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Evaluation of antidiarrheal activity of ethanolic extract of *Bauhinia variegate (*leguminosae) stem bark in wister albino rats

Rupa Sengupta[*,1] and Abdul Baquee Ahmed[2]

[1]* ROFEL, Shri G.M.Bilakhia College of Pharmacy, Namdha Road, Vapi, Gujarat, India.

[2] Girijananda Chowdhury Institute of Pharmaceutical Science, Hathkhowapara, Azara, Guwahati, Assam, India

***Corresponding Author:** Rupa Sengupta., Assistant Professor, Department of Pharmacognosy,ROFEL, Shri G.M.Bilakhia College of Pharmacy. Vapi, Namdha Road.PO Box No-11, Vapi west. Pin-396191, Gujarat. India.Cell No. +91 9904348668.
Email: rupasengupta222@rediffmail.com

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Abstract

The present study was performed to substantiate the traditional claim of the antidiarrheal activity of stem bark extract of *Bauhinia variegata* Linn. in rats. The effects of ethanolic extract of the stem bark of *Bauhinia variegata* on castor oil-induced diarrhea, castor oil magnesium sulphate-induced enteropooling, and gastrointestinal motility test using charcoal meal method were examined. The extract was initially assayed for its effects in castor oil-induced diarrhea at different doses (250, 500, and 1000mg/kg, p.o.) in which significant activity ($p<0.05$) was observed at a dose level of 500 mg/kg. Hence, this dose level was then used in other models. The extract was found to inhibit peristaltic movements in charcoal meal test, and intestinal fluid secretions in castor oil as well as magnesium sulphate induced enteropooling, confirming its antidiarrheal activity, which might be due to its high flavonoid and tannin content.The results provide evidence that the ethanolic extract of *Bauhinia variegata* stem bark possesses antidiarrheal activity.

1. INTRODUCTION

Bauhinia variegata also known as Kachnar (Hindi), is a medium sized deciduous tree distributed in sub-Himalayan tract and outer Himalaya of Punjab. There are two varieties Purple and White flowered. The present work has been done on white flowered variety that is *Bauhinia variegata* Linn var candida. The main chemical constituent present in bark is Lupeol which is a pharmacologically active triterpenoid found in variety of plants. It has several medicinal properties, one being anti-inflammatory which is supposed to be due to its action on interleukin system.[1]

Literature survey suggests that bark of purple flowered variety exhibits hepatoprotective, hypolipidemic, antioxidant, immunomodulatory, anti-inflammatory and anti microbial activities but very less work has been reported on the white flowered variety .This is the major reason behind selecting this plant for present work.[2-4]

This study aimed to investigate antidiarrheal activity of the ethanol extract from the stem bark of *Bauhinia variegata*, initially on castor oil-induced diarrhea (250, 500, and 1000mg/kg) and subsequently confirming its action in castor oil induced enteropooling, magnesium sulphate induced enteropooling, and gastrointestinal motility test using the charcoal meal method in rats.

2. MATERIALS AND METHODS

2.1 Plant materials and chemicals

The fresh stem bark of *Bauhinia variegata was* collected in June2014 from Valsad district of Gujarat. and Voucher herbarium specimen was deposited in the Department of Pharmacognosy of ROFEL.Shri G.M.B college of Pharmacy for future reference bearing a No-RPC/RS/35.The

following drugs and AR grade of chemicals were used: atropine sulphate and loperamide (standard reference antidiarrheal drugs), castor oil (laxative agents), normal saline solution (0.9% NaCl), charcoal meal (10% activated charcoalin, 0.5% w/v sodium carboxy methyl cellulose) and vehicle (0.5% w/v NaCMC) were used. All the treatments were given in a dose volume of 10 ml/kg, p.o. All the other chemicals and reagent used were of analytical grade, obtained from Priya Enterprise, Vapi, Gujarat.

2.2 Preparation of the extract from the stem bark of *Bauhinia variegata*

The shade-dried stem bark material was powdered. The coarse powder was subjected to extraction with ethanol in soxhlet apparatus at (60-80°C) The extract was concentrated and stored in amber bottles and refrigerated. The drug extract was suspended in sodium carboxymethyl cellulose (NaCMC 0.5%, w/v).

2.3 Phytochemical investigation

The dried extracts were weighed, and percentage yields were calculated. The methanolic extracts were used for preliminary phytochemical screening with a battery of chemical tests viz., Molisch's, Fehling's, Benedict's and Barfoed's tests for carbohydrates; Biuret and Millon's tests for proteins; Ninhydrin's test for amino acids; Salkowski and Liebermann-Burchard's reactions for steroids; foam test for saponin glycosides; Shinoda and alkaline tests for flavonoid glycosides; Dragendorff's, Mayer's, Hager's and Wagner's tests for alkaloids; and ferric chloride, lead acetate, potassium dichromate and dilute iodine tests for tannins and phenolics; tests for fats, oils and vitamin C.

