

ANTIDIARRHEAL ACTIVITY OF CHAKRANIKE (*Apama siliquosa.Lam*) IN WISTAR RATS - A FOLKLORE DRUG

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ABSTRACT

Objectives: To evaluate the anti-diarrheal effect of *Chakranike (Apama siliquosa.Lam)* root in Wistar albino rats by using the method castor oil induced diarrhea experimentally. **Methods:** Experimental Study was done to assess the antidiarrheal effect of drug in Wistar albino rats, AOT study was done to standardize the dose. **Results:** There was significant increase in the latency and significant decrease in the frequency of diarrhoea when compared to control group in the double dose group. Folklore dose group showed significant decrease in the 24hr fecal wet and fecal dry when compared to control group. **Conclusion:** Chakranike *Apama siliquosa. Lam* was found to possess antidiarrheal effect.

Keywords: *Apama siliquosa. Lam*, AOT, Folklore, Chakranike

INTRODUCTION

New drug discovery plays an important role in the world of Ayurveda. A number of medicinal plants which are not mentioned in the classics were effectively used by the folklore practitioners in various diseases. Trying with the information obtained, selecting and experimenting on the new drugs to produce scientific evidence is very important. All the drugs found in this world are having medicinal value. *Chakranike (Apama siliquosa.Lam)* is a plant used by the folklore practitioner in treating diarrhea.^{1, 2} It is necessary to establish scientific evidences for therapeutic use of folklore medicinal plants, as it may potentially be useful source of new effective therapies in drug development process. Hence this study is aimed at evaluating the folklore claim of antidiarrheal effect of *Apama siliquosa. Lam* in Wistar albino rats.

MATERIALS AND METHODS:

Test drug: Root of the plant sources were collected from local areas surrounding Udupi district and was authenticated by the Taxonomist, K. Gopalakrishna Bhat. The root was cleaned to remove the physical impurities and washed thoroughly with water. The root powder of *A.siliquosa.Lam* was prepared at SDM pharmacy, Udupi

Place of study: The experimental study was carried out in the Pharmacology department of S.D.M Centre for Research in Ayurveda and Allied Sciences, Udupi.

Selection of animals: Healthy wistar albino rats of either sex, weighing between 200-250gms were obtained from the Animal House of SDM Centre for Research in Ayurveda and Allied Sciences, Udupi to

conduct the animal experiment. The rats were housed in polypropylene cages with an environmental temperature, humidity and with light and dark cycle. Rats were fed with food pellets and water. They were acclimatized to laboratory conditions for at least one week before using them for experiment.

Inclusive criteria: Healthy Albino rats weighing 200g - 250g of either sex will be taken for the study.

Exclusion criteria:

- Rats subjected to other experiments.
- Rats with pathological conditions.
- The rats weighing less than 200g and more than 250gms.
- Albino rats which are pregnant.

Form of drug: Aqueous extract of the drug *A.siliquosa.Lam*.

Route of drug administration: The test drug, reference standard and castor oil were administered according to the body weight by oral route with the help of oral feeding needle sleeved on to disposable syringe.

Dose fixation: Dose fixation of the test drug was done according to Acute Oral Toxicity study. LD₅₀ is greater than 2000mg/kg. Hence Therapeutic effective dose is,

$$TED = 1/10^{\text{th}} LD_{50}, TED = 2000/10, TED = 0.2\text{mg/g.}$$

Castor oil induced Diarrhoea:³

Grouping: Total 30 rats weighing 200g - 250g were grouped in to five groups with 6 animals in each group and kept in separate cages.

Table 1: Grouping for castor oil induced diarrhoea

Group A	Control group	Distilled water
Group B	Standard group	Tab. Loperamide
Group C	Test group 1	Aqueous extract of <i>Apama siliquosa.Lam</i> in single dose.
Group D	Test group2	Aqueous extract of <i>Apama siliquosa.Lam</i> in double dose.
Group E	Test group 3	<i>Apama siliquosa.Lam</i> in folklore method.

Procedure:

30 Wister albino rats weighing 200g - 250g made into 5 groups (standard group, control group, Test group 1, test group 2 and test group 3). The dose is fixed on the basis of body weight. The study was carried out for 8 days. The test drugs were administered on all the 8 days. Rats of all the groups will be fasted overnight, provided with distilled water for drinking. On 8th day after one hour of drug administration the castor oil will be administered orally to the rats. After administering corresponding drug to each group, rats were kept individually in separate cages and observed for the parameters for the duration of 6 hours.

