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Research Article

Evaluation of Antidiarrhoeal Activity of the Leaves and Stem of *Bauhinia vahlii*

Divaker Shukla*, Kavita Gahlot

ABSTRACT

Objective: The present study aimed to investigate the antidiarrhoeal activity of the traditional claim of *Bauhinia vahlii* Wight and Arn. **Method:** Dried ethanolic extracts of leaves and stem of the plant were used at two dose level (200 mg/ kg and 400 mg/kg p.o) screened separately for their antidiarrhoeal activity by Castor oil induced diarrhea and enteropooling in rats model. **Result:** The leaves extracts showed significant (p<0.001) antidiarrhoeal activity at 400 mg/kg in both the tested models. **Conclusion:** The findings of the present study provide clear idea and prove the traditional claim that ethanolic extracts of the leaves and stem of *Bauhinia vahlii* (EEBVL and EEBVS).

Key words: *Bauhinia vahlii*, Antidiarrhoeal activity, Castor oil induced diarrhea, Castor oil induced enteropooling.

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INTRODUCTION

Diarrhea is one of the death causing diseases, especially in developing countries. It has been a very concerning issue as it causes millions of deaths. Diarrhea is a gastrointestinal disorder, which is characterized by an increase in frequency of stool with changed consistency. The incidence of diarrhea is still high, despite of all the efforts done by international organizations. As, antibiotics are used against diarrhea, sometimes it cause side effects, Therefore, there is need to search the effective and safe agents from plant origin.[1]

Additionally, Diarrhea includes the alteration in the normal bowel movement, characterized by increased in the water content in the intestine and enhanced frequency of stools.[2] It is caused by the organism including *Shigella flexneri*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Candida albicans* and also it is one of the primary causes of mortality especially in developing countries.[3] Diarrhea is the world's third highest killer disease about 7.1 million per year, despite of all the efforts made by international organizations to control this disease.[4] Some synthetic chemicals including diphenoxylate and loperamide are used for the treatment of diarrhea but their uses are restricted due to adverse effects produced by them.[5] Therefore, the search for safe and more effective agents for the treatment of diarrhea from plant origin has continued to be an important area of active research. At present, several naturally occurring compounds are enormously used for the treatment of diarrhoea because these herbs are readily available, affordable and an indispensable component of traditional medicine practice. For this reason, international organizations including the World Health Organization (WHO) have encouraged studies directly related with the treatment and prevention of diarrhea using traditional medicines.[6]

Therefore, it is essential and important to identify and evaluate the commonly available plant based drugs as an alternative of currently being used antidiarrhoeal drugs, which are not completely free from adverse effects.

Bauhinia vahlii Wight & Arn belonging to the family Caesalpiniaceae is used in Indian traditional medicine as antidiarrhoeal drug. The plant is a giant climbing shrub found throughout the India. It is commonly distributed in the Sub-Himalayan region up to 3,000 meters above the sea level and also found in Assam, Central India, Bihar, Eastern and Western Ghats. This plant is called as "Maljan" in Hindi and "Malanjhana" in Sanskrit.[7,8] Various part of *B.vahlii* are used as the seeds of this plant possesses tonic and aphrodisiac properties. The leaves are demulcent, antidiarrhoeal and mucilaginous. Stem bark of this plant is useful for skin disease and pods are taken orally as antidiarrhoeal and antidysentery agent and roots are used for the pulmonary tuberculosis and root juice are used to treat dysentery. The leaves and stem of *B.vahlii*, the villagers used as traditional medicine in various ailments such as Diarrhea, dysentery, stomachache, fever, wounds, cuts and skin diseases in the form of decoction, juice and infusion. Bark juice is given orally, immature seed is also used.[8, 9] The various major constituents of leaves of *B.vahlii* contain agathisflavone, betulinic acid, campesterol, quercetin, isoquercetrin, quercetin 3-glycoside, kaempferol, β-sitosterol and stigmasterol.[10] The flowers of *B.vahlii* contain flavanol glycoside quercitroside, isoquercitroside, rutoside, taxifoline and rhamnoside. The seed of *B.vahlii* contain rich source of crude protein (24.59%), crude lipid (23.26%), crude fibre (6.21%), carbohydrates (41.72%), minerals and essential amino acid.[8,11]

Therefore, in the present study the vacuum dried ethanolic extract of leaves and stem of *Bauhinia vahlii* were evaluated for their antidiarrhoeal activity by Castor oil induced diarrhea and enteropooling in rats method.