2.4 Animals

Wistar albino rats of either sex weighing 120-200 g were maintained at 25±2ºC Temperature, 50±15ºC relative humidity and normal photoperiod(12h dark/12h light) in plastic cages. The animals were fed standard pellet diet and water *ad libitum*. All the animal experiments were carried out in accordance with the guidelines of CPCSEA and were approved by the Institutional Animal Ethical Committee.

2.5 Antidiarrheal activities

2.5.1 Castor oil induced diarrhea

The antidiarrheal activity of ethanolic extract was evaluated according to the method described [5]. Rats were fasted for 18 hours and divided into five groups of five animals each group. Castor oil at a dose of 1 ml was given orally to all groups of animals for the induction of diarrhea. One hour prior to castor oil administration various treatments were given, Group I (control) and given 0.5 % sodium carboxymethyl cellulose (Na CMC), Group II (standard) was treated with loperamide (3 mg/kg p.o.), a positive control. Group III-V were administered ethanolic extract BDEE (250, 500, and 1000 mg/kg) respectively by oral route. Animals were placed separately in individual cages lined with filter paper. The filter papers were changed every hour and the severity of diarrhea was assessed hourly for 4 hours.

2.5.2 Gastrointestinal motility test

Wistar rats were fasted for 18 hours and divided into three groups of five animals each, group I animals served as control and was treated orally with 0.5% w/v Sodium CMC in distilled water. Group II were treated orally with atropine 5 mg/kg, a positive control. Group III received orally 500 mg/kg extract of ***Bauhinia variegate.*** After 1 h, each animal received charcoal meal 0.25 ml (10% charcoal in 0.5%w/v Sodium CMC) administered orally. Thirty minutes later, the animals were sacrificed. Total small intestine from pylorus to caceum was isolated and the total length and the length traveled by the charcoal meal were measured. This distance was expressed as a percentage of the length of the small intestine .

$$\% \text{ Inhibition} = Mc - Md/Mc \times 100$$

Mc: mean distance travelled by charcoal meal; Md: mean distance travelled by drug or extract.

2.5.3 Castor oil-induced enteropooling

Rats were fasted for 18 hours and divided into three groups of five animals each. Group I which received normal saline (2 ml p.o.), served as the control group. Group II received loperamide (3 mg/kg p.o.). Group III received ***Bauhinia variegate*** extract of 500 mg/kg p.o., one hour before the oral administration of castor oil (2 ml v/v). One hour later, the rats were sacrificed, and the small intestine was removed after tying the ends with threads and weighed. The intestinal content was collected into a graduated cylinder and its volume measured. The intestine was reweighed and the difference between the full and empty weights calculated.[6]

2.5.4 Magnesium sulphate-induced enteropooling

Rats were fasted for 18 hours and divided into three groups of five animals per group. Solutions of magnesium sulphate were made in the 10% w/v aqueous solution. Group I which received normal saline (2 ml p.o.) served as the control group. Group II received loperamide (3 mg/kg p.o.). Group III received ***B.variegata*** extract of 500 mg/kg p.o. Immediately after the extract administration, magnesium sulphate (10 % w/v) was administered. After 30 minutes following administration of magnesium sulphate the rats were sacrificed, the small intestine was removed after tying the ends with threads and weighed. The intestinal content was collected into a graduated cylinder and its volume measured.
The intestine was reweighed and the difference between the full and empty weights calculated [7].

2.5.5 Statistical analysis

Data were expressed in as the mean ± standard error of mean (S.E.M.) and statistical analysis was carried out employing one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test at $P < 0.05$ significance level using "Graphpad Instat" version 3.00 for Windows 95, Graphpad Software, San Diego, California, USA.

3. RESULTS

3.1 Phytochemical investigation

The percentage yields of the ethanolic extracts were found to be 12.36% w/w. The ethanolic extract showed the presence of flavonoid, tannin, saponin, and protein. The total flavonoid content in ethanolic was found to be 12.15 mg quercetin equivalents/g.

3.2 Effects of the ethanolic extract of *Bauhinia variegate* on castoroil-induced diarrhea

One hour after castor oil administration, all the rats in the control group of animals produced copious diarrhea. Pretreatment of rats with ethanolic extract of ***B. variegata***(BDEE 250, 500, and 1000 mg/kg, p.o.) dose dependently and significantly ($p<0.05$) delayed the onset of diarrhea, reduced the frequency of defecation and the wetness of the fecal droppings (reduction in the no. of wet stool and the general diarrheal scores including the hard and copious stool (Table 1). The standard antidiarrheal drug loperamide (3 mg/kg p.o.) produced a significantly greater ($p< 0.05$) inhibitory effect in all the diarrheal parameters.

