

## In-vivo diuretic and antiulcer activity in fruits of *Buchanania lanzan*

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Received: 28 Jul 2016

Accepted: 07 Aug 2016

Published: 28 Dec 2017

### Abstract

**Ethno pharmacological relevance:** The fruits of *Buchanania lanzan* are used traditionally in the treatment of skin afflictions, sores, ulcers, inflammation and as diuretic (1, 21, 35, 42). The aim of the study was to investigate the antiulcer and diuretic activity of the crude ethanolic and pet. Ether extract from the fruits of *Buchanania lanzan*.

**Materials and methods:** The antiulcer activity of the crude extracts was evaluated in ethanol and pylorus ligation induced model for gastric lesions in Wister albino rats (2,8,12,19). Parameters such as gastric volume, pH and acidity were determined in the pylorus ligation model. Furthermore the diuretic activity was evaluated in comparison of Standard drug i.e. Furosemide.

**Results:** The acute toxicity studies revealed that LD<sub>50</sub> > 2000mg/kg for the extract. The extract caused significant (p < 0.05) dose-dependent inhibition of ulcer in the ethanol and pylorus ligation induced ulcer models at the dose of 250mg/kg, respectively. And the diuretic activity of extract also found significant (p < 0.01) dose dependent increase in urine volume. Both ethanol and aqueous extracts have showed a significant dose-dependent increase in the excretion of electrolytes when compared to the control group.

**Conclusion:** Our data proved aeration albase for the folkloric use of *Buchanania lanzan* in the treatment of ulcers and as diuretic.

**Keywords:** Antiulcer, diuretic activity.

### Introduction

*Buchanania lanzan* is claimed to have diuretic and antiulcer activity in indigenous system of medicine [3,6,9]. A survey of the literature revealed the absence of any systematic study on diuretic and antiulcer activity of the plant [13,15,19,25]. Hence a study has been taken up to verify the claims made in the indigenous system of medicine. *Buchanania lanzan* is a tree which produces the seeds known as charoli. These seeds are used as a cooking spice primarily in India [7,16,24,27,33]. Charoli are tiny almond-flavoured dried seeds of a bush called *Buchanania lanzan*, which is cultivated across India, primarily in the northwest [33,38,49]. After the hard shell is cracked, the stubby seed within is as soft as a pine nut. The charoli seed is lentil-sized, is slightly flattened and has an almond-like flavour ( ). Though they can be eaten and used raw they are often toasted or roasted before use, as this intensifies the flavour. They are commonly used in sweets in India. However, they are also ground into powders for thickening savory sauces and flavoring batters, and stewed into rich, meaty kormas. Charoli seeds are used in the Ayurveda and Unani system of medicine [33,35,42].

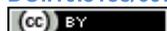


Figure.1 Charoli nuts

### Materials and Methods

#### Collection and Authentication of plant

DOI:10.5138/09750185.1920



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The fruits of *Buchanania lanzan* were purchased from local herbal market of Bhopal. The fruit was identified with the help of available literature and authenticated by Govt. MLB Girls PG College, Bhopal (M.P.), Affiliated to Barkatullah University, approved by AICTE and Government of Madhya Pradesh, India. The fruits were dried in the shade for 15 days prior to extraction and then powdered with mechanical grinder.

### Preparation of Extract

The powder was then extracted with Petroleum Ether and ethanol using cold maceration method separately in conical percolator at 4-15°C (39-50°F) temperature for 14 days and was shaken frequently. Then extracts were collected in a beaker and the dried at 55°C using water bath [23, 24, 31, 36]. The semisolid pastes were formed and transferred into air tight container for further use.

### *In vivo* Diuretic Activity

The method of Lipschitz *et al.*, was employed for the assessment of diuretic activity. [40, 42, 46, 49]

Animal were divided in five groups containing six in each all animal work being provided food and water 18 hours were scheduled as follows.

Group I: received normal saline (25 ml/kg) and drinking water (control).

Group II: received Furosemide (20 mg/kg b.w. i.p.) a reference diuretic (standard).

Group III: received Petroleum ether extract at the dose level of 200 mg/kg bodyweight.

Group IV: received Ethanolic extract at the dose level of 200 mg/kg body weight.

### Evaluation of Diuretic activity

Male rats (wistar albino strain) weighing 150 to 180gm were maintained under standard condition of temperature and humidity. They were fed with standard rat feed and water *adlibitum*.

The method of Lipschitz *et al.* was employed for the assessment of diuretic activity. The experimental protocols have been approved by the Institutional Animal Ethical Committee of College of Pharmacy, SSSUTMS, Sehore. Four groups of six rats in each and were fasted and deprived of water for eighteen hours prior to the experiment. The first group of animals serving as control received normal saline (25ml/Kg.); the second group received Furosemide (20mg/Kg, i.p.) in saline; the third and fourth groups received the petroleum ether and ethanolic extracts at the doses of 200 mg/Kg, respectively, in normal saline.

