International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.94

An Experimental Study to Evaluate Antidiarrheal Activity and Antimicrobial Activity of Ananda Rasa

Dr. Chaitra L V

Rajiv Gandhi University of Health Sciences

Abstract: ***Ananda rasa is a Khalveeya Rasaoushadi mentioned in Vaidya Chintamani, indicated in Atisara and contains Hingula, Saindhava, Varatika, Vatsanabha, Pippali, Sunti, Jatiphala and Dhattura Beeja. In present era people believe in proved facts and require rationality behind facts. Hence the facts have to be proved by scientific methods and establish the facts through experimental trail. So in the present study, Animal experimentation was chosen to validate scientifically that Ananda Rasa is effective in Atisara through Anti - diarrheal activity models. Objective: To evaluate antidiarrheal and antimicrobial property of Ananda Rasa. Methods: Experimental study was conducted for acute toxicity on 5 rats and antidiarrheal study in 4 groups – Control, Standard, Test (175mg), Test (2000mg) each group contains 6 rats. Antidiarrheal study was done by Castor oil induced diarrhea, Castor oil induced enteropooling and Gastro intestinal motility to evaluate latency, frequency, enteropooling and intestinal motility. Antimicrobial study was conducted on Staphylococusaures and E. coli. Result: Ananda Rasa was found safe even at high dose. Ananda Rasa shown efficacy in reducing latency, frequency, intestinal motility andintestinal secretion compared to standard and control group. Ananda Rasa showed significant Zone of inhibition for Staphylococusaures and E coli. Results were statistically significant. Conclusion: Ananda Rasa possess significant antidiarrheal activity in inhibition of diarrhea, intestinal secretion, intestinal motility and possess significant antimicrobial activity.***

Keywords: *Ananda Rasa*, castor oil induced enteropooling, intestinal motility, antidiarrheal activity, antimicrobial, acute toxicity activity

1. Introduction

Diarrhea is a problem, not only of the developing world, but also of the western world[1]. According to the world health organization, diarrhea affects 3 - 5billion people per year worldwide and cause 5 million death per annum. Diarrhea can be defined as a gastrointestinal disorder in which there is rapid transit of gastric contents through the intestine, which is characterized by abnormal fluidity and high frequency of fecal evacuation, usually semisolid or watery fecal matter, three or more times/day[2]. Intestinal infection is the most common cause of diarrhea world wide[1].

Atisara is characterized by "***Bahudrava mala sarana[3]***". With regard to the quotation of *Vagbhata*i. e "***RogahSarveApi Mandagno[4]***" the disease *Atisara*is said to be caused by *Agnimandhya*. *Atisara* is a universally found disease and can be compared to diarrhea, although diarrhea is not considered a disease on its own in conventional medicine, *Ayurvedic* literature records it as both a symptom and an independent disease. *Ananda Rasa* is a *Khalveeya Rasaoushadi* mentioned in *Vaidya Chintamani*, indicated in *Atisara* and contains *Hingula, Saindhava, Varatika, Vatsanabha, Pippali, Sunti, Jatiphala and Dhattura Beeja*[5].

In modern science anti - diarrheal drug (loperamide, bismuth subsalicylate) have been used to treat diarrhea, which may cause side effects like tinnitus, blackened stool/tongue, dizziness, constipation[6], that made the physician to look for safe and effective anti - diarrheal medicine, and also development of microbial resistance to the available antibiotics have led to investigate anti - microbial activity. Hence the study has under taken for the scientific validation of *Ananda Rasa* on its anti - diarrheal activity and antimicrobial activity.

2. Material and Methods

Preparation of *Ananda Rasa*: *Anandarasa* was prepared as per the classical reference of *Vaidyachintamani* in teaching pharmacy of Ramakrishna Ayurveda Medical College, Bangalore.

Experimental study: Acute toxicity and animal study was done in Pharmacology laboratory, SDM Centre for Research in Ayurveda and Allied sciences, Udupi.