3.3 Effects of the methanolic extract of *B. variegate* on gastrointestinal motility test

Compared with the control group, (BDEE 500 mg/kg p.o.) significantly ($p<0.05$) decreased the propulsive movement and transit of charcoal meal to the gastrointestinal tract. The standard antidiarrheal drug, atropine (5 mg/kg p.o.) produced greater antimotility effect than the higher dose of (BDEE 500 mg/kg p.o.) (Table 2).

3.4 Effects of the methanolic extract of *B.variegata* on castor oil-induced enteropooling

Oral administration of castor oil (2 ml p.o.) produced a marked and significant ($p<0.05$) increase in the intestinal fluid volume of castor oil-treated groups of rats compared to control group of animals treated with normal saline (2 ml p.o.) only. Compared with the control group of rats, pretreatment of the 'test' group of rats with BDEE (500 mg/kg p.o.)dose dependently and significantly ($p<0.05$) inhibited castor oil-induced enteropooling in rats (Table 3). The standard drug, loperamide produced a marked and significantly greater ($p<0.05$) inhibitory effect on castor oil-induced fluid accumulation than the higher dose of BDEE (500 mg/kg p.o.) (Table 3).

3.5 Effects of the methanolic extract of *B. variegate on* magnesium sulphate-induced enteropooling

The extract reduced the intestinal fluid secretion induced by magnesium sulphate, in a dose dependent fashion (Table 4). The standard antidiarrheal drug, loperamide (3 mg/ kg, p.o.), produced a more marked and significantly greater ($p<0.05$) inhibitory effect on magnesium sulphate-induced fluid accumulation than the extract (Table 4).

Table 1: Effect of *B. variegate* bark extract on castor oil induced diarrhea

Treatment	Dose (mg/ml)	No. of rats with diarrhea out of no-5	Mean defecation in 4 hrs	Percentage inhibitionof defecant
Control	-----	05	0.55±0.03	-----
Loperamide	03	00	0.001±0.004[a]	99.8
BDEE	250	05	0.067±0.031[a]	87.8
BDEE	500	05	0.024±0.025[a]	95.6
BDEE	1000	05	0.023±0.020[a]	95.1

BDEE= ***B. variegate*** ethanolic extract ; LOP = Loperamide

Results are expressed as mean ± SEM; n=5 in each group comparison made with control (0.5%NaCMC) group and with standard (loperamide 3 mg/kg) group. Data was analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test. [a]p <0.05= compared to control group, [c]p <0.05= compared to standard group (loperamide 3 mg/kg).

Table 2: Effect of *B. variegate* bark extract on gastrointestinal motility test

Treatment	Dose (mg/ml)	% Intestinal Transit
Control	----------	80.5±7.9
Atropine sulphate	3	29.4±0.9[a]
BDEE	500	48.0±6.2[a]

Results are expressed as mean ± SEM; n=5 in each group comparison made with control (0.5% NaCMC) group and with standard (atropine sulphate 5 mg/kg) group. Data was analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test. . [a]p <0.05= compared to control group, [c]p <0.05= compared to standard group(atropine sulphate 5 mg/kg).

Table 3: Effect of *B.variegata* bark extract on castor oil-induced enteropooling

Treatment	Dose (mg/ml)	Volume of fluid(ml)	Weight of intestinal contents (gm)	Percentage inhibition (%)
Control	----------	1.49±0.2	2.3±0.05	----
Atropine sulphate	3	0.53±0.50	0.76±0.03[c]	64
BDEE	500	1.37±0.50	1.01±0.45[c]	60

BDEE= ***B. variegate*** ethanolic extract ; LOP = Loperamide

Results are expressed as mean ± SEM; n=5 in each group comparison made with control (0.5%NaCMC) group and with standard (loperamide 3 mg/kg) group. Data was analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test. [a]p <0.05= compared to control group, [c]p <0.05= compared to standard group (loperamide 3 mg/kg).

Table 4: Effect of *B. variegate* bark extract on magnesium sulphate-induced enteropooling

Treatment	Dose (mg/ml)	Volume of fluid (ml)	Weight of intestinal contents (gm)	Percentage inhibition (%)
Control	----------	4.5±0.25	11.13±0.26	
Atropine sulphate	3	2.2±0.26	7.27±0.37[c]	33.6
BDEE	500	2.8±0.22	8.47±0.54[c]	13.9

BDEE = B. variegate methanolic extract

Results are expressed as mean ± SEM; n=5 in each group comparison made with control(2 ml normal saline) group and with standard (loperamide 3 mg/kg) group. Data wasanalyzed by one way ANOVA followed by Tukey Kramer multiple comparison test. [a]p <0.05= compared to control group, [c]p <0.05= compared to standard group (loperamide3 mg/kg).

