- The data was fed into AOT software to obtain LD50 value with confidence limit.
- Hence, the LD 50 is greater than 2000mg/kg.

Sub-Acute Study: 28days (AYUSH rule 170)

- Test drug: Malla sindūra.
- Dose selection: Human therapeutic dose of MS 62.5mg.
- The dose administered in different groups of sub-acute study. Table 9
- Route of administration : oral
- Duration of the study : 28 days
- Statistical analysis: one way ANOVA followed by Dunnett's multiple 't' test.
- Animals:-Wistar strain albino rats of either sex weighing between 150-250g.
- Drug Administration: 28 days

Parameters studied

Changes in body weight : recorded once a week

Ponderal changes: The weight of important organs like brain, heart, liver, spleen, lungs, kidney, jejunum, testis and uterus was recorded.

Hematology: The following parameters were measured using automated cell counter: hematocrit, hemoglobin concentration, erythrocyte count, total and differential leucocyte count and platelet count.

Biochemical parameters: Glucose, urea, creatinine, total protein, albumin, globulin, activity of the enzymes SGOT, SGPT and ALPase., serum cholesterol, triglycerides, direct bilirubin and total bilirubin were determined in plasma or serum.

Table 1
Heating schedule (Kramāgni tāpa)

Mṛdvagni			
Date	Time	Temperature in ^{0}C	Observations
Day 1	05.30am	26	Agni started.
	06.30am	247	No fumes inside kūpi, bottom of kūpi could be seen clearly with torch.
	07.30am	275	-
	08.30am	213	Slight white fumes were seen inside kūpi.
	09.30am	193	-
	10.30am	195	Thick dense whitish fumes inside the kūpi.
	11.30am	210	The fumes became dense, can't see the bottom of kupi with torch.
	12.30pm	252	Still dense whitish fumes found inside kūpi.
	01.30pm	253	Śīta śalāka inserted kajjaḷi started melting, it was sticky in consistency.
Madhyamāgni			
Day 1	02.30pm	253	Mild sulphur smell appreciated.Yellowish dense fumes ++
	03.30pm	265	-
	04.30pm	315	Strong sulphur odour appreciated.
	06.30pm	340	Śīta śalāka was inserted, kajjaḷi was slight sticky.
	07.30pm	349	-
	08.30pm	329	Dense yellow fumes observed. Bottom of kūpi was not visible.
	11.30pm	350	Yellowish particles deposited around the neck of kūpi.
Day 2	12.30am	368	Dense gandhaka fumes found. Bottom cannot be seen with torch.
	04.30am	408	Melting of kajjaḷi started.
	05.30am	452	Orange yellow fumes ++
	06.30am	470	Dense yellow fumes + + +
Tīvrāgni			
Day 2	10.30am	488	Śīta śalāka inserted at half the level of kūpi. At this level liquified kajjaḷi adhered- kajjaḷi is boiling.
	11.00 am	488	Hot śalāka was inserted to clear the block. Whitish fumes observed with sulphur smell.
	11.30am	491	Adhering of liquefied kajjaḷi at the mouth of kūpi.
	12.30pm	517	Deposition of kajjaḷi at the mukhabhāga.
	01.30pm	540	Hot śalāka inserted. Blue flame partially observed as the kajjaḷi started adhering to the mouth of the kūpi.
	02.30pm	555	Hot śalāka was inserted three times block was cleared off. Bluish flame at the mouth was found.
	03.30pm	582	-
	04.30pm	578	-
	05.30pm	573	Onset of sūryodaya lakṣaṇa.
	06.30pm	567	Sūryodaya lakṣaṇa +, Sulpher fumes +++
	07.30pm	582	-
	08.30pm	592	Hot śalāka inserted. Blue flame seen at the kaṇṭha bhāga.
	09.30pm	565	-
	10.30pm	583	Hot śalāka inserted. Blue flame seen at the kaṇṭha bhāga.
	11.30pm	580	Sulpher fumes reduced