Immediately after administration the animals were placed in metabolic cages (1 per cage), specially designed to separate urine and faeces, kept at room temperature of 25± 0.5°C throughout the

experiment. The urine was collected in measuring cylinders up to 5hrs after dosing. During this period, no food or water was made available to animals.

The parameters taken for individual rat were body weight before and after test period, total concentration of Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> in the urine. Na<sup>+</sup>, K<sup>+</sup> concentrations were measured by Flame photometry and Cl<sup>-</sup> concentration was estimated by titration with silver nitrate solution (N/50) using three drop of 5% potassium chromate solution as indicator.

Results are reported as mean ± SD, the test of significance (p<0.01) was statistically.

### Statistical analysis

Data are expressed as mean ± S.E.M (standard error of mean). Statistical analyses were performed with one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

### Induction of Ulcer

#### Gastic lesions induced by Pylorus – ligation

Rats were fasted for 36 h with access to water *ad libitum* before pylorus ligation under either anesthesia was carried out. Care was taken not to cause bleeding or to occlude blood vessels. Extract was administered intraperitoneally immediately after pylorus ligation. The rats were sacrificed 6 h after treatment. The stomachs were removed, the contents were collected, volumes measure, centrifuge and analyzed for titratable acidity against 0.01 mol/L NaOH at pH-7.

### Determination of acidity

Acidity = Volume of NAOH × Normality of NAOH 100/ .01 mEq / L

## Results and Discussion

### Plant material identification and extraction

*Buchanania lanzan* Spreng fruits were purchased from local market in March 2015. The fruit were subjected to size reduction to make coarse powder and passed through 40-mesh sieve and stored in an airtight container for further use. The dried and powdered fruits were subjected to hot extraction in Soxhlet apparatus with solvents.

### Percentage Yield of *Buchanania lanzan*

In extraction procedure we have taken 150 gm powder of *Buchanania lanzan* after extraction extract was weighed after



solvent elimination under reduced pressure. The percentage yield of extract was calculated as:

$$[\text{Percentage (\%)} \text{ Yield} = (\text{Extract value in grams} / \text{Actual value taken in grams}) * 100]$$

### Percentage (%) Yield of Petroleum Ether extract

The weight of Petroleum Ether extract was 33gm so the percentage yield of was found to be 22% (w/w).

### Percentage (%) Yield of Ethanolic extract

The weight of ethanolic extract was 16gm so that the percentage yield was found to be 10.6 % (w/w).

### Preliminary Phytochemical Screening

The extracts and powder were found to contain alkaloids, carbohydrates, glycosides, fixed oil and fats, tannins and phenolic compounds, saponins, flavonoids, proteins and terpenoids.

### Animals

Swiss albino mice weighing 25–30gm and Wister albino rats weighing 180-200 gm of either sex were used in the study. Animals were procured from Laboratory Animal House of College of Pharmacy, SSSUTMS, Sehore. 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 for seven days. Food was provided in the form of dry pellets and water *ad libitum*.

### Acute toxicity assay

Acute toxicity assay was performed in mice according to OECD guidelines. Animals were divided into different groups of six each. After an overnight fast, the test drug was administered orally in graded dose (100–2000 mg/kg). In further, they were observed continuously for the first 2 h for toxic symptoms and up to 24 h for mortality. There was no lethality in any of the groups after treatment.

**Table No.1:** Preliminary phytochemical constituents present in various extracts of *Buchanania lanzan* fruit

S. NO.	Chemical constituents	Petroleum ether extract	Ethanolic extract
1	Alkaloids	-	+
2	Carbohydrates	+	+
3	Glycosides	+	++
4	Fixed Oil and Fats	+	-
5	Tannins and Phenolic Compounds	-	++
6	Saponins	-	++
7	Flavonoids	-	++
8	Proteins	-	+
9	Terpenoids	++	+

**Table No. 2:** Diuretic activity of petroleum ether and ethanolic extracts of fruits of *Buchanania lanzan*. Electrolyte excretion

S.No.	Treatment	Dose	Urine volume (ml) 24 hr.	Na+ (m m/l)	K+ (m m/l)	Cl- (m m/l)	Na+/K+ ratio
1	Control	25ml/kg	6.5±1.02	71.0±0.2	32.5±0.7	65.2±3.12	2.18±0.004
2	Standard(Furosemide)	25mg/kg.I.P.	13.8±2.02bb(a)	94.9±1.22bb(a)	46.3±0.9bb(a)	98.9±3.06bb(a)	2.08±0.008
3	Pet. Ether extract	250mg/kg Suspension	7.5±1.01	87.8±0.15	43.0±0.60	76.4±2.96	2.04±0.09
4	Ethanolic extract	250mg/kg Suspension	8.3±1.18bb(b)	89.9±0.15bb(b)	42.5±0.6bb(b)	69.3±2.06bb(b)	2.11±0.07bb(b)

