

EVALUATION OF ANTIDIARRHEAL ACTIVITY OF ETHANOLIC
EXTRACT OF *TAXUS BACCATA* IN WISTAR RATS

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ABSTRACT

The present study was undertaken to evaluate the antidiarrheal activity of alcoholic leaf extract of *Taxus baccata*. The models used in this study were castor oil induced diarrhea in rats, castor oil induced enteropooling, charcoal meal test and the effect of *Taxus baccata* on isolated rat ileum. Each model consisted four groups, control, standard (loperamide), *Taxus baccata* leaf extract 100 mg/kg and 200 mg/kg were used. It was noticed that the fecal weight was reduced in the doses 100 and 200 mg/kg significantly in comparison to control. The

mean intestinal fluid volume was reduced only in the group dosed with *Taxus baccata* leaf extract 200 mg/kg, and at this dose it also decreased the propulsion of charcoal meal through the gastrointestinal tract in comparison to the control. On the isolated rat ileum the leaf extract of *Taxus baccata* demonstrated inhibitory effect to the acetylcholine induced intestinal contractions. Due to its effect of decreasing intestinal secretions and also decreasing the intestinal propulsive movement, the leaf extract of *Taxus baccata* could be a potential antidiarrheal agent in herbal medicine

KEYWORDS: charcoal meal test, enteropooling, intestinal fluid volume.

INTRODUCTION

Diarrhea is one of the most common cause of death in infants and in children less than 5 years accounting to about 5-8 million. Diarrhoea is an alteration in normal bowel movement characterized by an increase in the water content, volume, or frequency of stools.^[1] One of the main processes of diarrhea is the secretory process. Hence there has been a need for the development of the drugs that inhibit this process.^[2]

The present antidiarrheal drugs are known for their adverse effects like abuse potential of codeine, loperamide induced common side effects like abdominal cramps and rashes. In young children serious complications like paralytic ileus and toxic megacolon was seen due to loperamide, probable cause being absorption of toxins.^[3]

By evaluating herbal medicines which have antispasmodic effects, suppress gut motility and stimulate water absorption, we can find a potential anti-diarrheal agent.^[2]

Taxus baccata is an evergreen tree with leaves in two rows, needle like, used as an ornamental plant in gardens.^[4] The leaves are found to possess anticancer action, bronchodilating effect and also decreases bronchial hyperreactivity, also has abortifacient and antimalarial action.^[5]

In present study, the anti-diarrheal activity of ethanolic extract of leaves of *Taxus baccata* was evaluated in experimental animals.

MATERIAL AND METHODS

Plant material

The dry powder of the *Taxus baccata* leaves was procured from an ayurvedic shop from tamil nadu.

Preparation of extract

The extract was prepared by soxhlet extraction for about 30-40 h with 95% ethanol. The solvent was distilled and the extract was concentrated under reduced pressure on a water bath at a temperature below 50°C to a syrupy consistency and was dried in dessicators. The yield was about 7%.

Chemicals- Castor oil, loperamide (Durga chemicals), normal saline, charcoal meal (10% activated charcoal in 100 ml of 5% aqueous gum acacia), acetylcholine (Sigma Aldrich).

Experimental animals

The project was started after obtaining Institutional Animal Ethical Committee approval (IAEC /KMC/52/2012). Male inbred albino rats of Wistar strain weighing 200-250 g were used in this study. They were fasted for 24 h, were kept in polypropylene cages (U.N.Shah Manufacturers, Mumbai) and maintained at 27° C. They were fed with standard rat feed procured from (Amrut lab animal feed, Pranav Agro industries Ltd., Sangli, Maharashtra) and water ad libitum was provided.

Four experimental models were used to study the anti-diarrheal effect of the plant extract

1. Castor oil induced diarrhea in rats
2. Castor oil induced enteropooling
3. Charcoal meal test.
4. To evaluate the effect of *Taxus baccata* on isolated rat ileum.

In the first three models, animals were divided in four groups, each group containing six animals.

Group I-control (distilled water)

Group 2-loperamide 3mg/kg, p.o (standard)

Group 3-*Taxus baccata* leaves extract 100mg/kg, p.o

Group 4- *Taxus baccata* leaves extract 200mg/kg, p.o

Induction of experimental diarrhea

Each animal was given 0.5 mL of castor oil 30 min following the drug treatment. Later they were placed in separate cages to observe for the mean weight of the feces for 4 h. Feces was collected with an absorbant paper placed in the cages. Results were expressed as percentage of inhibition of diarrhea.