Day 3	12.30am	582	Śīta śalāka was introduced into kūpi and kajjaḷi adhesion was seen.
	01.30am	607	-
	02.00am	605	-
	02.30am	601	Sūryodaya lakṣaṇa ++, Thick fumes decresed, śīta śalāka inserted and upper 1/3rd was adhering and lower 2/3rd was dry product when rubbed in khalva it showed reddish colour.
	03.30am	597	-
	04.30am	589	-
	05.00am	615	Product when rubbed in khalva reddish color seen.
	05.30am	600	Copper coin test was done. It was positive.ie copper coin was kept over the mouth of the bottle, the surface of the coin turned into greyish white in colour. No flames observed.
	05.45am To 06:45 am	570	Preparation for corking - wood removed, vālukā surrounding the neck region of kūpi was removed. Corking was done with the help of gopīcandana smeared cloth. It took about 45min.
	07:00 am	343	Fire given after corking
	08.00am To 11.30pm	423 To 180	As corking was done, no visible observations were possible.

Table 2
Physical tests of MSK and MS

Physical test	MSK	MS
Colour	Black	Reddish orange
Odour	Odourless	Odourless
Taste	Slightly pungent (characteristic)	Slightly pungent
Touch	Amorphous	Amorphous

Table 3
Parameter tests of MSK and MS

Parameter	MSK	MS
pH	5.61 ± 0.10	6.00 ± 0.10
Total Ash value	0.16 %	0.13 %
Acid Insoluble Ash	0.50%	0.75%
Water Soluble Ash	1.45%	1.50%
Loss on Drying	2.70 %	Nil

Table 5
X-Ray diffraction

Parameters	MSK	MS
Name	Arsenic oxide, metacinnabar	Cinnabar, Arsnolite
Composition	As_2O_3, HgS	HgS, As_2O_3
Crystal system	Cubic ,Cubic	Hexagonal, Cubic

Table 4
Chemical tests of MSK and MS

Contents	MSK	MS
Total Mercury	45.25 %	51.55 %
Mercurous mercury	13.45%	11.45%
Mercuric mercury	30.50%	40.10%
Free Mercury	1.30%	Nil
Total Sulphur	22.13%	13.55%
Free Sulphur	1.25%	Nil
Sulphide	18.35%	12.80%
Sulphate	2.53%	1.75%
Total Arsenic	7.12%	9.45%
Arsanate	5.10%	6.50%
Arsanite	2.02%	2.95%

Table 6
Particle size of MSK and MS

Name of the sample	Mean particle size
MSK	550.0 nm
MS	393.3 nm

Table 7
EDX of MSK and MS

Elements Found	Concentration in %	
	MSK	MS
S	29.10	17.08
As	19.41	14.51
Hg	37.13	56.62

Table 8
The Namburi Phased Spot Test of MSK and MS

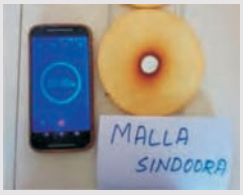
Sample Name	Phase I	Phase II	Phase III
MSK	MSK 1st phase 5min 	MSK 2nd phase 20min 	MSK 3rd phase 1hour 
MS	MS 1st phase 5min 	MS 2nd phase 20min 	MS 3rd phase 1hour 

Table 9
Dose administered in different groups of sub-acute study

Group	No. of animal	Drug	Dose	Duration	Purpose
Control group 1	10	Water	Sufficient	28 days	To serve as control group
Trial group 2	10	MS	5.62 mg/kg. (Therapeutic dose)	28 days	To serve as trial group
Trial group 3	10	MS	28.12 mg/kg. (5 times of Therapeutic dose)	28 days	To serve as trial group
Trial group 4	10	MS	56.25 mg/kg (10 times of Therapeutic dose)	28 days	To serve as trial group

Discussion

Hiṅgulottha pārada

- Citric acid helps for the disintegration of HgS and weaken the bond and hence, facilitates dissociation of mercury.
- Mercury has a low boiling point of 356.9°C. When heat is applied oxygen combines with sulphur to form sulphur dioxide and mercury is liberated in the vapour form at a temperature above its boiling point.
- Impurities like nāga, vaṅga, etc., having high boiling point do not sublimate and remain at the bottom.

