

Original Article

PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIDIARRHOEAL ACTIVITY OF *BUNIMUM BULBOCASTANUM* SEEDS EXTRACT IN EXPERIMENTAL WISTAR RATS

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ABSTRACT

Objective: The main objective of this study was to evaluate the antidiarrheal activity of *Bunium bulbocastanum* seeds extracts, to exploit the medicinal use of plant in the traditional system of medicine scientifically.

Methods: The adult Wistar albino rats were divided in four groups, i.e. Group M1 (control group) receiving normal saline, group M2 (test group 1) receiving the 250 mg/kg *Bunium bulbocastanum* extract, group M3 (test group 2) receiving the 500 mg/kg *Bunium bulbocastanum* extract and group M4 (reference) receiving 3 mg/kg P. O Loperamide. Each group of mice with a bodyweight of 1 ml/100 g received castor oil. Mice were sacrificed and the distance traveled by the charcoal meal and the total length of the intestine was then measured. The peristaltic index and percentage of inhibition were calculated by using the formula.

Results: It was found that in the castor oil-induced intestinal transit method extract produced a significant ($p < 0.0001$) dose-dependent reduction in the distance traveled by charcoal meal comparable to the control peak effect was at the dose of 500 mg/kg ($PI = 12.06 \pm 3.38$). Likewise, in the diarrheal dropping test, *Bunium bulbocastanum* extract causes a significant ($p < 0.05$) dose-dependent reduction in the number of wet feces i.e. the mean wet of feces was decreased from 2.3 ± 0.44 gm to 1.28 ± 0.36 gm i.e. significantly different from that elicited by control (0.80 ± 0.17 gm) ($p = 0.0081$). However, there were no significant differences in inhibition at a dose of 250 mg/kg of extract.

Conclusion: This study demonstrated that the crude methanol extract from *B. bulbocastanum* seeds possesses significant antidiarrheal property and the presence of various secondary metabolites. This justified the antidiarrheal use of plant in the traditional system of medicine.

Keywords: *B. bulbocastanum*, Phytochemical screening, Anti-diarrheal activity, Methanol extract

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INTRODUCTION

Diarrhea is an alteration of normal bowel movement characterized by an increase in the volume, fluidity, frequency and passage of loose stool at least three times a day. It is a common symptom of gastrointestinal infection due to the number of communicable factors [1, 2].

According to the WHO 3-5 billion cases of diarrhea are reported annually, of which 1 billion are less than 5 y. As a result, it accounts for 20% of infant mortality [3, 4]. Especially in developing countries like Nepal, it is an important health problem. So every year Nepal faces an outbreak due to the number of poor health hygiene factors. In year 2000, almost 67,000 people were affected, 371 of whom, died of diarrheal disease. Likewise, in the year 2014, total death from diarrheal disease was 3.89% [5]. Beside this reality in Nepal, 384 number of species are used to treat diarrhea [6, 7].

Lots of modern technology has been established for the synthesis of new molecules, despite the fact that a number of developing countries still use the herbal-based crude drug for diarrheal treatment [8]. *Bunium bulbocastanum* is one of the important medicinal plants that grows in mountainous to shurland of Iran, Pakistan, India, Nepal and Afghanistan [9, 10]. It is an herbaceous perennial plant with typical habitat, i.e. Grows in chalky and rough grassland in arrange of soil from light (sandy), medium (loamy) to heavy (clay) soil [10]. In the indigenous system of medicine seed of *B. bulbocastanum* is regarded as a stimulant and carminative and found to be useful in diarrhea and dyspepsia as well as essential oil fraction of the seed is used in perfumery as a carminative purpose.

Seed propagation and germination ratio is very low because of long seed cycles [2, 4]. Its plants vary from dwarf (30 cm) to tall (80 cm), compact or spreading, moderately to highly branch tuberous. It is a

perennial herb, the leaves are freely pinnate, finely dissected and filiform. Flowers are small and white in color [11, 12].

B. bulbocastanum traditionally used in the treatment of diarrhea from past decades. So the main aim of the study was to provide scientific justification for the use of *Bunium bulbocastanum* in modern medicine.