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MATERIALS AND METHODS

Plant material

The plant, *Bauhinia vahlii* leaves and stems were collected from nearby place of Chandi Devi Temple located at Haridwar District, Uttarakhand, India. The plant specimen was authenticated by Dr. S. K. Sinha, Scientist-E, Botanical Survey of India, Allahabad, (BSI). A voucher specimen (BSI/CRC/2015-16/603) of the plant specimen was deposited in the herbarium section of BSI, Allahabad for further reference.

Preparation of extract

Air dried coarsely powdered (40 mesh size)[12] leaves and stems of *B. vahlii* were successively extracted separately with ethanol (95%) by hot percolation method using soxhlet apparatus for two days at 45-50°C temperature.[13] The extracts were filtered and filtrate were distilled at low temperature (55-60°C) and finally evaporated under reduced pressure to obtain dried ethanolic extract of *Bauhinia vahlii* leaves (EEBVL) and ethanolic extract of *Bauhinia vahlii* stem (EEBVS).The two extracts viz EEBVL and EEBVS were made into fine suspension with 1% w/v aqueous normal saline as the suspending agent and used for in vivo antidiarrhoeal evaluation by two models.

Animal selection

Wistar albino rats of either sex weighing between 150-200g were selected for the toxicity evaluation and antidiarrhoeal activity. The animals were acclimatized to standard laboratory conditions (temperature 25 ± 2˚C) and maintained on 12 h light; 12 h dark cycle. The animals were fed with standard animal feed and water *ad libitum*.[14] The protocols for animal care and experimental procedures were approved and performed in accordance with Institutional Animal Ethical Committee (IAEC) with approval no.837/ac/04/CPCSEA.

Acute toxicity study

The acute oral toxicity study was carried out as per the OECD 423 guidelines set by organization for economic co-operation and development.[15] The ethanolic extract of leaves (EEBVL) and stem (EEBVS) were administered at the single dose level of 2000 mg/kg. One tenth and one fifth of the lethal dose (LD_{50}) was considered for further pharmacological evaluation. The doses were selected after oral acute toxicity study (LD_{50} determination).

In vivo antidiarrhoeal activity models

Castor oil induced diarrhea in rats

The antidiarrhoeal activity of EEBVL and EEBVS were studied by castor oil induced diarrhoea method. Wistar albino rats of either sex (150-200 g) were fasted for 18h.The selected rats for castor oil induced diarrheal test were divided into six groups (n =6). Group I was given normal saline (2 ml/kg) orally as control group and Group II received loperamide (5 mg/kg) as standard group. Groups III and IV were given EEBVL in dose of 200 and 400 mg/kg bw whereas group V and VI were given EEBVS 200 and 400 mg/kg bw respectively. After 1 h, all groups received castor oil 1ml each orally. Afterwards, they were placed in cages lined with adsorbent papers and observed for 4h for the presence of characteristic diarrheal droppings. The total number of feces of control group was considered as 100%.[16] The frequency of defecation and number of diarrhoeal feces excreted in the recorded time were scored and compared with control group. The results were expressed in percentage of inhibition.[17]

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

Percent (%) inhibition of defecation = $[(A - B) / A] \times 100$,

Where A is mean number of defecation time caused by castor oil and

B is mean number of defecation time caused by drug or extract.

Castor oil-induced enteropooling in rats

Castor oil-induced enteropooling test is used to determine the prevention of fluid accumulation ability of plant based extract. In the present study, also rats of both sexes (150–200 g) were fasted for 18 h. The selected rats for this test were divided into groups (n= 6). Animals of Group I (control group) was given normal saline (2ml/kg) orally while Group II (standard group) received standard drug loperamide (5 mg/kg).[18] The rest of the groups III and IV were given EEBVL in dose of 200 and 400 mg/kg bw whereas group V and VI were given EEBVS 200 and 400 mg/kg bw respectively. After 1 h, all groups received castor oil, 1ml orally per animal. Two hours later, all rats were sacrificed and the small intestine from the pylorus to the cecum was isolated. The intestinal contents were collected by milking into graduated tube and their volume was measured and recorded.[19]

Statistical analysis

The data were expressed as Mean ± SD (n=6). Statistical analysis of data was carried out by two-way ANOVA followed by Bonferroni test *($p<0.05$), ** ($p<0.01$) and ***($p<0.001$) when compared with control group was statistically significant. All the graph in the study were drawn with Graph Pad Prism.

