



Research Article

AN EXPERIMENTAL STUDY TO EVALUATE THE ANTI-DIARRHEAL ACTIVITY OF
BHUVANESHWARA RASA IN WISTAR ALBINO RATS

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ABSTRACT

Atisara (Diarrhea) is most commonly encountered disease of the present era, due to unhealthy and irregular habits. *Atisara* (Diarrhea) finds a place as important disease in individual's life as everyone suffers from it at least once in life time. *Bhuvaneshwara rasa* is a unique herbo-mineral formulation explained in *Bhaishajya ratnavali* indicated for all kinds of diarrhea cases. *Bhuvaneshwara rasa* is a potent formulation having *Saindhava*, *Triphala*, *Yamani*, *Bilva peshika* and *Gruhadhooma*.

Materials and methods: Raw materials were screened and collected and the formulation selected for the present study *Bhuvaneshwara rasa* was prepared accordingly. Anti-diarrheal activity of *Bhuvaneshwara rasa* was evaluated experimentally in albino rats. Experimental study was conducted in 3 groups of animals for anti-diarrheal study each. Anti-diarrheal study was done by castor oil induced diarrhoea and castor oil induced enter-pooling method. *Bhuvaneshwara rasa* (test drug) and Loperamide are effective in controlling diarrheal episodes. *Bhuvaneshwara rasa* has shown significant Anti-diarrheal activity in both Castor oil -induced diarrhea and castor oil induced enteropooling, test drug effective than control.

Result: *Bhuvaneshwara rasa* was prepared according to SOP. Test drug group have shown effect experimentally. **Conclusion:** *Bhuvaneshwara rasa* is a good anti-diarrheal drug. It can be administered in all types of *Atisara*.

INTRODUCTION

'Rasashastra' is the science specially deals with the mercury, other minerals and poisonous drugs. These drugs are pharmaceutically processed and rendered it for internal administrations.

Diarrhea is the condition of having three or more loose or liquid bowel movements per day [1]. The loss of fluids through diarrhea can cause dehydration and electrolyte disturbances such as potassium deficiency or other salt imbalance. According to the World Health Organization, diarrhea affects 3-5 billion people per year worldwide and cause 5 million death per year[2].

It is an important health problem in all age groups and is a major cause of death in socio-economical backward class of people.

In Ayurvedic classics Diarrhea is correlated with *Atisara*. *Atisara* is a condition where watery stools are passed in excess, several times a day, through *guda*[3]. Most important factor in the pathogenesis of *Atisara* is *Mandagni*. *Dalhana* commentator of *Sushrurha Samhitha* mentioned that passing of watery stools in increased quantity is the cardinal feature of *Atisara*. *Amadosha* results due to *Agnidushti* caused by *Mithyahara vihara*, ultimately manifesting as *Atisara*[4]. Drugs which are mainly of *Deepana*, *Pachana* and *Langhana* should be adopted in the *Amavastha* of *Atisara* and drugs which have *Sthambhana* properties are selected in *Niramavastha*[5]. Ayurvedic literature records it found as both a symptom and an independent disease.

In modern science, Loperamide and Bismuth subsalicylate are used to treat diarrhea. They may cause side effects like dizziness, tinnitus, blackened

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stools, constipation, fatigue, abdominal pain, hives, and wheezing^[6]. Number of *Atisarahara* drugs has been mentioned in our Ayurvedic classical texts. *Bhuvaneshwara rasa*^[7] is one such simple formulation explained in the *Atisara Chikitsa Prakaranam* of *Bhaishajya Ratnavali*, which is typical combination of drugs to combat the disease *Atisara*. The combinations of *Saindhava*, *Triphala*, *Yamani*, *Bilva peshika*, *Gruhadhooma* are capable to overcome the disease *Atisara*. In this formulation *Saindhava*^[8] and *Triphala*^[9] acts as *Agni deepaka*, *Bilva*^[10] and *Yamani*^[11] acts as *Deepana* and *Pachana karma*, *Bilva peshika* helps in *sthambhana*, and is also *Grahi*. *Triphala* and *Yamani* acts *Shoolahara*. *Triphala* also act as *Krimighna karma*. *Gruhadhooma* helps in *Vishahara*.