Acute toxicity study

The acute oral toxicity of *Ananda Rasa* was performed as per the OECD guidelines. A total of 5 healthy either sex of rats were selected according to AOT software. All selected rats were kept under acclimatization for 7days before dosing. Single dose per animal and all the animals were dosed constant dose volume of 175mg/kg, 550mg/kg 2000mg/kg (1ml/100g body weight). The animals was observed at ½, 1, 2, 3, 4, 24, 48h after dosing and there after daily once for mortality during the period of 14days[6].

Castrol oil induced diarrhea: -

Rats of both sexes (150 - 200 g) were been fasted for 18 hours. The rats for castor oil - induced diarrheal test were divided into four groups (24 rats). Group I was be given normal saline (2 ml/kg) orally as control group and Group II was be given Loperamide (5 mg/kg) as standard group. Groups III - IV - V was receive *Ananda Rasa* (low dose, high dose). After 1 h, all groups were given castor oil 1 ml each orally. Then they was placed in cages lined with adsorbent papers and observed initially, 6th hourly, 24th hourly for the presence of characteristic diarrheal droppings.100% was been considered as the total number of feces of control group[7]. The activity will be expressed as % inhibition of diarrhea.

The percent (%) inhibition of defecation will be measured using the following formula:

Percentage (%) inhibition of defecation= (A - B) /A X 100
A - number of defecation time caused by castor oil and B - number of defecation time caused by drug.

Castor oil induced enteropooling
Rats of either sex (150 - 200 g) was been fasted for 18h. They were divided into four groups. Castor oil (1ml) was administered orally to these animals. One hour later Group I (control group), was administered with Normal saline (2 mL/kg). The Group II (standard group) was administered with Loperamide (5 mg/kg) by oral route. Groups III received *Ananda Rasa*175mg and Groups IV received *Ananda Rasa* 2000mg. After 2 hour of treatment, rats were sacrificed by ether anesthesia. The edges of the intestine from pylorus to cecum were dissected out, its content collected in measuring cylinder and volume was measured. The intestine was reweighed and a difference between full and empty intestines was calculated[7].

Intestinal motility test: -
Selected 24 Mice (30 - 40g) were divided into four groups of 6 mice in each. At first, 1 ml castor oil was been given orally in every mice of each group to produce diarrhea. After 1 h, Group I (control group) received saline (2 ml/kg) orally. Group II received standard drug (Loperamide 5 mg/kg body weight) and Groups III received *Ananda rasa*175mg and Groups IV received *Ananda Rasa* 2000mg. After 1 h, all animals received 1 ml of charcoal meal (10% charcoal suspension in 5% gum acacia) orally. One hour after following the charcoal meal administration, all animals were sacrificed and the distance covered by the charcoal meal in the intestine, from the pylorus to the cecum, was measured and expressed as percentage of distance moved[7].

Intestinal transit (%) = (D/L) x 100 ……. (1) Where D = Distance covered by Charcoal and L = Intestinal length.

The intestinal motility was calculated by using following formula
% travel = 100 - B. Where B = Total length of intestine – Distance travelled by Charcoal / Total length of Intestine x 100.

Anti - microbial activity: -
Antimicrobial activity was done by cup plate method. The antibacterial activity of the extracts was determined by using the agar well diffusion technique. Nutrient agar or muller agar plates (Himedia Mumbai) were seeded with 0.1ml of overnight culture of Staphylococcus aureus and Escherichia coli allowed to incubate for 24hrs. Cups were made in petri plates using sterile cork borer (0.85 cm) and different concentrations of the trail drug were added into each well. Then bacterial plates were incubated at room temperature 24hrs for which zone of inhibition diameter and mean values are determined and recorded as diameter in mm [8].

Ananda Rasa was screened for their antibacterial activities against Escherichia coli and Staphylococcus aureus. The sets of six dilutions (100, 75, 50, 25, 10, 5µL) of *Ananda Rasa* and standard drug were prepared in double distilled water using nutrient agar tubes.

3. Results and Observation

Acute toxicity: - *Ananda Rasa* did not produce any mortality up to the dose of 2000mg/kg per oral. All the animals belonging to the treated group survived throughout the 14 days observation period after dosing.