4. DISCUSSION

Diarrhea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by hurry, leading to an excess loss of fluid in the feces [8]. Indeed, the oral administration of AESE provoked, like diphenoxylate, a significant dose-dependent increase in the latency time, a significant decrease in the frequency of defecation with a subsequent increase in the percentage of inhibition of defecation in castor oil treated animals.

Diarrhea induced by castor oil results from the action of ricinoleic acid which causes the irritation and inflammation of the intestinal mucosa leading to prostaglandins (PGE2α) release. The released PGE2 stimulates gastrointestinal motility and secretion of water and electrolytes[9], thus inducing an increase in the peristalsis and an intestinal hyper secretion of fluid. The inhibition of prostaglandins biosynthesis prolongs the time of induction of diarrhea by castor oil [10].

The antidiarheal activity of the alcoholic extract of the stem bark of ***B. variegate*** was evaluated by employing castor oil-induced diarrhea, gastrointestinal motility test, castor oil and magnesium sulphate-induced enteropooling methods. The results of the present study showed that the ethanolic extract of ***B. variegate*** stem bark in castor oil-induced diarrhea at 250, 500 and 1000 mg/kg body weight doses significantly lowered several typical parameters of diarrhea, producing a statistically significant reduction in the severity and frequency of diarrhea produced by castor oil. Furthermore, our preliminary investigations have revealed that the extract was safe up to 5 g/kg dose level in acute oral toxicity studies (data not shown in the manuscript), and there are no reports about any specific toxicity of the plant in literature.

In the evaluation of intestinal transit, atropine sulphate was used as the standard drug. Atropine is known to inhibit intestinal transit probably due to its anticholinergic effect[11]. The ethanolic extract of ***B. variegate.S***tem bark also appeared to act on all parts of the intestine. Thus, it reduced the intestinal propulsive movement in the charcoal meal treated model at a dose level of 500 mg/kg of body weight, and a transit period for sixty minutes, though this was not comparable to the effect of atropine sulphate. Nevertheless, this is but logical since atropine sulphate is pure compared to the extract which is mixture of many compounds. Studies made on activated charcoal showed that it prevents the absorption of drugs and chemicals into the system by adsorbing them on the surfaces of the charcoal particles. Activated charcoal was used in the gastrointestinal motility test to find out the effects of these extracts on the peristaltic movement. The results show that these extracts suppressed the propulsion of charcoal meal (probably in the same way as atropine sulphate) and thereby increased the time for absorption of water and electrolytes. Further, the experiments carried out on gastrointestinal tract motility after charcoal meal administration also showed a reduction in the propulsive movement of the small intestine after pretreatment with the extract of ***B. variegate.***

In the enteropooling study, the BDEE (500 mg/kg) significantly reduced the intestinal content of the rat. The intraluminal fluid accumulation was blocked significantly by the ethanolic extract of ***B. variegate***. The intestinal fluid secretion induced by castor oil was blocked by the test extract in a dose-related manner. Further, the experiments carried out on the gastrointestinal tract motility after charcoal meal administration also showed a reduction in the propulsive movement of the small intestine after pre-treatement with the extract of ***B. variegate***. Intestinal fluid secretion has been analyzed by enteropooling assay in rat, evoked by magnesium sulphate (a standard laxative agent). It is known that castor oil induces alteration in intestinal electrolyte transport. Our results suggest that the effects of ***B. variegate*** extract may be due to an increase in the absorption of electrolytes and or inhibition of the hypermotility of the intestine, thereby increasing its capacity to retain fluids, an action similar to that of loperamide.

The phytochemical analysis of the extract showed the presence of flavonoids and terpenes. These constituents may responsible for the antidiarrheal activity of ***B. variegate*** ethanolic extract. The antidiarrheal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretion, which are known to be altered in this intestinal condition [12].

Thus, the present study systematically investigated the antidiarrheal potential of ethanolic extract of ***B. variegate***, and supports its traditional use as antidiarrheal medicine.

5. CONCLUSION

The bark extract contains pharmacologically active substances with antidiarrheal properties.This antidiarrheal activity probably results from the spasmolytic or may be due to a possible antisecretory effect of the plant extract on the intestinal smooth muscle. Thus, this lends some credence to its widespread traditional use by the local population as an antidiarrheal agent. The plant seems safe based on the results of acute toxicity testing.

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