Malla śodhana

- The phytochemicals such as charantin, polypeptides, Ca , Fe, Mg, etc. present in kāravellaka svarasa might be reducing the toxicity of malla or be doing a chemical detoxification. Thus making malla a safe, non-toxic and bio acceptable drug.
- The bhedana property of kāravellaka might be having some role in converting viṣa guṇa of malla into medicinal values.

Discussion on toxicology study

It was observed that there is a significant decrease in the mean cell volume (MCV) in TED group and a significant decrease in the mean cell haemoglobin (MCH) in all the TED group.

Thirteen biochemical parameters were studied, out of which significant change was observed in Group TEDx5 and significant increase in SGPT and significant decrease in ALP were observed as compared to control group.

In Group TEDx10, significant increase in sugar, APL, albumin, cholestrol and triglycerides; and significant decrease in SGPT, urea were observed compared to control group.

Liver function test: SGOT and SGPT activity was found decreased in all groups. The observed effect may not be considered as indicative of any underlying pathology.

The weight of lungs, heart, liver, kidney and spleen were not affected to significant extent indicating good tolerance of these tissues. Jejunal weight was found significantly decreased. This may be indicative of loss of tissue. Histological observation showed epithelial layer disruption at all the dose levels but it was comparatively less at higher dose level. Decrease in uterine weight and increased testis weight was analyzed. Histology revealed normal profile of both.

Food intake: TED x10 dose significant increase was observed in all the 4 weeks. This shows the agnidīpana property of MS.

Conclusion

Even though malla sindūra has different pharmaceutical procedures in rasaśāstra classics, in the present study, it has been prepared according to Rasatantra sāra va Siddhaprayoga saṅgraha. It is a sāgni, sagandha, bahirdhūma and kaṇṭhastha kūpī pakva rasāyana, containing pārada, gandhaka and malla in 1:1:1/2 proportions. The kajjaḷi prepared out of them was subjected to kūpīpāka in vālukāyantra for 56hrs through kramāgni. Yield obtained was 71.11%.

The test drug MS was studied to elucidate the acute and sub- acute toxicity profile. Acute toxicity study showed a LD_{50} of greater than 2000mg/kg body weight. In sub-acute study MS was found to be well tolerated even at higher dose level.

Overall assessment was made by observing the hematological, biochemical and food conversion parameters. MS is safe at therapeutic dose, 5 times of therapeutic dose and up to the 10 times of therapeutic dose. Its careful administration is not likely to cause any serious toxic outcomes at the therapeutic dose levels.

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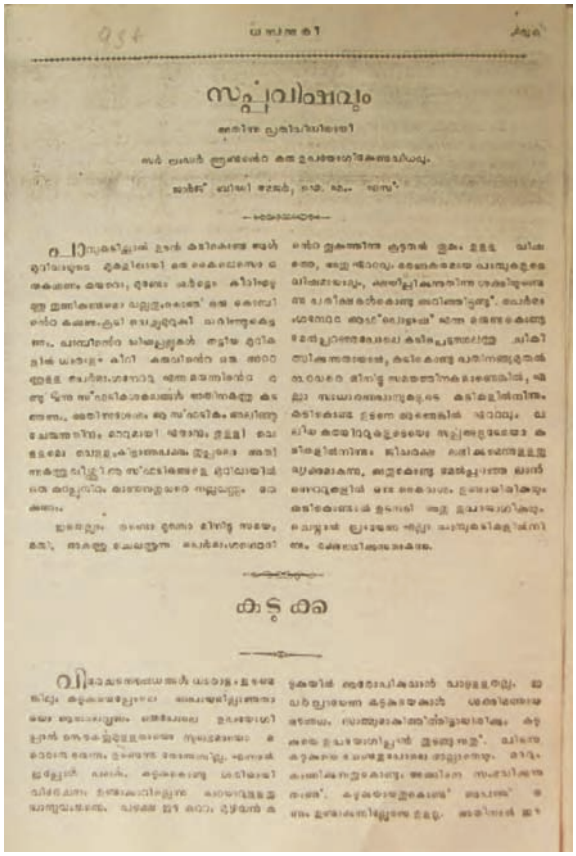
Kaṭukka (Harītakī)

Dhanvantari is the first medical journal in Malayalam published every month by Vaidyaratnam P. S. Varier from Arya Vaidya Sala uninterruptedly for 23 years from 1903 to 1926 . This clinical note was published in its column on Book No. 8, 1086 Minam Malayalam Era (1911 CE), Article No. 10, Page 93A.