Antidiarrheal activity

Castor oil induced diarrhea - In the present study it was observed that *Ananda Rasa* significantly reduced latency, frequency of diarrhea at low dose of trail drug (175mg/kg) and high dose (2000mg/kg), compared to control. Frequency of diarrhea at low dose (175mg/kg) shown significant reduction compared to standard. Frequency of diarrhea reduced significantly in high dose (2000mg/kg) compared to low dose (175mg/kg) (table no01).

Percentage of inhibition in high dose of trail drug was more compared to standard, but less in low dose of trail drug (175mg/kg) when compared with high dose of trail drug (2000mg/kg) (table no02 and graph 01).

Castor oil induced enteropooling: -
Ananda Rasa of low dose (175mg/kg) and high dose (2000mg/kg) showed significant effect in castor oil induced enteropooling activity. Intestinal volume and weight of the content of high dose (2000mg/kg) was decreased compared to standard drug and low dose (175mg/kg) (table no03 and graph 02).
Gastro intestinal motility test: -
The gastro intestinal distance traveled by the charcoal meal in mice given with *Ananda Rasa* of low dose was 46.06%, *Ananda Rasa* of high dose was 52.93% and standard was 46.08%. The distance travelled in *Ananda Rasa* of low dose was equalent with standard drug (table no04 and graph 03).

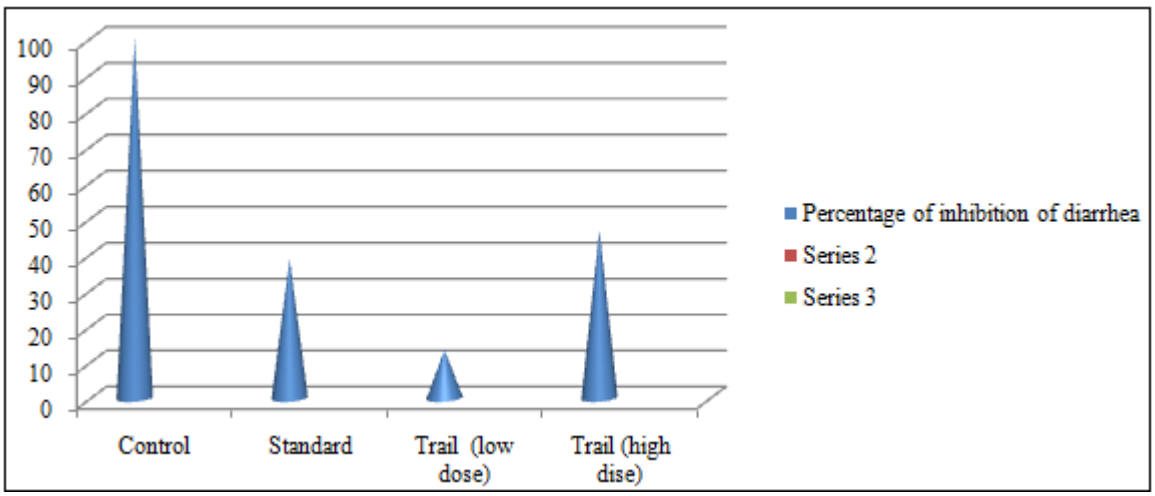
Anti - microbial study: -
Anandarasa has shown significant zone of inhibition in both Staphylococcusaureus (25 ±11.19) and Escherichia coli organism (25.42±11.50) in 6 different concentration, but less compared to standard drug in Staphylococcus aureus (30.85 ± 14.94) and Escherichia coli organism (30.28 ± 14.19) (table no 5)

Table 1: Mean of frequency and latency in four groups studied.