In the view to contribute a safe and effective anti-diarrheal formulation, the present study "An experimental study to evaluate the anti-diarrheal activity of *Bhuvaneshwara Rasa* in wistar albino rats" was planned.

AIMS AND OBJECTIVES: To evaluate the anti-diarrheal activity of *Bhuvaneshwara rasa* in wistar albino rats.

MATERIALS AND METHODS

Method of Preparation of *Bhuvaneshwara Rasa* (28/09/2020 -01/02/2020)

The ingredients are *Saindhava* (rock salt), *Triphala* [*Haritaki* (*Terminalia chebula* Retz.), *Vibhitati* (*Terminalia belerica* Roxb.)], *Amalaki* (*Phyllanthus emblica* Linn.), *Yamani* (*Carum copticum* Benth & Hook.), *Bilva peshika* (*Aegle marmalose* Corr.), *Gruhadhooma* (chimney soot). These were collected and authenticated. *Bhuvaneshwar rasa* was prepared Fig. 1 as per SOP in the Teaching pharmacy, Department of Rasa shastra and Bhaishajya kalpana, Ramakrishna Ayurvedic Medical College and Hospital, Yelahanka, Bangalore.

- All herbal drugs were washed with water and dried and powdered finely.
- All the ingredients are weighed accurately.
- The homogeneous mixture of *Saindhava*, *Triphala*, *Yamani*, *Bilva peshika*, and *Gruhadhooma* was taken.
- Required quantity of water was added and triturated until consistency to roll the pills.
- A *Vati* of one *Masha* (1gm) size was prepared and 100 such *Vatis* was prepared preserved in air tight container.

Figure: 1 Preparation of *Bhuvaneshwara rasa*





Bhavana process

Subhavitha lakshana Bhuvaneshwara rasa

Experimental study (14/12/2020-28/12/2020)

Test Drug Used

Bhuvaneshwara rasa: Prepared and was stored in clean air tight container.

Standard Drug Used

Loperamide

Loperamide, sold under the brand name Imodium classifies as an anti-diarrheal agent., it is a highly lipophilic synthetic phenylpiperidine opioid among others is a medication used to decrease the frequency of diarrhea. It is often used for this purpose in inflammatory bowel disease and short bowel syndrome.

Selection of Animals: Healthy wistar albino rats, which weigh Fig.2 in range of 150-250gm were taken from the animal house attached to the pharmacology laboratory of Invivo Biosciences, Bengaluru. The animals were housed in standard laboratory condition by exposure to natural day and night cycle with temperature of 25°C and 50-70% relative humidity in well-ventilated conditions kept in polypropylene cages. Animals were fed with rat pellet feed supplied by VRK Nutrition Solutions, and water ad libitum Fig.3.

The animals were randomly selected, marked with picric acid to permit individual identification Fig.4, and kept in respective cages Fig.5 for 7 days prior to start of the administration of medicine to allow for the acclimatization to the laboratory conditions. The experimental study was conducted after obtaining the permission from the institutional ethics committee in accordance with the guideline formulated by CPCSEA. Approval No: IAEC Invivo-106, 28-12-2020.

Route of Drug Administration

The test drug *Bhuvaneshwara rasa* was administered Fig.6 according to the body weight of animals by oral route, with the help of a gastric catheter of suitable size sieved to a syringe nozzle.

Inclusion Criteria

1. Healthy rats of either sex will be considered.
2. Weighing about 150g to 250g.

Exclusion Criteria

1. Infected, pregnant and diseased rats.
2. Rats which are under trial for other experiments.

Castor oil-induced Diarrhea in rats.^[12]

Total 18 Albino rats of either sex weighing between 150g to 250g will be taken and divided randomly into three groups Table: 1, each group containing six rats.