RESULTS AND DISCUSSION

Castor oil induced rats model

In case of castor oil induced diarrhoeal test, the EEBVL showed a marked antidiarrhoeal effect in the rats (Table 1). In both doses, 200 mg/kg and 400 mg/kg, extracts produced significantly ($p<0.001$) inhibition of defecation at 400 mg/kg of the EEBVL. The leaves and stem extracts decreased the total amount of diarrhoeal feces produced upon administration of castor oil (7.67 ± 1.21g and 6.83 ± 1.33 g) and (10.50 ± .1.05 g and 10.00 ± 1.10 g) at doses 200 mg/kg and 400 mg/kg respectively in dose dependent manner compared to control group (12.83 ± .1.72 g) while in case of loperamide decreased total amount of diarrhoeal feces (6.50 ± .1.52 g) at the dose of 5 mg/kg. The percentage inhibition of diarrhoeal in the EEBVL 200 mg/kg and 400 mg/kg showed the significant antidiarrhoeal effect. The EEBVL 200 mg/kg and 400 mg/kg were found more effective as compared to the EEBVS. The ethanolic extract of leaves 200 mg/kg and 400 mg/kg showed % inhibitions of diarrhoeal were found 40.22 and 46.77 respectively. The ethanolic extract of stem 200 mg/kg and 400 mg/kg showed % inhibitions of diarrhoea were found 18.16 and 22.06 respectively. The EEBVL showed higher % inhibition of diarrhoea as compared to EEBVS. The tannins and phenolic compounds were present in ethanolic extract of the leaves and stem of *B.vahlii* but ethanolic extract of leaves showed more prominent result as compared to ethanolic extract of stem. The antidiarrhoeal effect might be due to presence of the tannins and phenolic compounds.

The activity was expressed as % inhibition of defecation and % inhibition of diarrhoeal. The findings of castor oil induced diarrhea are presented in Table 1 and Figures 1 and 2 (A, B).

Castor oil induced enteropooling in rats model

In Castor oil induced enteropooling in rats test, EEBVL and EEBVS at both the doses of 200 mg/kg and 400 mg/kg, extracts produced significantly ($p<0.001$) % inhibited of intestinal volume at 400 mg/kg of the EEBVL. The ethanolic extracts of leaves showed most potent effect over intestinal content of weight and volume as compared to ethanolic extracts of stem (Table 2). Among the two extracts, EEBVL

Table 1: Effect of ethanolic extracts of leaves and stem of *B. vahlii* on castor oil induced diarrhea in rats.

Group	Treatment	Total number of feces (Mean ± SD)	% Inhibition of defecation	Total number of diarrhoeal feces (Mean ± SD)	% Inhibition of diarrhoeal
I	Castor oil and Saline (2ml/kg p.o.)	21.17 ± 1.94	-	12.83 ± .1.72	-
II	Castor oil and Loperamide (5 mg/kg p.o)	8.00 ± 1.63***	62.21	6.50 ± .1.52***	49.34
III	Castor oil and Leaves extract (200 mg/kg p.o.) (EEBVL)	12.00 ± 1.26**	43.32	7.67 ± 1.21**	40.22
IV	Castor oil and Leaves extract (400mg/kg p.o.) (EEBVL)	8.67 ± 1.37***	59.05	6.83 ± 1.33***	46.77
V	Castor oil and Stem extract (200 mg/kg p.o.) (EEBVS)	16.67 ± 1.03*	21.26	10.50 ± .1.05*	18.16
VI	Castor oil and Stem extract (400 mg/kg p.o.) (EEBVS)	15.83 ± 1.17*	25.22	10.00 ± 1.10*	22.06

All values were expressed as Mean ± SD (n=6). Statistical analysis of data was carried out by two-way ANOVA followed by Bonferroni test *($p<0.05$), **($p<0.01$) and ***($p<0.001$) when compared with control group.

Table 2: Effect of ethanolic extracts of leaves and stem of *B. vahlii* on castor oil induced enteropooling in rats.

Group	Treatment	Weight of intestinal content (g) (Mean ± SD)	Volume of intestinal content (ml) (Mean ± SD)	Inhibition of the intestinal fluid (%)
I	Castor oil and Saline (2ml/kg p.o.)	4.17 ± 0.98	3.67 ± 0.82	-
II	Castor oil and Loperamide (5 mg/kg p.o.)	1.83 ± 0.75***	1.50 ± 0.84***	59.12
III	Castor oil and Leaves extract (200 mg/kg p.o.) (EEBVL)	2.33 ± 0.82***	2.00 ± 0.89***	45.50
IV	Castor oil and Leaves extract (400 mg/kg p.o.) (EEBVL)	2.00 ± 0.89***	1.67 ± 0.82***	54.49
V	Castor oil and Stem extract (200 mg/kg p.o.) (EEBVS)	3.00 ± 0.89**	2.83 ± 0.98**	22.88
VI	Castor oil and Stem extract (400 mg/kg p.o.) (EEBVS)	2.50 ± 0.84**	2.33 ± 0.82**	36.51

All values were expressed as Mean ± SD (n=6). Statistical analysis of data was carried out by two-way ANOVA followed by Bonferroni test *($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$) when compared with control group.

