

Investigation of Antidiarrhoeal Activity of Ethanolic Extract of *Tamarindus indica* L. Seeds in Albino Wistar Rats

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Abstract

Context: Nowadays scientist increasing interest in the field of research and development to investigate the employ of Indian traditional medicine in the treatment and prevention of diarrhea. Frequent of passage of three or more loose or watery stools and unformed feces per day indicates diarrhea. Diarrhea is a major cause of ill-health, especially for children because, particularly, rotavirus responsible for it. Traditionally, *Tamarindus indica* seed is employed in the management of diarrhea. **Aims:** The aim of recent research to study the traditional claim of ethanolic (alcoholic) extract of *T. indica* L. (EETI) seed for antidiarrheal activity in albino Wistar. **Materials and Methods:** The extract of *T. indica* seeds was developed using successive solvent extraction using ethanol at room temperature in a soxhlet apparatus. The preliminary phytochemical study was carried out for the identification of active phytoconstituents that elicit antidiarrheal action in albino Wistar rats. **Statistical Analysis Used:** Yes. The results of *in-vivo* pharmacological studies were represented as mean \pm standard error of mean. The total variations present in data were evaluated using Graph Pad Prism 5 project software analysis of variance followed by Student's *t*-test. The result was considered statistically significant when the value of $P < 0.05$ versus control. **Results:** The preliminary phytochemical investigation of EETI seed indicated the presence of alkaloids, flavonoids, carbohydrates, glycosides, and tannins phytoconstituents. Acute toxicity test was investigated and performed the antidiarrheal activity in albino Wistar rats. EETI indicated neither any toxic effect nor mortality in albino Wistar rats up to the 2000 mg/kg, p.o. dose for 48 h and then up to 14 days. EETI seed showed a significantly antidiarrheal activity 78.23% at a dose of 400 mg/kg, p.o. in comparison to control group in the castor oil induced diarrhea in albino Wistar rats. Phytochemical investigation indicated the existence of flavonoids and tannins in the EETI, so the possible mechanism for the reduction of diarrheal episode may be due to the presence of these contents of the extract. **Conclusions:** It was concluded that the significant antidiarrheal activity have been shown by EETI seeds. EETI played a significant role in the management of diarrhea.

Key words: Albino Wistar rats, antidiarrheal activity, ethanolic extract, *Tamarindus indica*

INTRODUCTION

Frequency of passage of three or more loose or watery stools and unformed feces per day indicates diarrhea. Diarrhea is not a disease, but it may be associated with several diseases like abdominal pain and irritation within the lining of small or large intestine leads to diarrhea. Decrease in water absorption and increase in loss of water with stools also leads to diarrhea. Loss of fluid in the form of diarrhea causes electrolyte imbalance and dehydration. Food tolerance, food poisoning, intestinal disease, infection (parasitic, bacterial and viral), malnutrition, and sometime medication factors are responsible for diarrhea.^[1-6] Plants having medicinal activity are the source of antidiarrheal drugs.^[7]

The World Health Organization encourages the scientist for study of traditional medicine pertaining to prevention and treatment of diarrheal disease. Diarrhea is a major cause of ill health, especially for children because particularly rotavirus responsible for it.^[4,8,9]

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Tamarindus indica L. (Tamarind), a dicotyledonous belongs to family leguminosae, subfamily caesalpiniaceae, long-lived, slow-growing, massive tree achieve a trunk circumference of 7.5 m (25 ft) and may attain a spread of 12 m (40 ft) and a height of 24-30 m (80 or even 100 ft) under favorable conditions. Traditionally, *T. indica* L. is employed for the treatment of diarrhea, skin cleanse, fever, stomach disorder, and cold.^[10-12]

From literature survey, no relevant literature was found for the study of antidiarrheal activity of ethanolic extract of seeds of *T. indica* L. (EETI). Hence, the aim of recent research to study the traditional claim of EETI for antidiarrheal activity and to access acute toxicity studies.

MATERIALS AND METHODS

Plant material

In the month of May, seeds of *T. indica* L. were collected from local area of Mandasaur, MP and authenticated by botanist Dr. C. L. Ningwale, Government P.G. College, Mandasaur, MP, India.