1. **Latency:** Onset of diarrhea. Time in minutes between the drug administration and the 1st diarrheal output.
2. **Frequency:** Number of times the stool was passed by each animal in 24 hours

3. **Fecal wet weight:** Initial fecal wet weight, 6th hour fecal wet weight, 24th hour fecal wet weight is recorded.

4. **Fecal dry weight:** Initial fecal dry weight, 6th hour fecal dry weight, 24th hour fecal dry weight is recorded.

Stools were collected immediately after defecation to avoid the weight loss by sticking to the animal body and to the other part of the cage by the movements of the rats and also to avoid the consistency variation by mixing it with the urine of the animal. Rats were observed continuously for 6 hours and stools collected was weighed and kept for drying in oven at 80°C. Again after 24 hours the stools was collected, dried and weighed before and after drying. Total weight of the stools wet and dry in 24 hours collection was noted.

RESULT**Castor oil induced diarrhea:****Table 2:** Observations of control group albino rats

Control	Body weight (in g)	Dose of castor oil (in ml)	Time of castor oil administration (in AM)	Onset of diarrhoea (in min)
Head	216	2.1	10.29	10
Neck	177	1.7	10.30	82
Body	215	2.1	10.31	155
Tail	186	1.8	10.32	10
Fore limb	174	1.7	10.33	25
No mark	185	1.8	10.34	9

Table 3: Observations of standard group albino rats

Standard	Body weight (in g)	Dose of castor oil (in ml)	Time of castor oil administration (in AM)	Onset of diarrhoea (in min)
Head	233	2.3	10.25	210
Neck	260	2.6	10.26	70
Body	205	2.0	10.27	252
Tail	204	2.0	10.28	162
Fore limb	231	2.3	10.29	208
No mark	209	2.0	10.30	Not started till 4:30pm

Table 4: Observations of test drug single dose group albino rats

Standard	Body weight (in g)	Dose of castor oil (in ml)	Time of castor oil administration (in AM)	Onset of diarrhoea (in min)
Head	174	1.7	10.26	46
Neck	173	1.7	10.27	105
Body	186	1.8	10.28	50
Tail	210	2.1	10.29	110
Fore limb	287	2.8	10.30	45
No mark	239	2.3	10.31	105

Table 5: Observations of test drug double dose group albino rats

Standard	Body weight (in g)	Dose of castor oil (in ml)	Time of castor oil administration (in AM)	Onset of diarrhoea (in min)
Head	273	2.7	10.43	125
Neck	205	2.0	10.44	71
Body	228	2.2	10.45	68
Tail	264	2.6	10.46	340
Fore limb	244	2.4	10.47	265
No mark	245	2.4	10.48	194

Table 6: Observations of test folklore dose group albino rats

Standard	Body weight (in g)	Dose of castor oil (in ml)	Time of castor oil administration (in AM)	Onset of diarrhoea (in min)
Head	216	2.1	10.59	Not started till 5:00pm
Neck	206	2.0	11.00	267
Body	284	2.8	11.01	110
Tail	252	2.5	11.02	60
Fore limb	225	2.2	11.03	50
No mark	243	2.4	11.04	51

Statistical analysis:

All the values were expressed as mean \pm SEM (Standard error of mean) and the data was analyzed by one

way ANOVA. A level of $P < 0.05$ was considered as statistically significant. Level of significance was noted and interpreted accordingly.

Table 7: Effect of *Apama siliquosa.Lam* on the latency (onset of diarrhea)

GROUPS	MEAN \pm SEM
Control	13.5 \pm 2.51
Standard	270 \pm 24.44**
Test drug single dose	83.2 \pm 14.41
Test drug double dose	199 \pm 48.02**
Folklore dosage	107.6 \pm 41.35

Data: MEAN \pm SEM ** $p < 0.01$ in comparison to control group.

The data related to effect of test drug on the onset of diarrhea is summarized in Table no.7. The data shows there was increase in the onset of diarrhea in standard and double dose groups when compared to the normal control group, the observed increase was found to be statistically very significant.