MATERIALS AND METHODS

Collection and identification of plant material

The fresh seed of *Bunium bulbocastanum* 1000 gm was collected in September 2016 from the local market of Bhairahawa and the sample was identified in the Institute of Agricultural and Animal Sciences Paklihawa, Rupandehi, Nepal with a plant identification number (PKH3077).

Preparation of plant material

The collected seeds were separated from undesirable material. The whole seeds were kept in the shade for drying for three days. After drying, the seed was grounded into powder using an electronic grinder and the final grinded powder was weight.

Chemical and reagent

Methanol, Ethanol, distilled water, Sulphuric acid, Ferric chloride, Potassium hydroxide, Benzene, Lead acetate Glacial acetic acid, Picric acid, Activated charcoal, Loperamide, Castor oil, Ketamine Diethyl ether, Normal saline, Gum acacia, Sodium hydroxide.

Preparation of plant extract

The seeds were extracted by maceration. The grinded fruits powder (950 gm) was soaked in methanol for 10 d in a 2000 ml conical flask

i.e. 240-250 gm in each flask with 650 ml of methanol in each and mouth of the flask was covered with the aluminum foil. After 10 d, the mixture was filtered, then the filtrate was combined and then concentrated with a rotary evaporator at 40 °C. A black-green methanol extract of *B. bulbocastanum* obtained after concentration.

Phytochemical screening

A known volume of the sample of *B. bulbocastanum* was screened for the presence of some secondary metabolites as described for carbohydrates, alkaloids, Steroids, anthraquinones, cardenolides and dienolides, saponin, phenolic compound, flavonoids [13], cardiac glycosides, tannins and triterpenes [14].

Animals

Wistar albino mice of 73-159 gm of either sex obtained from Banaspati Bibhag, Kathmandu, were used. They were kept in a standardized environmental condition (22±2.5 °C), humidity 85%, 12 h light and 12 h dark cycle and feed with a standard rodent diet and water. Approval for the use of animals in the study was obtained from the Institute Review Committee of Universal College of Medical Science, Bhairahawa (IRC NO: UCMS/IRC/106/15).

Sample preparation

Appropriate weight of the extract was taken and the required concentration was made during the experimental process to make the standard solution of concentration 500 mg/ml and 500 mg/2 ml.

Preparation of standard

Loperamide standard solution was made by dissolving the appropriate amount of the drug.

Preparation of activated charcoal solution

Firstly 10 gm of activated charcoal was weight likewise 5 gm of gum acacia was also weighed. Afterward, the weighed amount of the gum acacia was transferred into the 100 ml volumetric flask and some amount of water was added to the flask to dissolve the gum acacia. And 10 gm of activated charcoal was added to the gum acacia solution and the volume was made up to 100 ml.

Castor oil-induced intestinal transit

Wistar albino rats were divided into four groups in such a way that 4 in each group, contained a total 16 of rats. All animal was fasted for

24 h prior to the experiment. Group M1 received distilled water 10 mg/kg P. O, group M2 and M3 were treated with *B. bulbocastanum* extract 250 mg/kg and 500 mg/kg P. O BBME respectively, whereas group M4 were treated with reference drug loperamide 3 mg/kg P. O. After 40 min castor oil was administered to each mouse by oral administration of a dose of 1 ml/100g body weight to each mouse. After 30 min of administration of castor oil, charcoal meal (10% activated charcoal in 5% gum acacia) 1 ml/100 gm body weight was administered to each animal. Then after 30 min each mouse was sacrificed and the abdominal cavity was dissected and entire small intestine was isolated and the distance travelled by the charcoal meal from the pylorus to the ileocecal junction was measured. The length of the entire small intestine was also measured. The peristaltic index of each rat was calculated by using the following data [15].

$$PI = \frac{Lm}{Lsi} \times 100$$

Where PI= peristaltic index Lm= length of charcoal meal

Lsi= length of small intestine

$$\% \text{ Inhibition} = \frac{(\text{control} - \text{test})}{\text{Control}} \times 100$$

Castor oil-induced diarrheal drooping test

Wistar albino rats were divided into four groups in such a way that 4 in each group contained a total 16 of rats. All animals had been fasted for 24 h prior to the experiment. Group M1 received distilled water 10 mg/kg P. O, group M2 and M3 were treated with *B. bulbocastanum* extract 250 mg/kg and 500 mg/kg P. O BBME respectively, whereas group M4 were treated with reference drug loperamide 3 mg/kg P. O. Each animal was placed in an individual cage and the floor of the cage was lined with blotting paper and diarrheal drooping was observed for 4 h. The initial weight and final weight of the filter paper were taken and the mean weight of the feces was calculated and evaluated by subtracting the final weight of filter paper and initial weight of filter paper [16].