Variables	Control (Group A)	Standard (Group B)	Trail Drug (Low Dose) (Group C)	Trail Drug (High Dose) (Group D)	Total	P Value
Latency	31.83±30.25	190.4±69.45	88.5±34.69	183.83±109.62	120.74±93.37	0.002**
Frequency	7.5±1.05	4.67±1.51	6.5±0.84	4±0.89	5.67±1.76	<0.001**

Table 2: Mean of frequency and percentage of inhibition

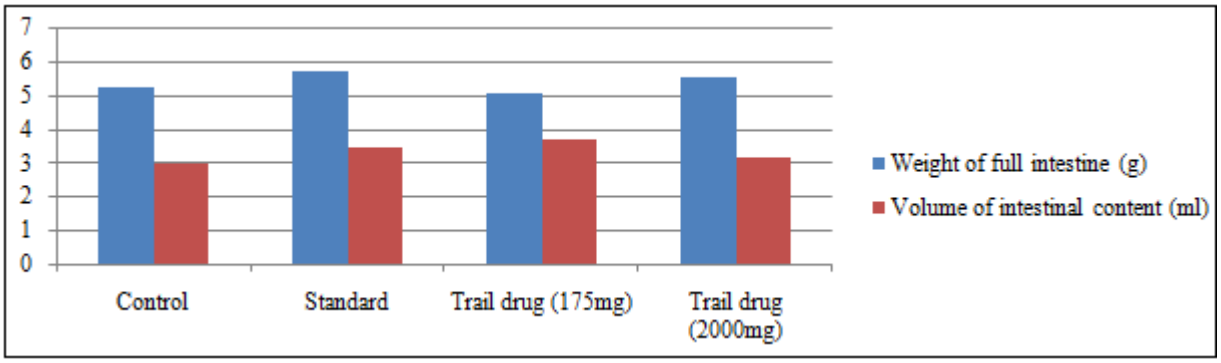
Group	Mean of frequency or number of stool	Percentage of inhibition
Control	7.5 ±1.05	-
Standard	4.6±1.51	38.66%
Trail drug (low dose)	6.5± 0.83	13.33%
Trail drug (high dose)	4±0.89	46.66%



Graph 1: Percentage of inhibition of diarrhea in the four groups studied

Table 3: Weight and volume of intestinal content in four groups studied.

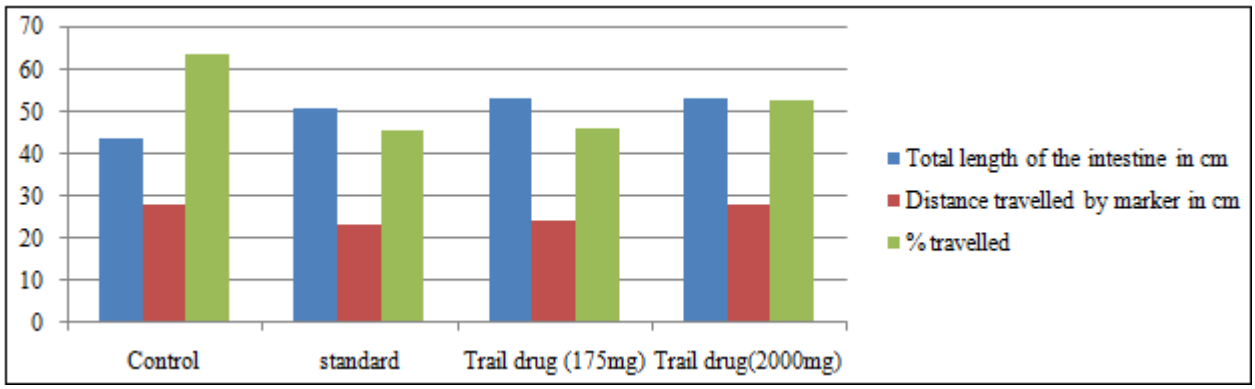
Variables	Control	Standard	Trail Drug (Low Dose)	Trail Drug (High Dose)	P Value
Weight of full intestine (g) (A)	5.3±0.58	5.74±0.37	5.09±0.35	5.54±0.38	0.079+
Weight of empty intestine (g) (B)	4.77±0.41	4.86±0.44	4.03±0.21	4.74±0.32	0.002**
Volume of intestinal content (ml)	3±0.94	3.52±0.54	3.72±0.48	3.22±0.28	0.213
Difference (g) (A - B)	0.53±0.26	0.88±0.14	1.06±0.3	0.8±0.1	0.004**
% Inhibition	-	27.95%	27.98	17.24	-



Graph 2: Weight and volume of intestinal content in four groups studied.