Table 1: Grouping of Animals in Castor Oil Induced Diarrhea Study

Group I	Normal saline (Control group)
Group II	Loperamide (Standard group)
Group III	<i>Bhuvaneshwara rasa</i> (Trial group)

All healthy Albino rats which will be selected for the experiments and kept under fasting for 18 hours, Group-I will be given Normal saline (2ml/kg) which served as control group. Group-II will be given Loperamide (5mg/kg) as standard group, Group III will be administered with *Bhuvaneshwara rasa*. All doses will be administered orally. After 1 hour, all groups received 1 ml of castor oil orally, and then animals were placed in the cages lined with absorbent papers Fig.7 and observed for 4 hour for the presence of diarrhea Fig. 8 defined as watery (wet), unformed stool. The control group result considered as 100%. The activity of each group will be

expressed as percent inhibition (%) of diarrhea. The percent inhibition of defecation will be measured by using following formula.

$$\text{Percentage inhibition of defecation} = \frac{[(A-B)/A] \times 100}{1}$$

A –Mean number of defecation time caused by castor oil

B- Mean number of defecation time caused by drug.

Castor oil Induced Enteropooling^[12]

Table 2: Grouping of Animals in Castor Oil Induced Entropooling Study

Group I	Normal saline (Control group)
Group II	Loperamide (Standard group)
Group III	<i>Bhuvaneshwara rasa</i> (Trial group)

Rats of either sex (150-250g) will be fasted 24h but allowed free access to water. They will be divided into three groups. Group I, which will be served as control group, will be administered with Normal saline (2ml/kg). The group-II, which will be served as standard will be administered with loperamide (5mg/kg) by oral route. Group-III will receive *Bhuvaneshwara rasa*, respectively 1 hour before the oral administration of castor oil. 2 hours following the administration of castor oil, each rat will be sacrificed by using specific instruments and whole length of the intestine from pylorus to cecum will be dissected out, its content collected in measuring cylinder and volume will be measured.

Castor Oil- Induced Diarrhea in Rats



Fig.2: Weighing of Rats



Fig.3: Providing Food And Water



Fig.4: Marking For Identification



Fig.5: Grouping The Rats



Fig.6: Dosing



Fig.7: Individual Housing of Animal for Feces Observation



Fig.8: Feces Weighing

OBSERVATIONS AND RESULTS

Anti-diarrheal Activity

After the induction of diarrhea, all the albino rats were closely observed for their behavior and symptoms. The observations noted are mentioned below:

- Half or complete eye closing
- Abnormal posture: For slightly arched back, for arched back or for very arched back
- Activity: Moderate or little activity
- For ruffled or very ruffled fur aspect

All these symptoms were used as pain indices in diarrhea.

The quantity and consistency of fecal output in rats confirmed that they were suffering from diarrhea.

After castor oil administration, the rats were individually placed in cages lined with adsorbent papers and observed for 4 h and the data is collected.

Castor Oil - Induced Diarrhea in Rats

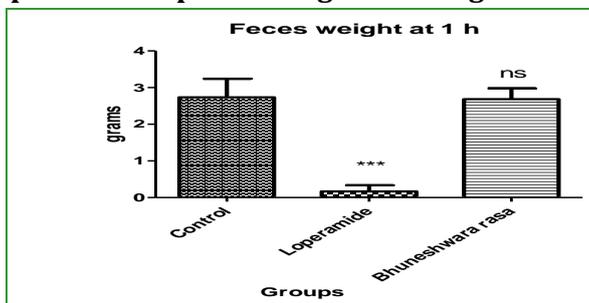
Observation at one hour

Here the faeces weight was recorded and observed at one hour.

Table 3: Mean Values of Fecal Weight at 0-1 hour

Group	Mean faeces weight (in gm)	SEM
Control	2.7	0.5
Loperamide	0.2	0.2
<i>Bhuvaneshwara rasa</i>	2.7	0.3

Graph No.1: Graph showing Feces weight at 1 hour



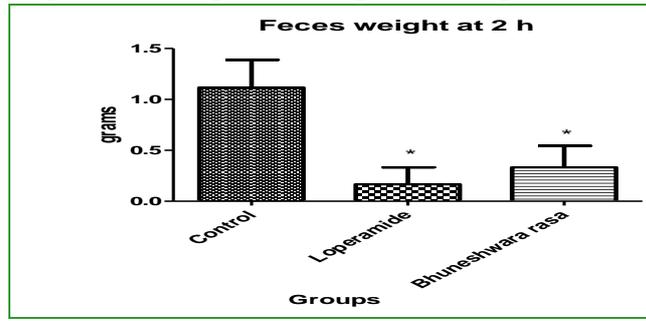
There was no markable changes observed at first hour.

