

Safety Evaluation of Instantly Prepared Three Samples of Madhuthailika Basti in Wistar Rats



Medical Science

KEYWORDS :

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ABSTRACT

Basti is regarded as the prime treatment modality among the five therapeutic measures in panchakarma. A huge number of Bastis are practicing in Ayurvedic hospitals with panchakarma centers. But to prepare the Basti formulation is a tedious process involving more manpower and time. Hence to overcome these difficulties, three methods of instant basti formulation had been developed. But as these are non-classical method, safety is to be evaluated. In the present study, three groups of animals were selected and instant basti was administered. 14 days of acute toxicity study was carried out as per OECD guidelines (420). All experimental animals were observed for the duration of 14 days. No mortality was found among any of the animals throughout the study. After washout period, animals were administered with yoga basti and organ histopathology of Brain, Lungs, Heart, Stomach, Spleen, Pancreas, Liver, Small intestine, Large intestine, Kidneys were carried out. No evident toxic signs in organs were observed in histopathology study. Hence the present study reveals that, instantly prepared three methods of Madhuthailika basti were safe as it doesn't show any mortality and toxic signs.

Introduction

Ayurvedic formulations are mainly prepared by basic classical methods explained. These traditional methods were dependent on the status of technology that was available at that time. Therefore the preparation methods can be modified using the present technologies to make them more acceptable for the present generation. Ever increasing urbanization and more dependency on readymade preparations has amplified the demand of Ayurveda products in many folds

Basti is regarded as the prime treatment modality among the five therapeutic measures in panchakarma. It is having a wider range of therapeutic applications and is considered as ardhachikitsa (Half of the treatment) as well as purnachikitsa (Complete Treatment) in the disease management. A Serial order of mixing of ingredients of niruha basti as described by charaka honey and rock salt are mixed together in the beginning followed by addition of sneha, kalka (fine paste obtained after wet grinding of the plant material), kashaya (decoction) one after the other with proper churning to form a homogenous mixture.

A huge number of Basti are practicing every day all over the country in Ayurvedic hospitals with panchakarma centers. But to prepare the Basti formulation is a tedious process involving more manpower and time. All this cause much inconvenience to physicians as well as patients. Its preparation is not easy to adopt in OPD basis. Hence to overcome these difficulties, instant three methods of basti preparation had been done such as Freeze dry method, Preservative method and Ghana method. But as these are non-classical method, hence safety is to be evaluated. Taking all these factors into consideration, in the present study, an attempt was made to evaluate safety of instantly prepared Madhuthailika basti in Wistar rats.

Materials and Methods:

Table Number: 1 Showing ingredients and proportion of classical Madhuthailika basti

SNo	Name of Drug	Latin name	Part	Quantity
1.	Makshhika	----	1 pala	50 ml
2	SaindhavaLavana	Sodium Chloride	½ karsha	12 gm
3	MoorchitaTilataila	----	1 pala	50 ml
4	Shatapushpa Kalka	AnethumsowaKurtz	1 karsha	25 gm
5	ErandamoolaKashaya	Ricinuscommunis.Linn.	2 pala	100 ml

Test formulation

The raw materials (Table-1) of Madhuthailika Basti3 were procured from GMP Certified KLE Shri BMK Ayurvedamahavidyalaya pharmacy Moorchitatilatailawas used as Sneha (oil) & Shatapushpa Kalka (Paste of hurb) as it is a commonly used Kalka for Basti. From these ingredients Madhuthailika Basti was prepared by classical method, and developed in to instant form.

Ghana basti

Instant basti packet containing of separate sachets of Makshhika (50 ml), Saindhalavana (12 gm), Moorchitatilataila (50 ml), Shatapushpachoorna (25 gm) and Erandakashaya Ghana tablet (1.25 gm) is prepared. Instead of preparing kashaya, by using Ghana, 100 ml of kashaya was prepared and basti was prepared classically.

Preservative basti⁵

2% (2gm) of Carboxy methyl cellulose (CMC), Potassium Meta bi sulphite was added to classically prepared basti.

Freezer dried basti⁵

230 ml of Madhuthailika basti was prepared by classical method. It was transformed in to special sterile air tight freezer drier bottles and was loaded to freezer drier instrument till it attains semi crystalline consistency. During the administration, 200 ml of hot water of temp 37°C was added to crystalline form of basti and shake well, homogenous mixture of instant Madhuthailika basti was prepared.

