

Assessment of Antidiarrheal Action of the Methanolic Extract of *Brassica nigra* Flower in Swiss Albino Mice



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ABSTRACT

The purpose of the present study was to evaluate the antidiarrheal activity of methanol extracts of *Brassica nigra* flower using castor oil – induced diarrhoea and gastrointestinal motility test using charcoal meal method. The methanolic extract was initially fractioned which was assayed for its effect in castor oil – induced diarrhoea at different doses (200 and 400 mg/kg body weight) followed by its evaluation on the peristaltic movements in charcoal meal test using loperamide

as a reference drug. The results of the present study indicated that, administration of the various fractions of methanolic extract of *Brassica nigra* induced dose-dependent percentages of inhibition of diarrhoea. The antidiarrheal potential of this plant might be due to its high contents of flavonoids and tannins. It could be claimed that, the remarkable antidiarrheal activity of *Brassica nigra* flower support to its utility in a wide range of remedies of diarrhoea.

Key words: *Brassica nigra*, antidiarrheal, gastrointestinal motility, castor oil, charcoal.

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INTRODUCTION

Nature is enriched with wide range of medicinal agents and many drugs are isolated from nature. Plants with medicinal values are a lucrative source of obtaining variety of drugs. Man uses a wide range of drugs to cure diseases traditionally.¹ The World Health Organization (WHO) reported that about 80% of the world's population depends primarily on traditional medicine that mainly involves the use of plant extracts.² Among them, *Brassica nigra* (Family: Brassicaceae) commonly known as mustard has both edible and medicinal value. This plant has been traditionally used in Africa for treatment of inflammation and rheumatism. It has also been used as simple rubefacient, diuretic, emetic, pneumonia, bronchitis, nerve stimulation and vesicant.³ Again, it has been cultivated for millennia as a spice.⁴ Judging by the amount of spice produced, mustard is the world's most important spice. *B. nigra* may decrease biomass and fecundity of co-existing species. It can generate huge amounts of biomass.¹ Again, it inhibits the germination of other species through allelopathy, supporting dense stands of nearby monotypic mustard.⁵ Brassica vegetables are belong to high nutritional value. They also supply high amounts of vitamin C and soluble fibres that contain nutrients with anticancer properties.⁶⁻⁷ Brassica fresh vegetables contain indole-3-carbinol, a compound which promotes DNA repair in cells in vitro and generally block any growth of cancer

cellular material in vitro.⁸⁻⁹ The hydro-alcoholic *B. nigra* exhibits anti-seizure potential in mice.¹⁰ The vegetables of *B. nigra* also contain goitrogens which suppress thyroid function. Goitrogens can initiates hypothyroidism and goiter in the absence of normal iodine intake.¹¹

Diarrhoeal disease is a leading cause of mortality and morbidity, especially in developing countries among children.¹² *Shigella flexneri*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* are the major causative agents of diarrhoea in humans.¹³ *Candida albicans* is also responsible for inducing diarrhoea in humans.¹⁴ Consumption of medicinal herbs is highly increasing over the past decades as alternative approach to improve the quality of life and maintain good health. World Health Organization (WHO) has encouraged studies for treatment and prevention of diarrhoeal diseases depending on traditional medical practices.¹⁵

So the present study was designed to explore the anti-diarrheal potential of *Brassica nigra* using castor oil induced diarrhoea and gastrointestinal motility test using charcoal meal method.

MATERIALS AND METHODS

Collection of plant materials

The plant part selected for present work was flower of *Brassica nigra* (Brassicaceae). For this present investigation *Brassica nigra* was collected from Noakhali Science and Technology University

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campus, Noakhali, Bangladesh in the month of December, 2015 and was identified in Bangladesh National Herbarium (BNH), Dhaka, Bangladesh. A voucher specimen (DACB Accession No 45837) of the plant has been deposited in the herbarium.