Observation at 2 hour

Table 4: mean values of fecal weight at 2 hour

Group	Mean faeces weight (in gm)	SEM
Control	1.1	0.3
Loperamide	0.2	0.2
<i>Bhuvaneshwara rasa</i>	0.3	0.2

Graph No.2: Graph showing feces weight at 2 hour

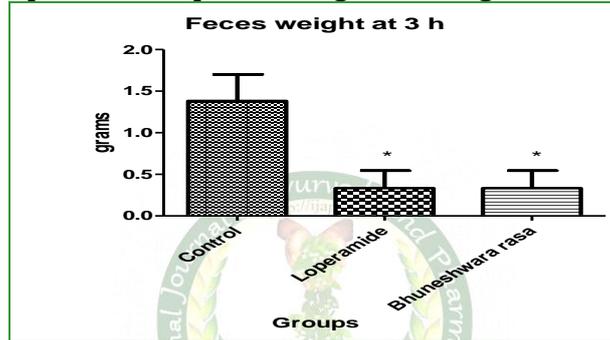


Observation at 3 hour

Table 5: Mean Values of Fecal Weight at 3 Hour

Group	Mean faeces weight (in gm)	SEM
Control	1.4	0.3
Loperamide	0.3	0.2
<i>Bhuvaneshwara rasa</i>	0.3	0.2

Graph No.3: Graph showing feces weight at 3 hour

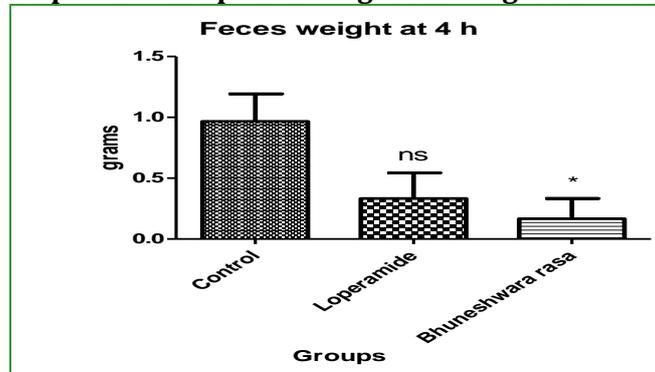


Observation at 4 hour

Table 6: Mean Values of Fecal Weight at 4 Hour

Group	Mean faeces weight (in gm)	SEM
Control	1.0	0.2
Loperamide	0.3	0.2
<i>Bhuvaneshwara rasa</i>	0.2	0.2

Graph No.4: Graph showing feces weight at 4 hour



Statistical Analysis

Average of all the data was compiled and SEM were calculated. The feces weight of the treated groups were compare with disease control groups by one way ANOVA followed by Dunnett’s multiple test. Values <0.05 were considered as statistically significant.

Here the significant values were denoted by stars (*) which is given above the bars in the graphs. The three stars (***) in the graph indicates statistically very highly significant and two stars (**) in the graph indicates highly significant value and the term ‘ns’ denotes the statistically non-significant value.

RESULT

In test drug at the dose of 18mg/kg body weight showed anti-diarrheal activity.

The fecal matter weights were measure periodically at 1h, 2h, 3h, and 4h time.

The average reduction in the fecal matter weights was observed at 4h. The percentage inhibition of the diarrheal is 43.54%.

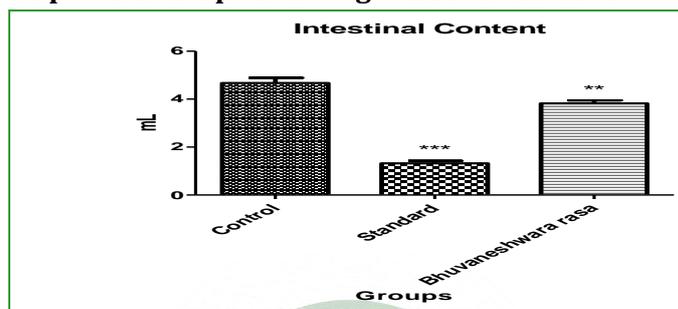
The anti-diarrheal activity of the test drug may be due to reduction of gastrointestinal motility or otherwise.