സുഖവിരേചനം

കടുക്ക

An effective purgative drug without any side effects that can be administered irrespective of age. But to our dismay, some are of the opinion that it is not as effective as it was earlier. This may be because of the image of stronger drugs or may be because of its faulty usage. One can in no way blame this drug for these reasons! Again, we should not underestimate the power of this drug.

An anonymous saying prevalent among some writers is that this harītakī (*Terminalia chebula* Retz.) was born from the elixir, that Lord Indra was consuming. So one need not be surprised if it is so because such are the properties that this drug displays.

The Sanskrit term harītakī means that this is seen in the abode of Lord Śiva and is green. These exaggerations cannot be ruled out because such great are the properties that this drug displays! A mention of this quote is very much appropriate here without which this article may not be complete.

Harītakī manuṣyāṇām
māteva hitakāriṇī |
Kadācit kupyati mātā
nodarasthā harītakī ||

The fact is clearly stated here. Normally a person who takes a purgative has the following symptoms such as fatigue, stomach ache and discomfort. Besides, a small variation in the dose (if it exceeds) may be a cause for concern. But it is safe to administer harītakī as one need not worry about these side effects. It is very safe to use harītakī. Unlike the other drugs even if one does not purge, there is no side effect to be alarmed of. There is no dought that the person who coined the quote has sufficient researches to his credit!

One can find innumerable formulae with harītakī but it is more effective as a mild laxative.Here I shall mention one or two important formulae with harītakī

that are used as a mild laxative.

Vaiśvānaram, Intuppukāṇam, Hutabhugādi, Pathyāmodakam and Gomūtraharītakī are very effective mild purgatives. Here again, Vaiśāvanaram and Intuppukāṇam are administered in agnimāndya (poor digestion) where as Hutabhugādi for arśa (piles) and Gomūtraharītakī for śopha (water retention/oedema). Pathyāmodakam, on the other hand can be administered in almost all kinds of diseases as a mild laxative. Laxatives are good appetizers. Harītakī of course boosts the digestive fire. It has the quality to alleviate all the three doṣa. It has all the rasa but for lavaṇa, in the ṣaḍrasa. Madhura-tikta-kaṣāya, rasa reduces the vitiated pitta. Likewise madhura-amḷa, reduces the vitiated vāta and kaṭu-tikta-kaṣāya reduces the vitiated kapha. Harītakī is of rūkṣaguṇa and uṣṇavīrya properties. Hence, aids in digestion and enhances the intelligence. Vipāka rasa is madhura. It has the qualities of rasāyana, thereby improving the eye sight and longevity and is anulomaka. It is bṛmhaṇa in nature. It also cures of the troubles due to santarpaṇa. It has been proved to be very effective in śvāsa, kāsa, arśas, prameha, śopha, kuṣṭha, kṛmi, mahodara, svarasāda, grahaṇī, jvara, gulma, ānāha, vraṇa, chardi, hikkā, amḷapitta, hṛdroga, kāmala, śūla, plīharoga, yakṛdroga, aśmarī, mūtrakṛchra, and so on. Again, this science advocates the use of seven types of harītakī for different ailments. It is said that vijaya variety is the best and can be used widely in all preparations. The commonly used variety is this, I guess, writes the author. But the common test to know the quality is to put harītakī in water and the one that is immersed/ the one that sinks is to be used. The exact weight of a harītakī is 6 kazañju.

Harītakī can be used as it is. One can chew it and it helps in digestion. It is good to enhance one's appetite. It can be used with food items (meals). It helps in excretions and improves one's enthuciasm. But decoction do not help in bowel movements, the physicians opines. But to take harītakī that is soaked overnight in buttermilk, early in the morning is good. But it is not advisable to chew harītakī in the following conditions; a person who is fatigued because of long walks, a weak person, rūkṣa and kṛśa individual, fatigued after laṅghana, diseased from pitta doṣa, pregnant and after raktamokṣaṇa.