The data shows there was increase in the onset of diarrhea in single dose and folklore dose groups when compared to normal control group, the observed increase was found to be statistically non-significant.

Table 8: Effect of *Apama siliquosa.Lam* on the Frequency

GROUPS	MEAN \pm SEM
Control	6.33 \pm 0.210
Standard	1.87 \pm 0.398**
Test drug single dose	6.2 \pm 0.489
Test drug double dose	4 \pm 0.707*
Folklore dosage	4.6 \pm 1.12

Data: MEAN \pm SEM ** $p < 0.01$, * $p < 0.05$

Data related to the effect of test drugs on frequency of stools in castor oil induced diarrhea is summarized in table no.8. The data shows there was decrease in the frequency of diarrhea in standard group when compared to normal control group, the observed decrease was found to be statistically very significant.

The data shows there was decrease in the frequency of diarrhea in test drug double dose group when com-

pared to normal control group, the observed decrease was found to be statistically significant.

The data shows there was decrease in the frequency of diarrhea in test drug single and folklore dose groups when compared to normal control group, the observed decrease was found to be statistically non-significant.

Table 9: Effect of *Apama siliquosa.Lam* on the fecal weight: Initial fecal wet

GROUPS	MEAN \pm SEM	%Change
Control	0.827 \pm 0.143	-
Standard	0.963 \pm 0.227	16.58 \uparrow
Test drug single dose	1.876 \pm 0.514	127.11 \uparrow
Test drug double dose	2.103 \pm 0.560	154.60 \uparrow
Folklore dosage	2.213 \pm 0.562	167.91 \uparrow

Data: MEAN \pm SEM

The statistical analysis of initial fecal (wet) weight of diarrhea is summarized in the table no.9. The data shows there was increased initial fecal (wet) weight in standard, test drug single, double dose group and folk-

lore dosage group when compared to normal control group, the observed increase was found to be statistically non-significant.

Table 10: Effect of *Apama siliquosa.Lam* on the fecal weight: Initial fecal dry

GROUPS	MEAN ±SEM	%Change
Control	0.263±0.048	-
Standard	0.245±0.058	6.84↓
Test drug single dose	0.093±0.134	64.63↓
Test drug double dose	0.783±0.627	197.71↑
Folklore dosage	0.253±0.081	3.80↓

Data: MEAN ± SEM

The statistical analysis of initial fecal dry weight of diarrhea is summarized in the table no.10. The data shows there was decrease in the initial fecal (dry) weight in standard, test drug single and folklore dosage groups when compared to normal control group,

the observed decrease was found to be statistically non-significant.

The data shows there was increase in the initial fecal (dry) weight in double dose group when compared to normal control group, the observed increase was found to be statistically non-significant.

Table 11: Effect of *Apama siliquosa.Lam* on the fecal weight: 6th hour fecal wet

GROUPS	MEAN –SEM	%Change
Control	0.848±0.109	-
Standard	2.265±0.683	167.09↑
Test drug single dose	0.706±0.127	16.74↓
Test drug double dose	0.795±0.203	6.25↓
Folklore dosage	1.465±0.567	72.75↑

Data: MEAN ± SEM

The statistical analysis of 6th hour fecal wet weight of diarrhea is summarized in the table no.11. The data shows there was increase in the 6th hour fecal (wet) weight in standard and folklore dosage groups when compared to normal control group, the observed increase was found to be statistically non-significant.

The data shows there was decrease in the 6th hour fecal (wet) weight in single and double dosage groups when compared to normal control group, the observed decrease was found to be statistically non-significant.

Table 12: Effect of *Apama siliquosa.Lam* on the fecal weight: 6th hour fecal dry

GROUPS	MEAN ± SEM	%Change
Control	0.027 ± 0.064	-
Standard	0.436 ± 0.149**	1576.92↑
Test drug single dose	0.085 ± 0.037	426.92↓
Test drug double dose	0.065 ± 0.069	350↓
Folklore dosage	0.027 ± 0.064	0

Data: MEAN ± SEM**p<0.01:

The statistical analysis of 6th hour fecal dry weight of diarrhea is summarized in the table no.12. The data shows there was increase in the 6th hour fecal (dry)

weight in standard group when compared to normal control group, the observed increase was found to be statistically very significant.