Preparation of plant extract

The collected plant parts were separated from undesirable materials or plants. They were sun-dried for two week. The plant parts were ground into powder with the help of a suitable grinder. Then the powder was stored in an airtight container and kept in a cool, dark and dry place. The parched and powdered flowers (500 g) were soaked in 1500 ml of 99% methanol for about 21 days at room temperature with occasional stirring. After 21 days the solution was filtered using filter cloth and what's man filter paper. The residue (40gm) derived from methanol extract was subjected to vacuum liquid chromatography using n-hexane, chloroform, ethyl acetate and water in order of increasing polarity.

Solvent-Solvent Partition of Crude extract

The solvent-solvent partition of crude extract was carried out according to modified Kupchan Partition.¹⁶

Preparation of Mother Solution

10 gram of dried methanol extract was mixed with 90 ml of methanol and 10 ml of distilled water. The crude extract was dissolved absolutely. This mother solution was partitioned off successively by three solvents of different polarity. Each of the fractions was analysed separately for the detection and identification of compounds having different pharmacological activities.

Partitioning with n-hexane

The mother solution was taken in a separating funnel in which 100 ml of the petroleum ether was added and the funnel was shaken carefully and kept undisturbed. The organic portion was collected. The process was repeated thrice. The n-hexane fractions were collected together and evaporated in Rota evaporator.

Partitioning with Chloroform

The mother solution that left after washing with n-hexane was taken in a beaker and 16 ml of distilled water was added and mixed uniformly. The mother solution was then taken in a separating funnel and extracted with CHCl₃ (100 ml X 3). The chloroform soluble fraction was collected together and evaporated.

Partitioning with Ethyl acetate

The mother solution that left after washing with chloroform and n-hexane was then taken in a separating funnel and extracted with Ethyl acetate (100 ml X 3). The Ethyl acetate soluble fraction was collected and evaporated. The remaining fraction was preserved as aqueous fraction.

Experimental Animal

For continuing my experimentation Swiss albino mice (both male and female) weighed between 20-25 g were used. These mice were amalgamated from Jahangirnagar University, Dhaka, Bangladesh. Before the experimentation, these animals were adjusted to standard animal house condition for five days. All the experiments were performed balancing with the permitted guideline and method of the Institution of Animal Ethics Committee of University of Development of Alternative, Dhaka, Bangladesh.

Drugs and chemicals

Standard Loperamide was purchased from Square Pharmaceutical Ltd., Bangladesh. Other reagents of analytical grade for conducting this research work were supplied from ethno pharmacology laboratory of pharmacy department of Noakhali Science & Technology University.

Evaluation of Anti-diarrheal activity

Castor oil induced diarrhea

The investigation of anti-diarrheal activity by castor oil induced method was carried out according to methods described by Billah.¹⁷ The experiment was performed by using Swiss albino mice (20-25 g). Mice were randomly divided into seven groups. Each group received a particular treatment. The animals were divided into control, positive, and test groups containing five mice in each group. Control group received vehicle (plain distilled water) at dose 10ml/kg orally. The positive control group received loperamide at the dose of 5 mg/kg orally. The test group received methanolic crude extract (MCE) and its fractions of *B. nigra* at the dose of 200 and 400 mg/kg body weight. After 60 minutes of administration of test samples the mice of all groups were orally treated with 0.5 ml of castor oil. 60 minutes interval was given between the administration of test samples and castor oil to ensure proper absorption of the administered samples. Then the mice were placed in a transparent cage observing the consistency of fecal matter and frequency of detection for 4 hours. Wet feces were counted at the end of the experiment by lifting the paper placed in the transparent beaker. Afterwards, percentage

of defecation and the percentage of inhibition of defecation was measured.

$$\text{Inhibition of defecation (\%)} = \frac{\text{NDC} - \text{NDT}}{\text{NDC}} \times 100$$

Here, NDC = mean number of diarrheal faeces of the control group.

NDT = mean number of diarrheal faeces of the treated group.