RESULTS

Phytochemical screening

The analysis of *B. Bulbocastanum* revealed the presence of tannins, carbohydrates, cardiac glycosides, flavonoids and phenol, while anthraquinones, triterpenes, cardinoline and dienolide were not detected. These observations are summarized in table 1 below.

Table 1: Secondary metabolites of *Bunium bulbocastanum* seeds

Secondary metabolite	Observation	Inference
Alkaloids	Cream color with Mayer's reagent Reddish-brown precipitate with Wagner's and Dragendorffs reagent.	Not detected
Carbohydrate	Presence of violet ring at the junction	Present
Tannins	White precipitate with ethanolic KOH	Present
Saponins	Stable, persistent froth with water	Present
Flavonoids	Dark yellow color with KOH	Present
Phenolics	Greenish precipitate with FeCl ₃ .	Present
Steroids	Red color with drops of concentrated H ₂ SO ₄ .	Not detected
Cardiac glycosides	Presence of reddish-brown color at the interface	Present
Anthraquinones	Absence of pink in the ammonical phase.	Not detected
Cardenolides and dienolides	Absence of brown ring at the interface.	Not detected

Castor oil-induced intestinal transit

The study from castor oil-induced intestinal transit revealed that the methanol seed extract of *Bunium bulbocastanum* (250 mg/kg and 500 mg/kg) produced a significant (p<0.0001) dose-dependent reduction

in the distance travelled by charcoal meal compared to the control. The peak anti-motility effect occurred at a dose of 500 mg/kg (PI=12.06±3.38). This effect was comparable to that produced by standard anti-diarrheal drug loperamide (PI=1.34±1.64).

Table 2: Peristaltic index of the different rat treated with control, loperamide, 250 mg/kg and 500 mg/kg of extract

Group	Castor oil	Treatment	Distance led by charcoal (cm)	Total length of small intestine	Peristaltic index	P-value (ANOVA)
I	+	Distilled Water	30.28±4.78	63.63±6.62	43.43±5.9	-
II	+	Loperamide	0.88±1.06	65±8.03	1.34±1.64**	0.000092
III	+	250 mg/kg	21.25±2.90	65.08±5.28	32.81±5.4**	0.000024
IV	+	500 mg/kg	7.38±1.45	62.28±9.05	12.06±3.38**	0.00051

**Indicates the p-value less than 0.0001 as compared to the control group, i.e. highly significant

Table 3: Mean weight of feces during 4 h of treatment and percentage inhibition of defecation of the different treatment group of rats

Group	Castor oil	Treatment	Meanwt. of feces during 4 h of treatment	Percentage inhibition	P-value (ANOVA)
I	+	DW	2.34±0.44	-	-
II	+	Loperamide	0.8±0.17*	62.88	0.000656
III	+	250 mg/kg	2.23±0.20	4.58	0.562
IV	+	500 mg/kg	1.28±0.36*	45.23	0.0081

*Indicates the p value less than 0.05 as compared to control i.e. highly significant.

Diarrheal drooping test

During 4 h after administration of castor oil, all the rats in the control group (distilled water 10 ml/kg P. O) produced copious diarrhea. Pretreatment of rats with the methanol seed extract of *Bunium bulbocastanum* causes a significant ($p < 0.05$) dose-dependent reduction in the amount of wet feces. The reduction exhibit of 500 mg/kg was higher than the next dose of 250 mg/kg.

At the dose of 500 mg, the mean weight of feces decreased from 2.3±0.44 gm (as observed in control group) to 1.28±0.36 gm which is significantly different from that elicited by control (0.80±0.17 gm), ($p = 0.0081$). However, there was no significant difference in diarrhea inhibition per 250 mg/kg of extract.

The highest percentage inhibition of 45.23% was obtained with the dose of 500 mg/kg body weight of extract which was comparable to 62.88% produced by loperamide (table 3).