The data shows there was decrease in the 6th hour fecal (dry) weight in single and double dosage groups when compared to normal control group, the observed decrease was found to be statistically non-significant.

The data shows there was no change in the 6th hour fecal (dry) weight in folklore dosage group when compared to normal control group and found to be statistically non-significant.

Table 13: Effect of *Apama siliquosa.Lam* on the fecal weight: 24 hour fecal wet

GROUPS	MEAN ± SEM	%Change
Control	4.494 ± 0.227	-
Standard	1.378 ± 0.169**	69.33↓
Test drug single dose	0.988 ± 0.194**	78.02↓
Test drug double dose	3.374 ± 0.793	24.92↓
Folklore dosage	1.988 ± 0.552**	55.76↓

Data: MEAN ± SEM **p<0.01

The statistical analysis of 24 hour fecal wet weight of diarrhea is summarized in the table no.13.

The data shows there was decrease in the 24 hour fecal (wet) weight in standard, single dose and folklore dosage groups when compared to normal control

group, the observed decrease was found to be statistically very significant.

The data shows there was decrease in the 24 hour fecal (wet) weight in double dosage group when compared to normal control group, the observed decrease was found to be statistically non-significant.

Table 14: Effect of *Apama siliquosa.Lam* on the fecal weight: 24 hour fecal dry

GROUPS	MEAN ± SEM	%Change
Control	1.733 ± 0.440	-
Standard	1.711 ± 0.212**	1.26↓
Test drug single dose	0.375 ± 0.091	78.36↓
Test drug double dose	0.862 ± 0.159	50.25↓
Folklore dosage	0.372 ± 0.099**	78.55↓

Data: MEAN ± SEM **p<0.01

The statistical analysis of 24 hour fecal dry weight of diarrhea is summarized in the table no.14. The data shows there was decrease in the 24 hour fecal (dry) weight in standard and folklore dosage groups when compared to normal control group, the observed decrease was found to be statistically very significant.

The data shows there was decrease in the 24 hour fecal (dry) weight in single and double dosage groups when compared to normal control group, the observed decrease was found to be statistically non-significant.

DISCUSSION

Experimental study was conducted at S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi. The animal experiment was conducted after getting prior permission from Institutional Animal Ethics committee.

Acute oral toxicity study was done to fix the dosage and result obtained as LD50 is greater than 2000mg/kg for the test drug.

30 Wistar albino rats weighing 200g - 250g were divided into 5 groups of 6 animals in each group. Control group were given with distilled water. Standard group were given with Tab. Loperamide. Test group-1 was given with aqueous extract of *Apama siliquosa.Lam* in single dose. Test group-2 was given with aqueous extract of *Apama siliquosa.Lam* in double dose. Test group-3 was given with *Apama siliquosa.Lam* in folklore method.

The study was carried out for 8 days where in the test drugs were administered for all the 8 days. On 8th day after 1 hour of drug administration rats were fed with castor oil orally. After administering corresponding drug to each group rats were kept individually in sepa-

rate cages and observed for the parameters such as latency, frequency, initial fecal wet, initial fecal dry, 6th hour fecal wet, 6th hour fecal dry, 24 hour fecal wet and 24 hour fecal dry.

The results obtained have been provided in the form of consolidated statement below:

Table 15: Consolidated statement of effect of *A.siliquosa.Lam* on the following parameters

No.	Parameters	compared to control group			
		Standard	T1	T2	T3
1.	Latency	SI	NSI	SI	NSI
2.	Frequency	SD	NSD	SD	NSD
3.	Initial fecal wet	NSI	NSI	NSI	NSI
4.	Initial fecal dry	NSD	NSI	NSD	NSD
5.	6 th hour fecal wet	NSI	NSD	NSD	NSI
6.	6 th hour fecal dry	NSI	NSD	NSD	-
7.	24 hour fecal wet	SD	SD	NSD	SD
8.	24 hour fecal dry	SD	NSD	NSD	SD

SI- Significant increase

NSI- Non significant increase

SD- Significant decrease

NSD- Non significant decrease

a. Effect of *A.siliquosa.Lam* on latency (onset of diarrhea):