Figure 1: Plant of *Bauhinia vahlii*.

and EEBVS showed castor oil induced enteropooling by reduction in weight of intestinal content (g) and volume of intestinal content (ml) (2.00 ± 0.89 and 1.67 ± 0.82) and (2.50 ± 0.84 and 2.33 ± 0.82) at the concentration of 400 mg/kg bw respectively in dose dependent manner compared to control group(4.17 ± 0.98 and 3.67 ± 0.82) .The EEBVL % inhibited the intestinal fluid accumulation showed by 45.50 % and 54.49 % at doses 200 mg/kg and 400 mg/kg respectively and the EEBVS, % inhibited the intestinal fluid accumulation showed by 22.88% and 36.51% at doses 200 mg/kg and 400 mg/kg respectively. The standard drug loperamide (5 mg/kg) also significantly (p<0.001) inhibited the intestinal fluid accumulation (59.12%). The findings of castor oil induced enteropooling in rats are presented in Table 2 and Figure 3.

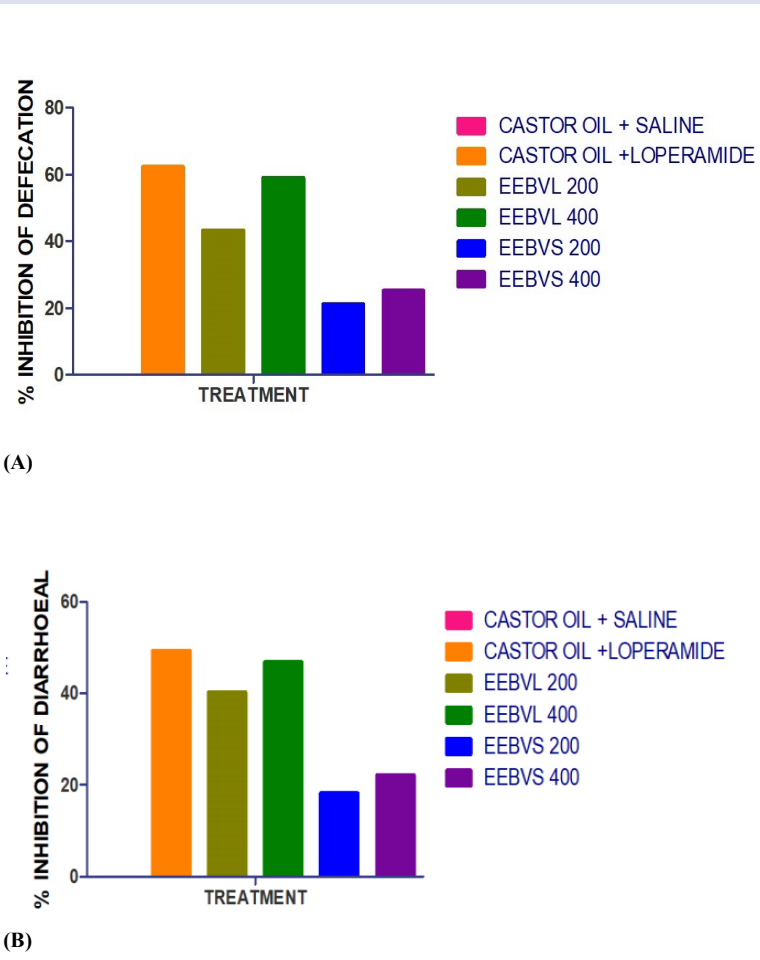

Figure 2: Effect of EEBVL and EEBVS on castor oil induced diarrhea in rats: (A) % Inhibition of defecation and (B) % Inhibition of diarrhoeal.

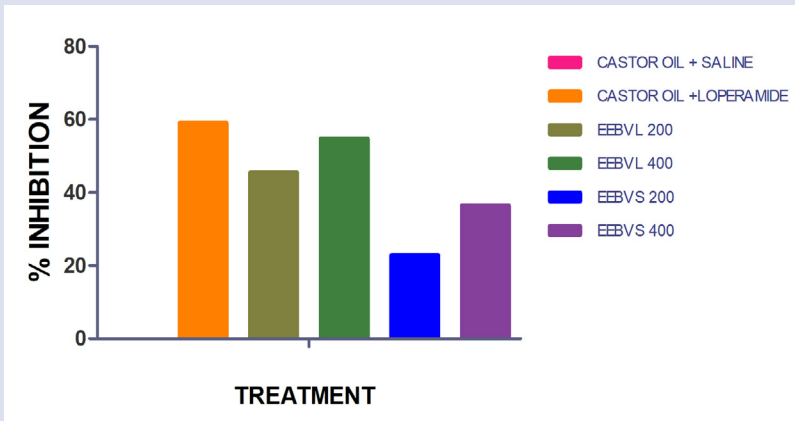

Figure 3: Effect of EEBVL and EEBVS of % inhibition of the intestinal fluid on castor oil induced enteropooling in rats.