Effect of the plant extract on castor oil induced intestinal fluid accumulation

After 30 min of 2 m L of castor oil administration, the rats were anaesthetized. The region of small intestine from pylorus to the cecum was dissected out and the contents were collected into a measuring cylinder to measure the volume of the fluid.

Effect of *Taxus baccata* on isolated rat ileum

Rats were fasted overnight, only access to water was allowed. After sacrificing the rats, the abdomen was dissected out, around 2 cm of rat ileum was removed and mounted in an organ

bath containing tyrode solution, which was aerated with oxygen. Controlled conditions like temperature and pH was maintained. The effect of the extract on acetylcholine induced contraction of rat ileum was demonstrated on a smoked drum.^[6]

Charcoal meal test for intestinal propulsion

Rats fasted for 24 h were treated with respective drugs .After 30 min 1ml of 10% activated charcoal suspension in in 100 ml of 5% aqueous gum acacia was given orally to each rat. The rats were sacrificed and the abdomen was dissected. The distance travelled by charcoal plug from the pylorus to the cecum was measured and peristaltic index calculated.^[7]

$$\text{Peristaltic index} = \frac{\text{length traversed by charcoal meal} \times 100}{\text{Total small intestine length}}$$

Acute toxicity studies

was not done as previous studies have reported no mortality or toxicity signs up to dose of 2000mg/kg.(PK Patel,2009).Hence ,the dose of 100mg/kg and 200 mg/kg were chosen.

Statistical Analysis

All the data was analyzed using SPSS 17 and was represented as Mean±SEM. A P value less than 0.05 was considered to be statistically significant.

RESULTS

1. Effect of alcoholic extract of *Taxus baccata* on Castor Oil Induced Diarrhoea in rats

Diarrhoea was seen in all the treated animals following administration of castor oil for next 4 h.The mean weight of the feces as significantly reduced in the loperamide group (12.75 ± 0.30) compared to control (30.00 ± 0.52).A similar reduction in weight of the feces was observed with *Taxus baccata* extract in doses of 100 and 200 mg/kg i.p. (Table 1)

2. Effect of alcoholic extract of *Taxus baccata* on castor oil induced enteropooling in rats

Due to castor oil administration there is accumulation of water and electrolytes in the intestinal loop.Loperamide has significantly reduced the mean volume of intestinal fluid in comparison to the control. The plant extract dose of 200mg/kg has significantly reduced the mean volume of intestinal fluid in comparison to control. (Table 2)

3. Effect of *Taxus baccata* leaf extract on gastrointestinal transit

The plant extract at a dose of 200mg/Kg significantly decreased the propulsion of charcoal meal through the gastrointestinal tract in comparison to the control. (Table 3)

4. Effect of *Taxus baccata* on isolated rat ileum

The acetylcholine induced intestinal contractions were reduced when *Taxus baccata* leaf extract 2mg/mL was added along with acetylcholine. The acetylcholine induced contractions were totally inhibited by *Taxus baccata* leaf extract 4mg/ mL (Figure 1).

DISCUSSION

The major mechanisms by which the antidiarrheal drugs act is by decreasing the secretions or reducing the gastrointestinal motility. Castor oil and its active component ricinoleic acid are known for their irritant action on the intestinal mucosa, contributing to the release of prostaglandins causing the diarrheal effects.^[8,9,10]

According to the phytochemical screening report of a previous study, leaves of *T.baccata* showed the presence of lignans and flavonoids. Both lignans and flavonoids possess various biological activities like antibacterial, spasmolytic and anti-inflammatory.^[5] Flavonoids are also known to inhibit the prostaglandin induced intestinal secretions.^[11]

From our study it is seen that *Taxus baccata* leaves extract inhibited the castor oil induced diarrhea and reduced intestinal transit of charcoal. A dose dependent effect was seen and results were comparable to the standard drug loperamide, which is a most widely used antimotility drug in diarrhoea. Its effect as shown on the isolated rat ileum, has inhibited the acetylcholine induced intestinal contractions, thus demonstrating the possible antimuscarinic effect it may possess. The antisecretory and the antipropulsive effects could be suggested as the possible mechanism of the antidiarrheal activity.