Analysis of data shows that there was delay in the onset of diarrhea in the standard group and test drug double dosage group when compared to control. Standard drug Loperamide inhibits secretion which may be the reason for delay in the onset of diarrhea. It was found that *A.siliquosa.Lam* is having *Ushna Veerya* because of which it might have done *Drava Shoshana*. Hence the delay in the onset may be because of *Sangrahi Karma* of the test drug *A.siliquosa.Lam*. Test single and folklore dose group showed mild to moderate changes which were found to be non-significant.

b. Effect of *A.siliquosa.Lam* on frequency:

It was observed that there was a significant decrease in the frequency of diarrhea in the standard group as well as test drug double dosage group when compared to control group. Loperamide appears to be the most effective and most suitable of the anti-motility drug. It may be the reason for the decrease in the frequency of diarrhea. *A.siliquosa.Lam* because of its *Grahi Karma* it helps in proper absorption by acting on the mucosal transport processes and also decreases secretion which may be the reason for the decrease in the frequency of diarrhea. Folklore dose group showed less frequency

followed by single dose group when compared to control which was statistically non-significant.

c. Effect of *A.siliquosa.Lam* on initial fecal wet:

There were no significant changes observed in initial wet fecal weight of standard, test single, test double, folklore dose groups when compared to control.

d. Effect of *A.siliquosa.Lam* on initial fecal dry:

It was observed that decreased initial fecal dry weight of standard, test drug single and folklore dose group, increased initial fecal dry weight of double dose group when compared to control but statistically non-significant.

e. Effect of *A.siliquosa.Lam* on 6th hour fecal wet:

It was observed that decreased 6th hour fecal wet weight of single and double dose group, increased 6th hour fecal wet weight of standard and folklore dose group when compared to control group but statistically non-significant.

f. Effect of *A.siliquosa.Lam* on 6th hour fecal dry:

There was increase in the 6th hour fecal (dry) weight in standard group when compared to control group and found to be statistically significant and decreased 6th hour fecal (dry) weight of test single, test double, folklore dose groups when compared to normal control group and found to be statistically non-significant.

g. Effect of *A.siliquosa.Lam* on 24 hour fecal wet:

Analysis of data shows that there was decrease in the 24 hour fecal (wet) weight in standard group, single dose and folklore dosage groups when compared to control group which was found to be very significant. Loperamide works by slowing down the movement of the gut and improves faecal continence by enhancing anal sphincter tone, decreases the number of bowel movements and makes the stool less watery. It may be the reason for decrease in the 24 hour fecal (wet) weight in the standard group. *A.siliquosa.Lam* was found to have *Tikta Rasa* and *Kashaya Anurasa*. *Tiktha Rasa* does *Pureesha Upashoshana* and *Kashaya Rasa* is having *Sangrahi*, *Stambhana*, *Shoshana* property⁴ which may be the reason for decrease in the 24 hour fecal (wet) weight in single dose, folklore dosage group and double dose group. But decrease in the 24 hour fecal (wet) weight in double dose group when compared to control group was found to be statistically non-significant.

h. Effect of *A.siliquosa.Lam* on 24 hour fecal dry:

It was observed that decrease in the 24 hour fecal (dry) weight in standard and folklore dosage groups when compared to control group which was found to be very significant. Loperamide is one of the anti-motility drugs and also inhibits secretion which may be the reason for the decrease in the 24 hour fecal (dry) weight in standard group. *A.siliquosa.Lam* was found to have *Ushna Veerya* because of which it might produce *Dravashoshana*. It regulates absorption of water and electrolytes by *Dravashoshana* property. It may be the reason for the decrease in the 24 hour fecal (dry) weight in folklore dosage group while decrease in the 24 hour fecal (dry) weight in single and

double dosage groups when compared to control group found to be statistically non-significant.

CONCLUSION

Animal experimental study was conducted to know the antidiarrhoeal activity of *Chakranike (Apama siliquosa.Lam)*. The dosage of test drug was obtained as LD₅₀ is greater than 2000mg/kg from AOT study. Analysis of the result obtained in this study indicates that there was significant decrease in the 24hr fecal wet weight in single dose group when compared to control group, there was significant increase in the latency and significant decrease in the frequency of diarrhoea in double dose group when compared to control group, there was significant decrease in the 24hr fecal wet and dry weight in folklore dose group when compared to control group.

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Photos:

Separate cages for the procedure – Castrol induced diarrhea



Drug administration



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Conflict Of Interest: None Declared

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