ANTIDIARRHOEAL ACTIVITY OF ALCOHOLIC EXTRACT OF *Buchanania lanzan* Spreg. ROOTS

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Summary

Buchanania lanzan Spreg is used in India especially in rural communities by traditional medicine practitioners to treat diarrhoea. However, scientific evidence does not exist in any literature. The present study designed to investigate the antidiarrhoeal activity of the leaf aqueous extract of *Buchanania lanzan* Spreg in mice. Castor oil induced diarrhoeal test was used to assess the antidiarrhoeal activity and gastrointestinal tract transit of charcoal meal test was used to assess the antipropulsive activity of the alcoholic extract of *Buchanania lanzan* Spreg roots alongwith phytochemical analysis were carried out using well established protocols and methods. The alcoholic extract of *Buchanania lanzan* Spreg roots significantly reduced faecal output in castor-oil induced diarrhoea and also significantly reduced the number of diarrhoeal episodes. *Buchanania lanzan* Spreg significantly delayed the onset of diarrhoea induced by castor oil and significantly reduced the number of animals exhibiting diarrhoea. *Buchanania lanzan* Spreg significantly reduced the intestinal propulsion of charcoal meal in mice. The phytochemical screening of the roots revealed the presence of tannins, saponins particularly steroidal saponin, and flavonoids. The data obtained indicate that the alcoholic extract of *Buchanania lanzan* Spreg roots has antidiarrhoeal activity. Tannins present in *Buchanania lanzan* Spreg probably contribute to its antidiarrhoeal activity.

Key Words: Diarrhoea, *Buchanania lanzan* Spreg, castor oil, gastrointestinal transit, Charcoal, alcoholic extract

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Introduction

Diarrhoeal disease is a major cause of mortality and morbidity, especially among children in developing countries resulting in a serious health care problem. Diarrhoea is defined as an increase in the frequency, fluidity, or volume of bowel movements and is characterized by increased frequency of bowel sound and movement, wet stool, and abdominal pain. In clinical terms it is used to describe increased liquidity of stool, usually associated with increased stool weight and frequency (1). Medicinal herbs constitute an indispensable component of the traditional medicine practised worldwide due to the economical viability, accessibility and ancestral experience. Despite the availability of a vast spectrum of approaches for diarrhoeal management, a vast majority of the people of the developing countries rely on herbal drugs for the management of diarrhoea (2). To combat the problem of diarrhoea in developing countries, the world health organization (WHO) has constituted a diarrhoea disease control programme (DDC) which includes studies of traditional medicine practices together with the evaluation of health education and prevention approaches (3).

***Buchanania lanzan* Spreng** (family: Anacardiaceae) commonly known as *Char, Chironji and Priyal* in hindi. It is a subdeciduous tree, founds throughout the hot dried parts of India. The plant used in ayurveda and the Unani system of medicine as a nervine tonic, anticough, antileprotic and and oleation (4). .Plant also reported to contain a rich amount of fixed oils, tannins and starch. The oil from the seeds is used to reduce granular swelling of the neck. *Buchanania lanzan* Spreng *kernel* also found to posses antioxidant and anti-inflamatory activity. Ointment is made from the kernel which is used to relieve itch and prickly heat. The gum from the bark used for treating diarrhea and intercostals pains and leaves are used for promoting wound healing (5, 6). Tribal people of Jharkhand and Chhattisgarh are using *Buchanania lanzan* Spreng mainly for wound healing, anti-diarrhoeal, analgesic and antiulcer activity but no scientific study has been carried out regarding its pharmacological activities. Therefore present study designed to evaluate the **anti-diarrhoeal** activity of *Buchanania lanzan* Spreng.

Materials and Methods

Plant material and extraction

Buchanania lanzan Spreng roots were collected in September 2008 from B.I.T. Mesra, Ranchi India. Further taxonomic identification was done Dr. S. Jha, Associate Professor, Dept. of Pharmacognosy, BIT, Mesra, Ranchi. The roots were dried in shade for 15 days and then roots were subjected to size reduction to make coarse powder and passed through 40-mesh sieve. The dried and powdered roots (200 g) were subjected to hot extraction in Soxhlet apparatus with ethanol. A crude etanolic extract of *Buchanania lanzan* Spreng roots (EBL) was obtained which was freez dried to obtain powder and stored in refrigeration for the experimental use. About 12.6 g powder was obtained after freez drying. The precentage yield of *Buchanania lanzan* Spreng roots was found to be 6.3%. This extract subjected to Preliminary Phytochemical Screening.

***Preliminary Phytochemical Screening of Buchanania lanzan* Spreng Roots**

The methods of Harborne (1984) were used to determine the groups of chemical compounds present in the dried powder of the roots of the *Buchanania lanzan* Spreng (7).