Harītakī is advised after food and it is very effective in the foresaid ailments. Powdered harītakī mixed with ghee is very good for people with vāta constitutions where vātadoṣa dominates; with sugar for pitta constitutions and with saindhava (rock salt) for kapha constitutions. Harītakī mixed with jaggery is advised for all. For rejuvenation the following is advised.

- Harītakī with rock salt in rainy season.
- Harītakī with sugar in autumn.
- Harītakī with śuṇṭhī (dried ginger/ *Zingiber officinale* Roscoe) in early winter.
- Harītakī with pippalī (*Piper longum* L.)in late winter.
- Harītakī with jaggery in summer.

[Varṣam- Rainy, Śarat- Autumn, Hemantam- Early winter, Śiśiram- Late winter and Grīṣmam- Summer.]

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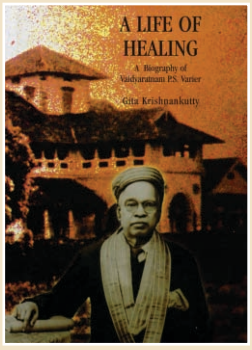

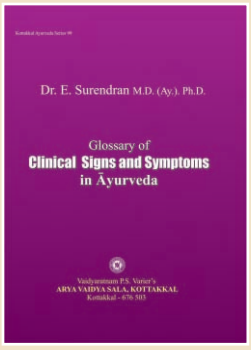

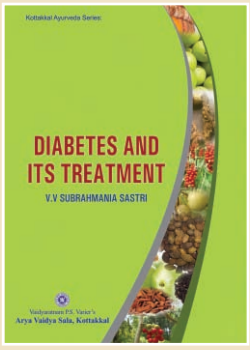