Castor Oil Induced Enteropooling

Table 7: Mean Values of Intestinal Content in ml.

Groups	Mean (Intestinal content in ml)	SEM
Control	4.67	0.216
Standard	1.32	0.104
<i>Bhuvaneshwara rasa</i>	3.82	0.127

Graph No.5: Graph showing intestinal content in ml.



Statistical Analysis

Average of all the data was compiled and SEM was calculated. All the data were compiled using one-way ANOVA followed by Dunnett’s multiple comparison tests. P values <0.05 were considered as statistically significant.

RESULT

The test drug at the dose of 18m/kg body weight showed anti-enteropooling activity.

The contents of the intestine were collected in measuring cylinder and volume was measured.

The anti-enteropooling activity of the test substance may be due to enhanced water and electrolyte secretion in small intestine.

DISCUSSION

The main aim of the present study was to evaluate the Anti-diarrheal activity of *Bhuvaneshwara rasa*. The objective is to assess whether the preparation possess anti-diarrheal activity. In this study two in-vivo models are used i.e., Castor oil-induced diarrhea and Castor oil induced enteropooling. The induction of diarrhea with castor oil results from the action of ricinoleic acid formed by hydrolysis of the oil. Ricinoleic acid produces changes in the transport of water and an electrolyte resulting in a hyper secretory response in addition to hypersecretion, ricinoleic acid sensitizes the intramural neurons of the gut.

Castor Oil Induced Diarrhea in Rats

Castor oil is a practical inducer that had been used in experimental models of screening anti-diarrheal agents. Following the administration of

castor oil, it is hydrolyzed by lipase in the upper part of the small intestine to ricinoleic acid (the active form) which causes local irritation, inflammation of the gut, release of prostaglandin leading to intestinal hyperactivity, and fluid hyper-secretion. This will prevent water and electrolytes absorption, reduction in activity of sodium potassium-ATPase in the intestine, and finally, diarrhea manifestation.

Agents that can inhibit electrolytes permeability and secretion can prevent castor oil-induced diarrhea. The acute toxicity study was conducted in rats as per OECD guidelines 420 and it was found that 18mg/kg body weight dose is safe as the toxicity study shows the test substance was not show any toxic clinical signs even at the dose of 2000mg/kg body weight. In this study it was found that test substance at the dose of 18mg/kg body weight showed anti-diarrheal activity. The faecal matter weights were measure periodically at 1h, 2h, 3h and 4h time. The average reduction in the faecal matter weight was observed at 4h. The percentage inhibition of the diarrheal is 43.54%.The anti-diarrheal activity of the test substance may be due to reduction of gastrointestinal motility or otherwise.

Castor Oil Induced Enteropooling

The technique is similar to that of castor oil-induced diarrhea. The difference is that animals used in the model are sacrificed and the fluid and electrolyte content of the small intestine are measured. It is not based on the intestinal hyper motility but

enteropooling (accumulation of fluid in the small intestine). The lesser volume of intestinal content, the more the anti-diarrheal activity produced by the test agent. Rats or mice can be used in this model.

This technique is simple to carry out and does not require the use of metabolic cages when compared to castor oil-induced diarrhea.

In present study observed that test substance at the dose of 18m/kg body weight showed anti-enteropooling activity. The contents of the intestine were collected in measuring cylinder and volume was measured. The anti-enteropooling activity of the test substance may be due to enhanced water and electrolyte secretion in small intestine.

Probable Mode of Action of Bhuvaneshwara Rasa

In order to know the mode of action of *Yogas*, it is necessary to analyse the action of each ingredient of the formulation, the individual property and the action of each constituents are collectively responsible for the action of *Yogas*.