Animals

Swiss albino mice weighing 25–30gm either sex were used in the study. Animals were procured from Laboratory Animal House of Birla Institute of Technology, Mesra (Reg. no.: 621/02/ac/CPCSEA). All animal experiments strictly complied with the approval of institutional animal ethical committee. The animals were kept in polyacrylic cages and maintained under standard housing conditions of temperature (24-27°C) and humidity (60-65%) with 12:12 light:dark cycles. They were acclimatized to experimental conditions for seven days before starting of the experiment. Food was provided in the form of dry pellets and water *ad libitum.*

Antidiarrhoeal activity test by castor oil-induced diarrhoea

The experiment was performed according to the method described by Yegnanarayan and Shrotri (1982). Briefly, mice fasted for 24 h were randomly allocated to four groups of six animals each. All the animals were screened initially by giving 0.5 ml of castor oil. Only those showing diarrhoea were selected for the final experiment (8). Following pre-treatment was given before induction of diarrhoea:

Group I (Control) received 1% Tween (10 ml/kg, p.o.);
Group II (Standard) was given Loperamide (3 mg/ kg, p.o.) in suspension;
Groups III and **IV** (Test) received EBL (200 and 400 mg/kg, p.o.), respectively.

After 60 min, each animal was given 0.5 ml of castor oil, each animal was placed in an individual cage, the floor of which was lined with blotting paper which was changed every hour, the latent period was noted and examined hourly for the presence of diarrhoea till 6 h after the castor oil challenge. Diarrhoea was defined as the presence in the stool of fluidy material, which stained the absorbent paper placed beneath the cage. The number of respondents and the number of stools passed during the 6-h period were noted for each mouse and following parameters were calculated:

$$\textbf{\% inhibition of defecation} = \frac{[Mc - Md]}{[Mc]} \times 100;$$

Mc: mean number of defecation caused by castor oil;
Md: mean number of defecation caused by drug or extract (EBL)

$$\textbf{\% inhibition of Diarrhoea drops (Wet Stool)} = \frac{[Mcw - Mdw]}{[Mcw]} \times 100;$$

Mcw: mean number of wet faeces caused by castor oil;
Mdw: mean number of wet faeces caused by drug or extract (EBL)

$$\textbf{\% Purging index (PI)} = \frac{[\text{Respondents\%} \times \text{average number of stools}]}{[\text{Average latent period}]} \times 100$$

Effect on gastrointestinal motility

Animals were divided into four groups of six mice each and each animal was given 1 ml of charcoal meal (5% activated charcoal suspended in 1% CMC) orally; 60 min after an oral dose of the test drugs and vehicle.
Group I was administered 1% CMC (10 ml/kg; p.o.);
Group II received atropine sulfate (0.1 mg/kg; p.o.,) as the standard drug;
Groups III and **IV** received EBL (200 and 400 mg/kg, p.o.) respectively.
After 30 min, animals were killed by light ether anaesthesia and the intestine was removed without stretching and placed lengthwise on moist filter paper. The intestinal transit was calculated as a percentage of the distance travelled by the charcoal meal compared to the length of the small intestine (9).

Statistical Calculations

The data expressed are mean ± standard error of mean (SEM). All statistical comparisons between the groups are made by means of One Way Analysis of Variance with post hoc Dunnett's test using Graphpad Prism 5 software. The p value less than 0.01 is regarded as significant.

Results

Preliminary Phytochemical Screening

The phytochemical analysis of the roots of *Buchanania lanzan* Spreng revealed the presence of tannins, saponins particularly steroidal saponin, and flavonoids.

Antidiarrhoeal activity test by castor oil-induced diarrhoea

Castor oil (0.5 ml, p.o.) induced diarrhoea promptly within 6 h in all the animals and also produced a considerable amount of stool in control group. EBL (200–400mg/kg, p.o.) significantly ($p < 0.05$–0.01), reduced the faecal output produced by castor oil. At doses of 200–400mg/kg (p.o.), the plant extract significantly ($p < 0.05$–0.01) and dose dependently delayed the onset of diarrhoea induced by castor oil.