Gastrointestinal Motility Activity

The effects on intestinal propulsion in Swiss albino mice were conducted using charcoal method.¹⁸ The animals were divided into control, positive, and test groups containing five mice in each group. Control group received vehicle (distilled water) at dose 10ml/kg orally. The positive control group received Loperamide at a dose of 25mg/kg orally. The experiment group received different fractions of *Brassica nigra* at the doses of 400 mg/kg body weight. Before the experiment, animals in all groups were fasted for 24 hours, but allowed free access to water. The animals were treated with distilled water, plant extract and standard drug. After 30 minutes, each animal was administered 1 ml of charcoal meal (which was prepared with 3% suspension of activated charcoal in 0.5% aqueous methyl cellulose) orally to all groups. All the animals were sacrificed after 30 minutes of charcoal meal administration and the small intestine was rapidly dissected out and placed on a clean surface. The intestine was carefully checked out and the distance journeyed by charcoal meal plug from the pylorus to caecum was measured by a measuring scale. The size of the

whole intestine was also measured. The distance travelled by the charcoal plug from the pylorus to the caecum was represented as a percentage of the total length of the small intestine.

The percentage of inhibition was determined by using the following equation.¹⁹

$$\text{IP \%} = (\text{LM}/\text{LSI}) \times 100$$

$$\% \text{ of Inhibition} = \{ \text{IP \% (control)} - \text{IP \% (treatment)} \} / \text{IP \% (control)}$$

Where,

PI = Peristaltic index

LM = Length of charcoal meal

LSI = Length of small intestine

RESULT

Castor oil-induced diarrheal test

The data obtained from castor oil-induced diarrhea are represented as the number of defecation and percent of inhibition compared with the control group in Table 1. After 30 minutes of administration of castor oil, diarrhea was clinically apparent for the next 4 h in the control group. This condition was markedly reduced by 46.77% by loperamide at a dose of 5 mg/kg. All of our extracts also demonstrated statistically significant ($P < 0.05$) inhibition of castor oil-induced diarrhea in a dose-dependent manner. Amongst five extracts, the chloroform soluble fraction (CSF) showed better activity against diarrhea and produced 74.19% and 79.03% inhibition at a dose 200mg/kg and 400 mg/kg

Table 1 Anti-diarrheal activity of MCE and its fractions against castor oil induced diarrhea in mice

| Sample | Dose (p.o) | No. faeces in 0-4 hours (Mean \pm S.E.M) | % inhibition of diarrhea |
|------------|------------|--|--------------------------|
| Control | 10 ml/kg | 12.40 \pm .51 | – |
| Loperamide | 5mg/kg | 6.60 \pm .51 | 46.77 |
| MCE | 200 mg/kg | 3.80 \pm .37** | 69.35 |
| | 400 mg/kg | 3.00 \pm .32*** | 75.81 |
| n-HSF | 200 mg/kg | 3.40 \pm .51** | 72.58 |
| | 400 mg/kg | 2.80 \pm .37** | 77.41 |
| CSF | 200 mg/kg | 3.20 \pm .37*** | 74.19 |
| | 400 mg/kg | 2.60 \pm .24*** | 79.03 |
| EASF | 200 mg/kg | 5.40 \pm .55 | 57.14 |
| | 400 mg/kg | 4.20 \pm .37 | 66.12 |
| ASF | 200 mg/kg | 4.20 \pm .37 | 66.12 |
| | 400 mg/kg | 3.40 \pm .51** | 72.58 |

Here, MCE= Methanolic Crude Extract, EASF=Ethyl Acetate Soluble Fraction, n-HSF=n-Hexane Soluble Fraction, CSF=Chloroform Soluble Fraction, ASF= Aqueous Soluble Fraction, S.E.M= Standard Error Mean. Each value represents as the mean \pm SEM (n=5). * $p < 0.05$ compared with standard. (One way ANOVA followed by Dunnett's 't'-test).