Preparation of EETI seed

The dried seed of *T. indica* were collected, washed, dried (oven 60°C), crushed by employing blender and convert in to powder after sieving through sieve no.80 then subjected to successive solvent extraction using ethanol at room temperature in a soxhlet apparatus. The extract was vacuum dried and kept in desicator for further studies.^[13,14]

Preliminary phytochemical investigation

For qualitative screening, the preliminary phytochemical investigation was performed on the EETI seed.^[13-20]

Experimental animals

The *in-vivo* antidiarrheal activity was performed in albino Wistar rats of weighing between 120 and 200 g of either sex and used for investigation of acute toxicity study. The protocol of the present work for conduct of animal experiments was approved by the Institutional Animal Ethical Committee (Ref.No.1291/09/ac/CPCSEA). The procure animals were grouped and housed in poly acrylic cages (38 cm × 23 cm × 10 cm) with not more than four animals per cage and maintained under the standard laboratory conditions with natural dark and light cycle (14 h light/10 h dark) at 27±2°C and relative humidity 44-56%. They were allowed free access to standard diet (Golden Feeds, India) and tap water *ad libitum* for 1 week before and during the experiments. The

animals were acclimatized 1 week before start the activity in laboratory.^[21,22]

Acute toxicity study

For acute toxicity study, the EETI was administered orally for lethal dose 50% to albino Wistar rats employing the fixed dose method as per Organization for Economic Co-operation and Development (OECD) guideline and recorded the intake of water and food, mortality, signs and symptoms of toxicity for 48 h and then up to 14 days.^[23]

In-vivo antidiarrheal activity study

Effect of EETI seed on castor oil induced diarrhea

In-vivo antidiarrheal activity was studied using castor oil induced model in albino Wistar rats.^[2-4] The animals were grouped into three groups after random selection, each group contain six albino rats Wistar strain of 6-8 weeks of age of either sex, each weighing 120-200 g. However, third group was further divided into three sub groups of each containing six rats. The following treatments were received by the rats as mention below:

- Group 1 (Control): Animals were received application of 1 ml of castor oil per oral (p.o.)
- Group 2 (Standard): Animals were received application of loperamide (3 mg/kg, p.o.), Yashica Pharmaceuticals Ltd., Thane, Maharashtra, India
- Group 3 (Test): EETI seed
- Group 3A: Animals were treated with EETI seed (100 mg/kg, p.o.)
- Group 3B: Animals were treated with EETI seed (200 mg/kg, p.o.)
- Group 3C: Animals were treated with EETI seed (400 mg/kg, p.o.).

After 1 h administration of drug/EETI, the animals Groups 2 and 3 (A, B, C) received 1 ml of castor oil by oral route then antidiarrheal activity was determined in terms of percentage of protection (inhibition) by following formula.

$$\text{Percentage of inhibition} = (1 - TWS_t / TWS_c) \times 100$$

TWS_t - Total weight of stool of test, TWS_c - Total weight of stool of control.

Statistical analysis

The results of *in-vivo* pharmacological studies were represented as mean ± standard error of mean. The total variations present in data were evaluated using Graph Pad Prism 5 project software analysis of variance followed by Student's *t*-test. The result were considered statistically significant when $P < 0.05$ versus control.

RESULTS AND DISCUSSION

Preliminary phytochemical investigation

The preliminary phytochemical investigation of EETI seed indicated the presence of carbohydrates, alkaloids, flavonoids, glycosides, tannins and absence of saponins, terpenoids, steroids phytoconstituents, results are shown in Table 1.

Table 1: Preliminary phytochemical study of EETI seed

Phytochemical constituents	EETI
Alkaloids	+ve
Carbohydrates	+ve
Flavonoids	+ve
Glycosides	+ve
Tannins	+ve
Terpenoids	-ve
Steroids	-ve
Saponins	-ve

EETI: Ethanolic extract of *Tamarindus indica* L., +ve: Presence, -ve: Absent

Acute toxicity study

It was investigated that EETI seed do not show any sign of mortality and weakness up to 2000 mg/kg, p.o. dose in albino Wistar rats.

In-vivo antidiarrheal activity study

Effect of EETI seed on castor oil induced diarrhea

In Indian traditional medicine system, traditional medicinal practitioners are employing *T. indica* L. in the therapy of diarrhea. EETI seed showed significant antidiarrheal activity at a dose of 400 mg/kg, p.o. in comparison to control group in the castor oil-induced diarrhea model in albino Wistar rats, as result showed in Table 2 and Figure 1. The percentage inhibition of EETI at various 100, 200 and 400 mg/kg, p.o. dose levels was found to be 42.03%, 51.94%, and 78.23% ($P < 0.01$), respectively, and the percentage inhibition by standard drug loperamide was found to be 87.44%.