Table 1: Antidiarrhoeal activity of EBL against castor-oil induced diarrhoea in mice

Treatment Group	% Respo-dents	Latent Period (min)	Total No of Faeces	Purging Index	% Inh. Defaecetion	Total No of wet Faeces	% Inh. Wet Stool
Control (1% Tween)	100	59.6±3.26	10.2±0.6	17.11	0.00	7.6±0.5	0.00
Standard (Loperamide)	33.3	234.4±9.13**	1.6±0.4**	0.23	84.31	0.4±0.2	94.7
Test (EBL 200mg/kg)	66.6	78.8±3.39*	8.0±0.3*	6.76	21.56	3.2±0.37**	57.9
Test (EBL 400mg/kg)	50.0	99.2±4.73**	4.8±0.28**	2.42	52.94	1.8±0.39**	76.3

*Values are expressed in mean ± SEM (n=6); *p<0.05; **p<0.01 compared with control group.*

EBL (200 mg/kg, p.o.) protected 33.3% of mice against the diarrhoea while the dose of 400 mg/kg (p.o.) significantly ($p < 0.01$) reduced the number of animals suffering from diarrhoea by protecting 50.0% of them. Loperamide (20 mg/kg, p.o.) profoundly ($p < 0.01$), reduced the faecal output produced by castor oil. The onset of castor oil-induced diarrhoea profoundly prolonged ($p < 0.01$) and fecal output were also reduced ($p < 0.01$), respectively by loperamide. The number of animals suffering from diarrhoea was also significantly ($p < 0.05$), reduced by loperamide by protecting 66.6.3% of them **(Table 1)**.

Effect on gastrointestinal motility

In gastrointestinal transit experiment, it was observed that in the control group of animals pretreated with vehicle only, the mean length of intestine travelled by the charcoal meal was 72.58±1.84%. EBL (200–400 mg/kg, p.o.) significantly ($p < 0.05$-0.01) reduced the mean length travelled by the charcoal meal in a dose-dependent manner. The propulsion of the charcoal meal was inhibited by 12.34–30.83% at the doses of 200–400 mg/kg (p.o.) of EBL. Atropine (0.1 mg/kg, p.o.) significantly ($p < 0.01$) reduced the mean length travelled by the charcoal meal. Atropine inhibited the propulsion of the charcoal meal by 40.33% **(Table 2)**.

Table 2: Effect of EBL on gastrointestinal transit of charcoal in mice

Treatment Group	Intestinal charcoal transit (%)	Inhibition of intestinal charcoal transit (%)
Control (1% Tween)	72.58 ± 1.84	0.00
Standard (Atropine)	43.31 ± 2.47**	40.33
Test (EBL 200mg/kg)	62.88 ± 2.12*	12.34
Test (EBL 400mg/kg)	56.20 ± 1.82**	30.83

*Values are expressed in mean ± SEM (n=6); *p<0.05; **p<0.01 compared with control group.*

Discussion

In the present investigation, ethanolic extract of *Buchanania lanzan* Spreng roots has shown good antidiarrhoeal activity in a castor oil induced model in albino mice. Castor oil, an irritant or stimulant laxative, which hydrolysed in the upper small intestine to active metabolite ricinoleic acid, that irritates the mucosa of the gastrointestinal tract resulting in increase in the peristaltic activity of small intestine leading to changes in electrolyte and water permeability of intestinal mucosa (10, 11). Induction of diarrhoea by castor oil involves elevated prostaglandin biosynthesis (12). From these observations, it can be deduced that the extract may act through inhibition of prostaglandin synthesis and intraluminal fluid accumulation. loperamide, used as a standard antidiarrhoeal agent in this study, promtly inhibited castor oil-induced Diarrhoea. Loperamide is an opioid derivative, has been shown to slowdown intestinal motility by its action on μ receptors in the submucosal neural plexus of the intestinal wall and its antimuscarinic activity

in the gastrointestinal tract (10, 13). The experiments carried out on gastrointestinal tract motility after charcoal meal administration has shown a reduction in the intestinal transit of charcoal meal after pre-treatment with EBL or atropine. It is probable that the plant extract may be exerting its antidiarrhoeal and antipropulsive activities by slowing intestinal motility same as atropine.

Furthermore, the standard chemical tests carried out in this study showed that the roots of *Buchanania lanzan* Spreng contain tannins, saponins and flavonoids. It is important to emphasize here that tannins have been reported in several studies to have antidiarrhoeal effect. Frei et al. (1998) and Bruneton (1999) reported that tannin containing drugs are widely used for the treatment of diarrhoea and related disorders (14, 15). Farthing (2000) reported that astringents such as tannins have been known to have antisecretory activity in the gastrointestinal tract and have been used to treat diarrhoea (16). In light of the above literature, It may be interpreted that the presence of tannins in *Buchanania lanzan* probably contribute to its antidiarrhoeal activity.

Conclusion

The data obtained in this study suggest that the ethanolic extract of *Buchanania lanzan* Spreng roots has antidiarrhoeal activity thus justifying its folk and traditional use in diarrhoea.

Acknwoledgement

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