Values are expressed as Mean ± SEM, Values are find out by using ANOVA followed by Newman level's multiple range tests. bb(a) values were significantly different from control at (P<0.01) bb(b) values were significantly different from standard at (P<0.01)

### Experimental Procedures

#### Ethanol-induced ulcer model

Swiss albino mice of either sex were divided into five groups, each group consists of six animals. All groups of animals received following treatments for 5 days: groups 1 (Normal) and 2 (Control)



received vehicle 10 ml/kg, groups 3 and 4 (Test) were given EBL 200 and 400 mg/kg, respectively, and the group 5 (Standard) given reference drug Sucralfate at the dose of 100 mg/kg. On the 5th day, 1h after final dose of treatment, the gastric ulcers were induced in rats by administering 96% ethanol (5ml/kg). After 1h animals were sacrificed by cervical dislocation and stomach was incised along the greater curvature and examined for ulcers index [4]. Percentage ulcer inhibition was calculated for each group on comparison with vehicle control group.

### Pylorus ligation model

Wister albino rats of either sex were divided into five groups, each group consists of six animals. All groups of animals received following treatments for 5 days: groups 1 (Normal) and 2 (Control) received vehicle 10 ml/kg, groups 3 and 4 (Test) were given EBL 200 and 400 mg/kg, respectively, and the group 5 (Standard) given reference drug ranitidine (RAN) at the dose of 100 mg/kg. All the doses calculated with respective body weights of animals and administered orally. Ulceration in rats was induced by method of Shay et al, 1945. On 5th day pylorus part was ligated following 36 h fasting. After the pretreatment period of 1h animals were anaesthetized using pentobarbitone (35 mg/kg, i.p.), the abdomen was opened and pylorus ligation was done without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen wall was closed in two layers with interrupted sutures. After 4 h of pylorus ligation, stomachs were dissected out and cut open along the greater curvature and examined for ulcers index. The gastric juice was titrated against 0.01N sodium hydroxide using Topfer's reagent as indicator to find out the free acidity and total acidity.

Calculation of ulcer index and Percentage ulcer inhibition

Ulcer index has been calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach. A score for the ulcer was made as follows:

- 0: normal colored stomach.
- 0.5: red coloration.
- 1: spot ulcers.
- 1.5: haemorrhagic streak.
- 2: ulcers.
- 3: perforation.

Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer inhibition was determined as follows:

$$\% \text{ inhibition of Ulcer Index} = \frac{[\text{Control mean ulcer index} - \text{Test mean ulcer index}]}{[\text{Control mean ulcer index}]} \times 100$$

### 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 (ANOVA) with post hoc Dunnett's test or by Student's t-test using Graph pad Prism 5 software. The p value less than 0.01 is regarded as significant

EBL was found to non-toxic as it did not show any toxic symptoms and mortality up to the dose of 2000mg/kg. Effects of EBL at dose of 200 and 400 mg/kg body weight, twice a day for 5 days prevented the acute gastric ulcers in a dose related manner. Administration of EBL 1 h before the induction of gastric lesions by ethanol showed significant activity, and inhibited the total ulcer index by 65.8±3.1 to 72.6±2.8 percent in dose dependent manner (Figure. 1). Results for EBL are comparable to RAN at the dose of 100 mg/kg.

**Table 3:** Effect of ethanolic extract of *Buchanania lanzan* fruit on gastric secretion, acidity and pH in plus pylorus ligated rats

Treatment	Normal	Control	PBL 200mg/kg	EBL 200mg/kg	Standard 100mg/kg
Volume of gastric secretion (ml/100 g)	1.28±0.02	2.37±0.06 a	1.52±0.04 b, c	1.43±0.03 c	1.36±0.02 c
Free acidity (mequiv./l/100 g)	114±4.23	220±6.21 a	137.8±5.12 c	128.3±3.60 c	118.4±4.90 c
Total acidity(mequiv./l/100 g)	255±8.20	510±10.50 a	380±7.90 a, c	342±7.35 a, c	354±8.64 a, c
pH	3.20±0.04	2.0±0.12 a	2.95±0.07 c	3.00±0.06 c	3.20±0.10 c

Values are expressed in mean ± SEM (n=6) a p<0.001 compared with normal group; b p<0.01 compared with normal group; c p<0.001 compared with control group; d p<0.01 compared with control group.

The oral administration of EBL at 200-400 mg/kg in pylorus ligature inhibited the total ulcer index by 60.4±2.9 to 69.5±3.7 percent in dose dependent manner as compare to control. In pylorus ligation induced gastric ulcer the EBL showed significant reduction in gastric volume, free acidity, total acidity and ulcer score (Table 1).

### Conclusion

It has been concluded that the ethanolic and Pet. ether extracts of *Buchanania lanzan* fruit showed a dose dependent activity in ethanol induced ulcer in rats. The active constituents can be isolated from *Buchanania lanzan* fruit and further studies on their gastro protective and diuretic function can be analyzed.



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