Acute toxicity study (OECD 420)

Experimental Animals: 15 Wistar rats of 150-200g (6 males, 9 Females)

Dose schedule – 300 mg to 5000mg/kg body weight

Total Duration – 14 days

Three groups containing Five Wistar rats (3 female, 2 male) were taken for the toxicity study. To easy identification each animal marking was given by the saturated picric acid as follows 1) Head 2) Neck 3) Body 4) Tail. 5) Limb.

Animals

Wistar rats weighing 150-200 gm were procured from animal house, K.L.E.U., Jawaharlal Nehru Medical College, Belgaum, Karnataka and Experimental study was conducted at the Animal house K.L.E.U., ShriB.M.K. Ayurveda Mahavidyalaya Belgaum. All

animals were housed in colony cages at an ambient temperature 22°C ± 3°C and 45-55% relative humidity with 12/12 hr natural light & dark cycle. All animals were acclimatized in the laboratory about a week before commencement of the study. They fed with free access of standard pellet diet (Amruta feeds, VRK's Scientist's Choice Laboratory Animal Feed, Baramati, supplied by SaiDurga Feeds and Foods, Bangalore) and fresh water *ad libitum*. Floor bed was changed every day, to maintain hygienic condition. The experiment protocol has been approved by the Institutional Animal Ethics Committee (BMK/IAEC/Res No 08/2014).

Dose fixation

The quantities of ingredients in liquid form were determined in ml by considering the specific gravities of the basic component of the formulation and converting the grams to ml. Thus, the final prepared 240 ml (Which is the dose of Madhuthailika basti as per Sharangdharasamhita). The dose of Madhuthailika basti *Basti* was calculated by extrapolating the therapeutic dose to rat dose on the basis of body surface area ratio by referring to the table of Paget and Barnes (1964).

Prior to the experiment proper, to rule out any possible practical difficulty while administering this particular dose of *Niruha Basti* (Decoction Enema) and to ascertain up to what region of colon this dose of *Basti* will reach, a pilot study was carried out in which two rats were used. Rats were administered with above mentioned dose of *Niruha Basti* and observed for any ill effect. Further the animal was observed for basti pratyagamana.

Administration of the test drug

Instant Madhuthailika Basti prepared of three methods was administered to the overnight fasted animal after doing local *Abhyanga* (Oleation) at lower abdomen and lower back with luke warm *Moorchitha Taila* (medicated oil) and *Swedana* (Sudation) with hot water steam. To administer the test drug, simple rubber catheter (No. 4) attached to 20cc plastic syringe was used.

Experimental protocol:

Table Number: 2 Showing Experimental protocol of acute toxicity study.

Groups	Treatment
Group A (n=5) (3female, 2male)	Receives freezer dried basti
Group B (n=5) (3female, 2male)	Receives Preservative basti
Group C (n=5) (3female, 2male)	Receives Ghana basti

The selected rats were grouped as Group A, Group B and Group C and were given *Instant Madhuthailika Basti*. 1st day one female rat was selected from each group and basti was administered in a dose of 300mg/kg body weight. The animals were observed for 24 hours for mortality and toxic signs. 2nd day one female animal and one male animal were selected from each group and basti was administered in a dose of 2000mg/kg body weight. The animals were observed for 24 hours for mortality and toxic signs. On the third day one female animal and one male animal were selected from each group and basti was administered in a dose of 5000mg/kg body weight. The animals were observed for 24 hours for mortality and toxic signs. All the animals were observed for duration of 14 days for mortality and toxic signs as per the guidelines.

The animals were observed for the following criteria's as per the OECD guide lines.

- 1) Changes in skin
- 2) Changes in Fur
- 3) Changes in Eyes
- 4) Behav-

- ioral pattern
- 5) Salivation
- 6) Respiration
- 7) Increased motor activity
- 8) Decreased motor activity
- 9) Central nervous system
- 10) Convulsions
- 11) Basti pratyagamana
- 12) stool and its consistency

Histopathology Study:

Table number: 3 showing Yogabasti schedule.