DISCUSSION

With effective ethnopharmacological value, *B. Bulbocastanum* is widely used by different tribes as an effective herbal medicine. Literature surveys have revealed that leaves of *B. Bulbocastanum* are being used for diseases like diarrhea [17]. The study aimed to examine the antidiarrheal effect of *B. Bulbocastanum*, to ascertain scientific rationales behind its ethnical practice. Our study validates the efficacy of methanol extract of *B. Bulbocastanum* on the castor oil-induced gastrointestinal transit model. These useful biological activities may be attributed by the presence of bioactive compounds like tannins, flavonoids, alkaloids, glycosides, phenols, steroids, saponins and terpenoids as reported on phytochemical screening.

A lethal dose (LD 50) of the plant extract could not be acquired, as no mortality was detected up to the maximum dose 4000 mg/kg and the extract was found to be safe with a wide therapeutic range. Therefore, two comparative doses of 250 mg/kg and 500 mg/kg were used in *in vivo* study reported by previous acute toxicity study [17].

Diarrhea results from an imbalance of the absorptive and secretory mechanism within the bowel. In absorptive/osmotic diarrhea a large number of osmotic particles on the gut wall compared with absorptive capacity, whereas in secretory diarrhea, excessive secretion mucosal fluid [18, 19]. Some studies indicate that in watery diarrhea mortality was mentioned as a major contributing factor i.e. rapid intestinal transit reduces the contact time between absorptive solute and normal epithelium, as well as other contributing factor like 5-HT present in the gut stimulate intestinal motility, secretion and vasodilatation promote for development of diarrhea [20]. In our experiment, extract produced a significant dose-dependent reduction in the amount of wet feces in castor oil-induced intestinal transit test and diarrheal drooping test. Most of the articles mention that ricinolic acid, an active component of castor oil, is responsible for inducing permeability changes in the mucosal membrane and alteration of ion [4, 21, 22]. Besides these purposed mechanisms, castor oil inhibits the intestinal Na⁺ and K⁺ATPase activity and stimulates the release of Nitric Oxide (NO) hereby NO causes the release of prostaglandin like PGE₂ that are responsible for hypersecretory action [4, 15, 23, 24]. Thus the extract could have been shown an antidiarrheal activity by reducing intestinal permeability or by minimizing hypersecretion.

In the animal experiment, Loperamide was used as a reference drug and sample extract had a statistically comparable effect to the reference drug. It has been reported that loperamide increases the intestinal absorption rate by epithelial cell and also increase intestinal transit time by the slowdown of intestinal motility.

Similarly, in animal model experiment it was found that loperamide inhibits the intestinal secretion induced by *E. Coli*, and Cholera toxin [25]. Beside this loperamide is an opiate analog, the drug shows its mechanism of action by decreasing the gut muscle tone, decrease the PGE₂ mediated fluid secretion, slow down transit time and increase the colonic water absorption [26, 27].

When the sample is phytochemically tested, the extract reveals the presence of phytochemical compounds like tannins and flavonoids. Some of the previous study mentions the antidiarrheal activity of Phyto-constituents like tannins and flavonoid [28, 29]. In terms of flavonoid, they may act by inhibition of intestinal motility and hydroelectric secretion to slow down the diarrheal effect [28]. Likewise, tannins is well known for its astringent property, hereby it may cause precipitation of intestinal protein on the intestinal lining as a resultant effect form a protective layer over it with inhibition of toxin from abdomen, which are responsible for diarrheal effect [28, 30]. So this result suggests that these phytochemicals may have contributed to antidiarrheal activity.

CONCLUSION

The methanol extract of *B. bulbocastanum* seeds showed antidiarrheal activity in several models of diarrheal conditions in test animals. The obtained result thus provided the experimental basis for understanding the use of *B. bulbocastanum* in traditional medicine as an antidiarrheal agent. Phytochemical screening revealed the presence of various secondary metabolites such as tannins, glycosides, flavonoids and carbohydrates in the seeds of experimental plants.

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Nil

CONFLICT OF INTERESTS

We declare that there is no conflict of interest.

AUTHORS CONTRIBUTIONS

All the authors are equally contributed

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