Table 2 Effects of MCE and its fractions on charcoal meal-stimulated gastrointestinal transit in mice

| Group | Dose(mg/kg) | Total length of intestine (cm) | Distance travelled by charcoal (cm) | % Intestinal transit | % Inhibition relative to control |
|------------|-------------|--------------------------------|-------------------------------------|----------------------|----------------------------------|
| Control | 0.2ml/10g | 50.17 ± 0.48 | 39.67 ± 0.33 | 79.07 | - |
| Loperamide | 25 | 53.67 ± 0.67** | 9.83 ± 0.48** | 18.32 | 76.83 |
| CSF | 400 | 55.67±0.61*** | 28.33±0.33*** | 50.89 | 35.64 |
| EASF | 400 | 51.33±0.49 | 30.67±0.99 | 59.75 | 24.43 |
| MCE | 400 | 53.67±0.67** | 30.33±0.49** | 56.51 | 28.53 |
| n-HSF | 400 | 50.67±0.21 | 27.67±0.49 | 54.61 | 30.93 |
| ASF | 400 | 50.33±1.20 | 29.67±0.49 | 58.95 | 25.45 |

Values are represented as mean ± SEM (n = 5). Data was analyzed by one-way ANOVA followed by Bonferroni's test.*p<0.05, **p<0.01***p<0.001 when compared to control.

respectively, which was significant when compared with the percent of inhibition of the standard drug (46.77%).

GI motility Activity

The result of gastrointestinal motility test was revealed in Table 2. From the result it was observed that charcoal meal had moved 79.07% of the total length of the intestine in animal treated with normal distilled water after 30 minutes of ingesting the meal. Furthermore, the standard drug loperamide significantly (p < 0.05) reduced the distance travelled by charcoal meal to 18.32%. Treatment with CSF, EASF, MCE, n-HSF and ASF also reduced distance traveled by charcoal meal 50.89%, 59.75%, 56.51%, 54.61% and 58.95% respectively at a concentration of 400mg/kg.

DISCUSSION

Diarrhoea is responsible for millions of deaths in the world annually. Most people who die from diarrhoea are actually die from severe dehydration and fluid loss.²⁰ In order to address this condition, human being are using different types of plants from the beginning of civilization which generates different adverse effects. As a result, the treatment of diarrhoea is an important medical topic.

Castor oil is usually used to induce diarrhoea in animal model research.²¹ The ingestion of castor oil results in release of ricinoleic by lipases in the intestinal lumen. This acid generates irritation and inflammation in intestinal mucosa by releasing the inflammatory mediators, such as prostaglandins, nitric oxide and histamine which in turn stimulate gastrointestinal motility, epithelial permeability, secretions and edema of the intestinal mucosa that inhibit the re-absorption of Na⁺, K⁺ and water.²² This situation is significantly reduced antidiarrheal agents (loperamide). In this investigation, all fractions of *Brassica nigra* flower also improve this

condition which may be due to the presence of anti-diarrheal agent in the flower of the plant.

Using activated charcoal as a marker, the intestinal motility test has been implemented for over 60 years as a simple and effective method of assessing the effects of laxatives on the gastrointestinal tract.²³⁻²⁴ This investigation also revealed that different fractions of *Brassica nigra* flower reduced intestinal motility. Among the different fractions, CSF showed the highest percentage of gastrointestinal motility inhibition (35.64%). This might suggest that *Brassica nigra* flower has the ability to relax intestinal muscles. Many drugs and medicinal herbal extracts that are used in the treatment of diarrhoea possess the ability to reduce intestinal contraction and motility.²⁵ The extract containing phytochemicals such as tannin, alkaloids, flavonoids, and terpenoids are responsible for generating antidiarrheal activity which was confirmed by chemical screening.²⁶ Thus, considering the result of above test we may claim that the flower of *B. nigra* could be a viable future target for diarrheal management.

CONCLUSION

On the basis of the findings of the present study it can be concluded that methanolic extract of *Brassica nigra* flowers possesses antidiarrheal activity and it could be a lucrative source of discovering new antidiarrheal agents.

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CONFLICT OF INTEREST STATEMENT

We proclaim that we have no conflict of interest.

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