Table 1. Effect of alcoholic extract of *Taxus baccata* on Castor Oil Induced Diarrhoea in Rats

Groups	Mean weight of feces after 4 h	Percentage inhibition
Control + castor oil	30.00 ±0.57	
Loperamide -2mg/kg+ castor oil	12.75±0.30*	65.50%
<i>Taxus baccata</i> extract - 100mg/kg + castor oil	18.41± 3.21*	38.63%
<i>Taxus baccata</i> extract - 200mg/kg + castor oil	15.33± 0.24*	48.90%

*P < 0.05 statistically (Mean±SEM) significant from control group (n=6)

Table 2. Effect of alcoholic extract of *Taxus baccata* on castor oil induced Enteropooling in Rats

Groups	Mean volume of intestinal fluid (ml) \pm SEM	Percentage inhibition
Control + castor oil	2.21 \pm 0.11	
Loperamide -2mg/kg + castor oil	0.85 \pm 0.04*	75.56%
<i>Taxus baccata</i> extract -100mg/kg + castor oil	1.73 \pm 0.06	21.71%
<i>Taxus baccata</i> extract -200mg/kg + castor oil	1.18 \pm 0.05*	46.60 %

*P < 0.05 statistically (Mean \pm SEM) significant from control group (n=6)

Table 3. Effect of alcoholic extract of *Taxus baccata* on gastrointestinal transit

Groups	Peristaltic index
Control + castor oil	51.65 \pm 0.96
Loperamide-2mg/kg + castor oil	28.75 \pm 0.72*
<i>Taxus baccata</i> extract -100mg/kg+ castor oil	34.39 \pm 1.65
<i>Taxus baccata</i> extract -200mg/kg+ castor oil	22.72 \pm 0.62*

P < 0.05 statistically (Mean \pm SEM) significant from control group (n=6)

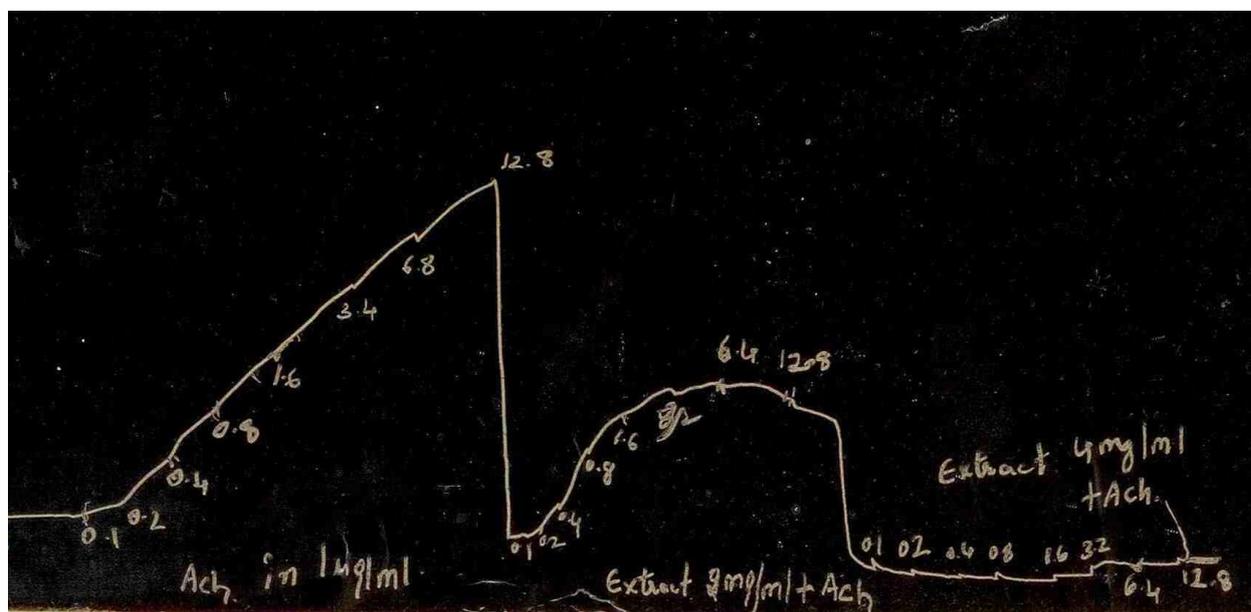


Figure 1. Effect of *Taxus baccata* leaf extract on acetylcholine induced contractions of isolated rat ileum

CONCLUSION

We conclude that *Taxus baccata* leaf extract has found to possess antidiarrheal property; further research is required to elucidate the various chemical compounds responsible for this property.

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