Table 4: Mean of total intestinal length, distance covered by charcoal meal in four groups studied

Variables	Control	Standard	Trail Drug (Low Dose)	Trail Drug (High Dose)	P Value
Body Weight (g)	34.17±3.66	37.33±3.01	30.83±5.12	31.5±5.75	0.083+
Total length of Intestine (cm)	43.88±2.7	51.15±6.62	53.3±1.77	53.43±4.49	0.003**
Distance travelled by Charcoal (cm)	27.98±3.49	23.22±9.97	24.45±7.62	28.12±6.1	0.553
% Travelled	63.96±7	46.08±19.27	46.06±14.78	52.93±10.41	0.109
% Inhibition	45.87	45.39	52.62	51.42	-



Graph 3: Total intestinal length, distance covered by charcoal meal in four groups studied

Table 5: Showing the zone of inhibition according to the trail drug concentration and standard drug

Micro organisms	Drug	1	2	3	4	5	6	7	Total Mean ± SEM
Concentration	-	100µL	75 µL	50 µL	25 µL	10 µL	5 µL	No drug	-
Staphylococcus aureus	Gentamycin	46mm	41mm	37mm	34mm	30mm	28mm	NZ	30.85 ± 14.94
Staphylococcus aureus	*Ananda Rasa*/trail drug	32mm	31mm	29mm	29mm	28mm	26mm	NZ	25 ± 11.19
Escherichia coli	Gentamycin	43mm	39mm	37mm	33mm	31mm	29mm	NZ	30.28 ± 14.19
Escherichia coli	*AnandaRasa*/trail drug	34mm	32mm	29mm	29mm	27mm	27mm	NZ	25.42± 11.50

NZ - No zone.

4. Discussion

In *Ayurveda* classics many formulations has been indicated for *Atisara*, among them *Ananda Rasa* was selected from *Vaidya Chintamani* to validate scientifically the effect of *Ananda Rasa* as antidiarrheal, antimicrobial property. Here study was carried out on wister albino rats for castor oil induced diarrhea, castor oil induced enteropooling and mice for gastro intestinal motility. Study has observed for latency, frequency, percentage of inhibition of diarrhea, intestinal secretion and intestinal motility. Antimicrobial study was carried in in - vitro on E. coli and Staphylococcus aureus.

Present study has proved that *Ananda Rasa* has potent antidiarrheal property at a low dose of 175mg/kg and high dose of 2000mg/kg by inhibiting frequency, latency, fluid secretion (enteropooling) and intestinal motility.

Ananda Rasa has shown significant zone of inhibition on Escherichia coli and Staphylococcus aureus.

Probable mode of action of Ananda rasa as antidiarrheal and antimicrobial

Ananda Rasa is a unique combination of drugs documented to treat *Atisara*. It contains *SodhitaVatsanabha, Pippali, Sunti, Sodhita Dhatturabeeja, Jatiphalachurna, Saindhava, Varatika Bhasma, Sodhita Hingula*. All these drugs have *Ushna Veerya, DeepanaPachana, VataKaphahara* properties. *Deepana* and *Pachana* property helps in correcting the *Ama* which is the prime cause of *Atisara*, *Ushna Veerya* and *Vatakaphahara* properties helps in normalizing the intestinal motility and absorption which in turn pacifies the *Atisara*. *Jatiphala* and *Dhatura* have *Krimighna* property. Thus we can conclude that *Ananda Rasa* is a typical synergistic combination which alleviates diarrhea and shows antimicrobial property.

5. Conclusion

Ananda Rasa statistically showed significant effect as antidiarrheal drug in the inhibition of diarrhea by both enteropooling and gastro intestinal motility and shown significant zone of inhibition in both Staphylococcus aureus and Escherichia coli organism.

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