Table 8: Ingredients of Bhuvaneshwara Rasa with Its Karma

Karma	Ingredients of Bhuvaneshwara rasa
Deepana	Saindava, Triphala
Deepana-pachana	Yamani, Bilva
Sthambhaka	Bilva
Grahi	Bilva
Shoolahara	Yamani, Triphala
Krimighna	Triphala
Vishahara	Gruhadhooma

- As *Agnimandhya* and formation of *Ama* are prime cause for *Atisara*, the balancing of *Agni* is achieved and relieving of *Ama* takes place by *Deepana* and *Deepana -pachana karma* done by *Saindava*, *Triphala*, *Yamani* and *Bilva*.
- *Atidrava malapravrutti* will be a one of the symptom of *Atisara*, *Bilva* helps in controlling of *Atidrava mala pravrutti* and helps in *Amapachana* and controls the *Atidrava malapravrutti*.
- *Shoola* will be a one of the symptom in *Atisara*, *Yamani* and *Triphala* will helps in relieving of *shoola*.
- *Krimi* is one of the causative factors for *Atisara*, *Triphala* helps to eradicate the *Krimi*.
- *Gruhadhooma* is chimney soot that has a role in the management of various poisons in Ayurveda. Used commonly in combination with other herbs. As it is a carbon compound it may probably bind with the poison and act as an antidote. The processing of it with other herbal drugs using different media increase its anti-poisonous effect. also used in

complications of poison and disease conditions like *Atisara*.

- Thus *Bhuvaneshwara rasa* will helps in treating, reducing causative factors and relieving the symptoms of *Atisara*.

Saindhava

- Antioxidant- Helps in get rid of toxic materials.
- It plays an important role in replenishing the body's electrolyte and maintaining the pH balance.
- Helps in maintaining the flow of digestive juices.

Triphala

- *Triphala* act as an excellent analgesic without any gastric damage.
- Ethanol extracts of *Triphala* confirmed the antibacterial activities.

Helps to eradicate Bacteria's and reduces all types of pain.

Yamani

- Essential oil significantly acts as analgesic.
- Ethanolic extract of *Ajwain* possessed antibacterial activity against eight strains of *Helicobacter pylori* also methanolic extract of *Ajwain* exhibited bactericidal activity.

• As *Ajwain* may have large amounts of Thymol or Carvacrol in its total essential oil, mentioned phenolic compounds are reported to be either bactericidal or bacteriostatic agents depending on the concentration.

• Total essential oil extracted from seeds was subjected for fungicidal effect and showed proper effect on *Aspergillus niger* and *Curvularia ovoidea*.

- The aqueous- methanolic extract of the seeds prove the potential antispasmodic activity.
- *Ajwain* can increase the secretion of gastric acid, bile acids and activity of digestive enzymes. It may also reduce the food transient time. *Ajwain* reinforced the pancreatic lipase and amylase effectiveness, which may support the digestive stimulant activity.

Thus this drug will help reducing the symptoms of diarrhoea.

Bilva

• Antidiarrheal activity- The unripe or half ripened fruit is the most effective remedy for chronic diarrhea. Dried fruit powder is the best one for this purpose. The astringency of fruit is the key to treat chronic diarrhea. Ethanolic extract of the fruit also act effectively.

• Bael is reported to be extraordinary in protection against a wide range of pathogenic organisms which include antibacterial, antiviral, anti-fungal. Marmelide extracted from bael shown antimicrobial activity. Bael powder has the ability to prevent

intestinal parasites like *Ascaris lumbricoides* and *Entamoeba histolytica*.

By the above explanation it can be inferred that *Bhuvaneshwara rasa* can effectively combat all factors in the causation of the Diarrhea.

CONCLUSION

The present study "An experimental study to evaluate the anti-diarrheal activity of *Bhuvaneshwara Rasa* in wistar albino rats." has put forth the following concluding statement.

- *Atisaragna* property of *Bhuvaneshwara rasa* was studied with respect to anti-diarrheal activity.
- *Bhuvaneshwara rasa* was prepared under established Standard operative procedure.
- *Bhuvaneshwara rasa* (test drug) is significantly effective in controlling diarrheal episodes.
- *Bhuvaneshwara rasa* has shown significant Anti-diarrheal activity in both Castor oil- induced diarrhoea and castor oil induced enteropooling, test drug effective than control.
- *Bhuvaneshwara rasa* is a good anti-diarrheal drug which established through this experimental study using castor oil-induced diarrhoea in rats and castor oil induced enteropooling experimental model.

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