Hence, in the present study, EETI seed showed significant antidiarrheal activity in the experimental rats. The extract

Table 2: Antidiarrhoeal activity (percent inhibition) of EETI and standard loperamide by employing castor oil induced diarrhea model in albino Wistar rats

Group	Treatment	Dose (mg/kg, p.o.)	Mean weight of feaces (g) [#]	Percentage of inhibition
Group 1	Control	-	5.223±0.748	-
Group 2	Loperamide	3	0.656±0.145**	87.44
Group 3A	EETI	100	3.028±0.091*	42.03
Group 3B	EETI	200	2.510±0.082*	51.94
Group 3C	EETI	400	1.137±0.144**	78.23

EETI: Ethanolic extract of *Tamarindus indica* L. [#]N=6 ± SEM, Data were expressed as mean±SEM when compared to control; * $P < 0.05$, ** $P < 0.01$. Student's test was used for statistical analysis. SEM: Standard error of mean

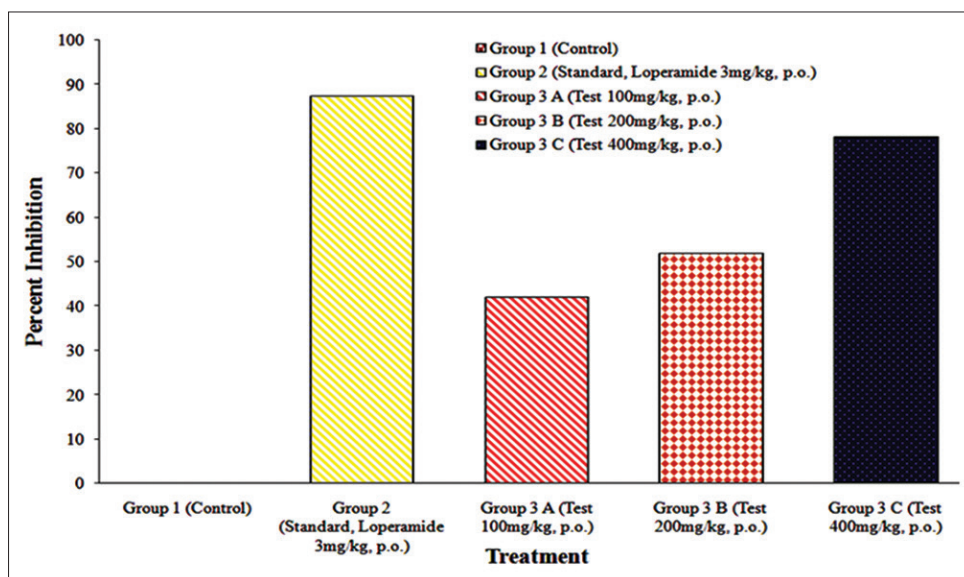


Figure 1: Antidiarrhoeal activity (percent inhibition) of ethanolic (alcoholic) extract of seeds of *Tamarindus indica* L. and standard loperamide by employing castor oil induced diarrhea model in albino Wistar rats

inhibited significantly the frequency of defecation and abatement rapidly the wetness of fecal excretion. The evidence presented here revealed that EETI seeds have dose dependent decrease in diarrhea induced by castor oil in a similar fashion like standard antidiarrheal agent loperamide. The action of castor oil induced diarrhea is due to its most active phytoconstituent ricinoleic acid, which produces an irritating activity of the small intestine. The peristaltic activity become enhanced by employing castor oil and changes the permeability of intestinal mucosal membrane to water and electrolytes.^[6,24,25]

The tannic acid and tannin present in the plant extract may be responsible for mechanism of antidiarrheal action by which extract produced relief in diarrhea because the intestinal mucosa becomes more resistant due to denature proteins forming protein tannate and decrease the intestinal secretion by virtue of which it becomes an excellent remedy for treatment of diarrhea.^[26,27]

Release of autacoids and prostaglandins became inhibited by in the presence of flavonoids, result in decrease of secretion and motility caused by castor oil.^[28]

Phytochemical investigation indicated the presence of tannins, flavonoids, glycosides, and carbohydrates in the EETI so the possible mechanism for the reduction of diarrheal episode may be due to existence of flavonoids and tannins contents in the extract.

CONCLUSION

It was concluded that the significant antidiarrheal activity have been shown by EETI seeds. Further studies on isolation and characterization of phytochemicals constituents of EETI of seed should be done that lead to a development of a potential and promising antidiarrheal age.

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