Days	1	2	3	4	5	6	7	8	9
Basti	A	N	A	N	A	N	A	A	N

Anuvasana Basti (A) (Oil Enema) was given with *Moorchitha-Tilataila Niruha Basti* (N) (Decoction Enema) was given with *Madhuthailika basti* [Group A- Freeze drier basti, Group B – Preservative basti, Group C – Ghana basti, Group D (control group) – Normal classical basti] After 14 days of observation no toxic effect is noticed in any of the animals. After the wash out period of 20 days, two animals from each group had been taken. From the Ghana group total 4 animals has been taken, among that two animals given with normal basti and considered as control group (Group D). For all the animals yoga basti is given for duration of 8 days as per classical method. 9th day *niruha basti* is given and animals were dissected to collect organs (Brain, Lungs, Heart, Stomach, Spleen, Pancreas, Liver, Small intestine, Large intestine, Kidneys) for histopathology study.

Results and discussion:

Instant basti by above mentioned methods are newly developed non classical formulation, hence safety is to be evaluated.

As per OECD 420 guidelines, for acute toxicity study, female Wistar rats are used. But, as per the ethical committee suggestion in the present study both male and female Wistar rats were used.

All experimental animals were observed for the duration of 14 days. No mortality was found among any of the animals throughout the study. No evident toxic signs were observed throughout the study. No changes found in Fur, Eyes, Skin, Sleeping, Respiration throughout the study. No effect on gross behavior at any dose level studies (300mg/kg, 2000mg/kg and 5000mg/kg body weight) were noticed. All animals had taken food and water normally within a range of 10-14gms and 18-21 ml respectively throughout the study. All animals gradually gained body weight within a range of 7-15 gms throughout the study. Urine was found normal in colour and consistency throughout the study. Stool was found normal in colour, with oily nature throughout the study. During *Basti pratyagamana*, stool was liquid in consistency it was observed after 5-10 min of basti administration in most of the animals. In some animals *basti pratyagamana* was not observed up to 30 minutes.

Basti as a treatment procedure administered, for a minimum duration of 8 days. Hence by administration of basti in a single dose for one day exactly may not reveal the organ toxicity. So in the present study basti was administered for a minimum duration of 8 days (*Yoga basti*) to assess the organ toxicity by histopathology study. As the basti is administered in the rectal route and maximum absorption is taking place in intestinal part, to rule out the organ toxicity, histopathology study of intestine is selected. Histopathology study of selected organs reveals no significant abnormality or organ toxicity.

Conclusion:

From the results obtained from acute toxicity study, it becomes clear that the instantly prepared three methods of *Madhuthailika basti* were safe as it doesn't show any mortality and toxic signs.

It is necessary to undertake detailed study of chronic toxicity using more number of animals (8-10 in a group) along with all

organ histopathology. So that, more accurate results concern to toxicity and safety can be obtain in detail. As well as to rule out the efficacy of instant basti, a comparative study with classically prepared basti can be undertaken.

From primary evidence obtained it can be said that, the instantly prepared Madhuthailika basti is safe in all aspect as per the study.

Figure 1,2,3,4 Showing Histopathology study of large intestine of single animal of all groups.



Figure (1) Group A

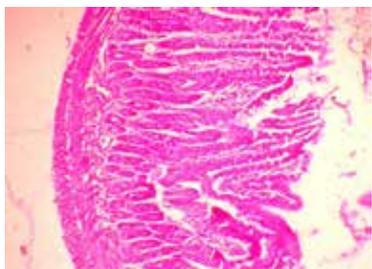


Figure (2) Group B

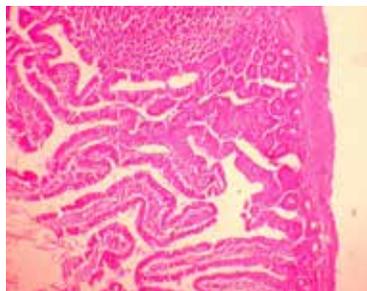


Figure (3) Group C

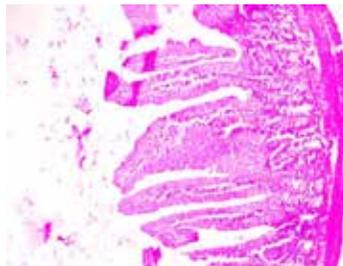


Figure (4) Group D

Acknowledgement:

The authors acknowledge Dr K S Gudaganatti, Dr R RHiremath for guiding throughout the study. Authors are thankful to Dr Ammanagi, Pathologist for his direction and final reading of histopathology study.

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