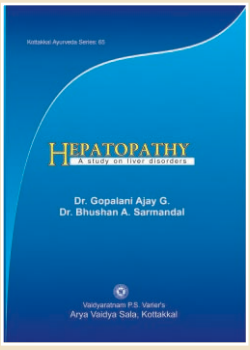

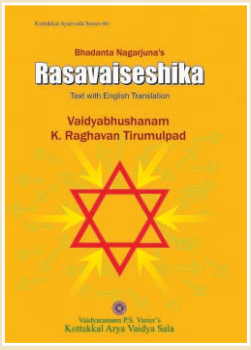

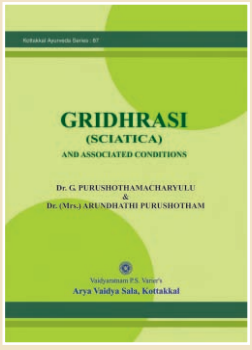

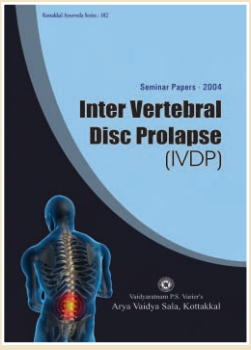

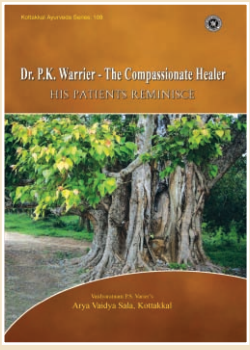

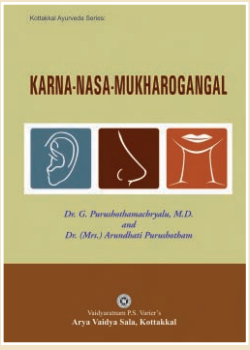

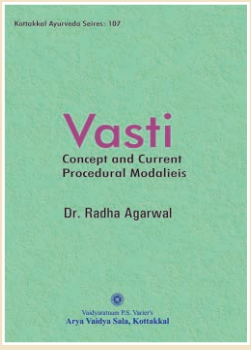

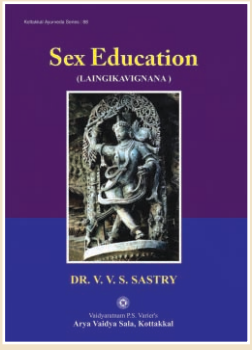

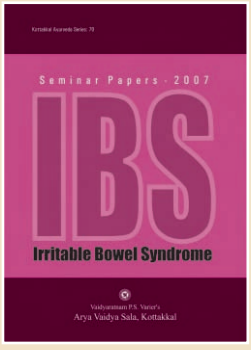

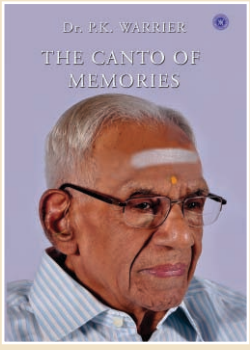

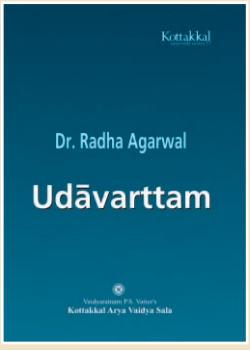

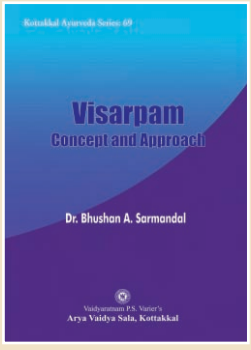

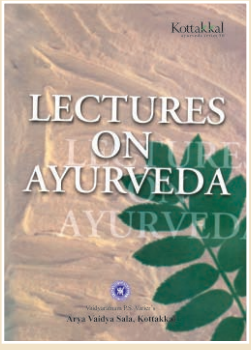

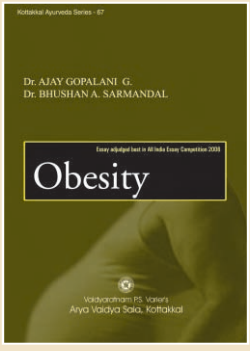

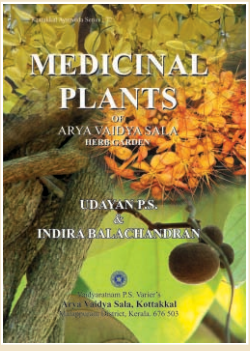

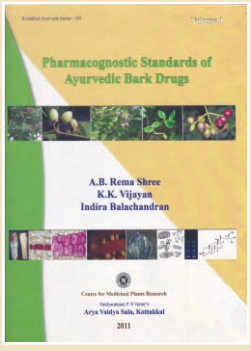

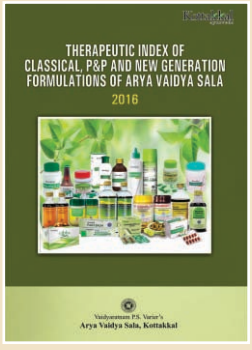

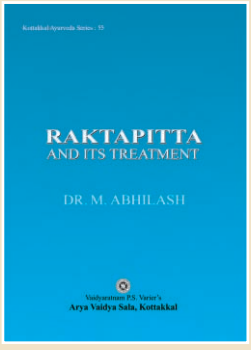

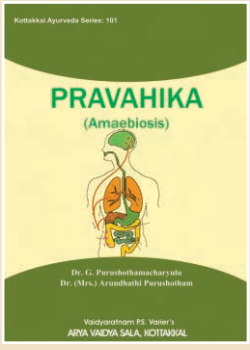

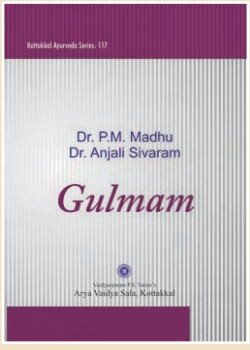

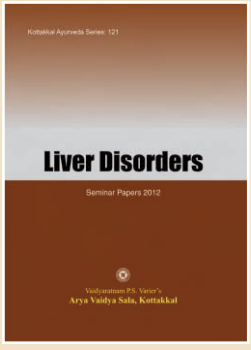

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