CONCLUSION

The findings of the present study provide clear idea and prove the traditional claim that ethanolic extracts of the leaves and stem of *Bauhinia vahlii* (EEBVL and EEBVS). Antidiarrheal effect is rapid, long lasting and statistically significant at both 200 and 400 mg/kg doses but remarkable antidiarrhoeal activity at 400 mg/kg was observed by ethanolic extracts of the leaves. Determination of antidiarrhoeal effect in Castor oil induced diarrhea as well as enteropooling in rats model may give a clear idea about the mechanism(s) of antidiarrhoeal activity. However, further chemical and pharmacological studies are required to isolate the bioactive compounds and elucidate the precise mechanisms responsible for the observed pharmacological activities of this plant.

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CONFLICTS OF INTEREST

The authors declare that there is no conflicts of interest.

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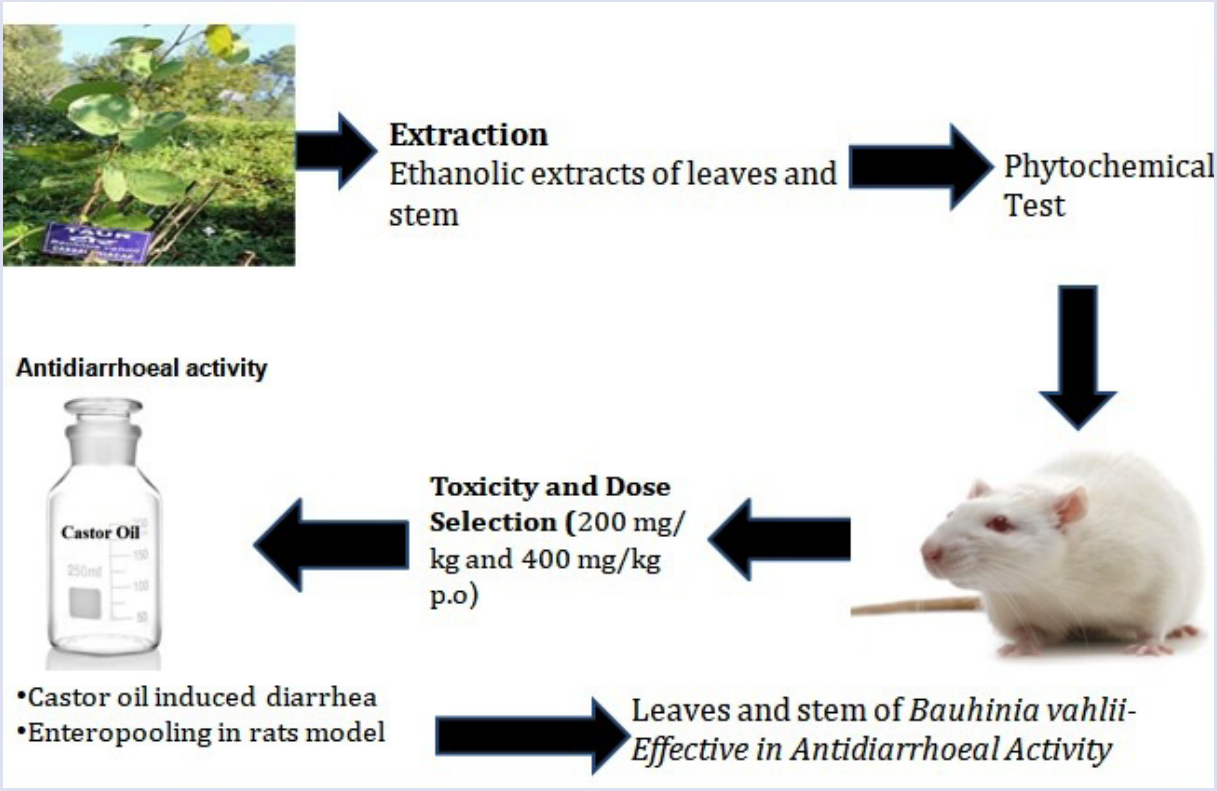
GRAPHICAL ABSTRACT



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