EVALUATION OF ANTI-INFLAMMATORY ACTIVITY AND TOTAL TANNIN CONTENT FROM THE LEAVES OF BACOPA MONNIERI (LINN.)

Hemayet Hossain*1, Abdullah Al-Mansur 1, Sanjida Akter 2, Umme Sara 3, Md. Ranzu Ahmed 4 and Abu Anis Jahangir 1

BCSIR Laboratories, Dhaka, Bangladesh Council of Scientific and Industrial Research 1, Dr. Qudrat-E-Khuda Road, Dhaka-1205, Bangladesh
Department of Chemistry, Jahangirnagar University 2, Dhaka-1315, Bangladesh
Department of Pharmacy, The University of Asia Pacific 3, Dhaka-1205, Bangladesh
Department of Chemistry, Bangladesh University of Health Sciences 4, Dhaka-1216, Bangladesh

ABSTRACT: The methanolic leaf extract of Bacopa monnieri Linn. (Family: Scrophulariaceae) was evaluated for its anti-inflammatory activity, and total tannins content in different in-vivo and in-vitro methods. The anti-inflammatory activity was studied using carrageenan and histamine-induced rat paw edema test at different doses (200 and 400 mg/kg body weight) of the methanol extract. At the dose of 400 mg/kg body weight, the extract showed a significant anti-inflammatory activity both in the carrageenan and histamine-induced oedema test models in rats showing 62.73% and 61.99% reduction in the paw volume comparable (P<0.01) to that produced by the standard drug indomethacin (67.08% and 70.76%) at 5 h respectively. The percentage inhibition of the oedema paw volume by the 400 mg/kg body weight of the extract was also statistically significant (P<0.05; P<0.01) compared favorably with the indomethacin treated animals at 1, 2, 3 4 and 5 h in both models. The total tannins content was calculated high and significant in methanol extract (105.90 mg of tannic acid equivalent per g of dry extract). Acute toxicity test showed that the plant part might be safe for pharmacological uses. Therefore, the obtained results tend to suggest the acute anti-inflammatory activity of the methanol extract of Bacopa monnieri leaves and thus provide the scientific basis for the traditional uses of this plant part as a remedy for pain, and inflammations.

INTRODUCTION: Bacopa monnieri (L) Penn. (B. monnieri) commonly known as ‘Brahmi’ is an ancient and renowned medicinal plant and has important applications in Ayurveda system 1.

Several phytochemical compounds such as brahmine and herpestine, saponins d-mannitol, hersaponin, betulinic acid, stigmasterol, β-sitosterol, bacosides A and B were isolated from the plant 2.

The plant possesses significant antiulcerogenic 3 and cardioprotective activities 4 and has wide applications in the treatment of anxiety, depression, mental fatigue 5, 6, asthma 7, antinociceptive, anti diarrhoeal and antioxidant activities 8, 9, 10. Moreover, in vitro research has revealed the

<table>
<thead>
<tr>
<th>Keywords:</th>
<th>Bacopa monnieri, Anti-inflammatory, Carrageenan, Histamine, Total tannins</th>
</tr>
</thead>
</table>

Correspondence to Author:
Md. Hemayet Hossain
Senior Scientific Officer, Chemical Research Division, BCSIR Laboratories, Dhaka, Bangladesh
E-mail: hemayethossain02@yahoo.com

DOI: 10.13040/IJPSR.0975-8232.5(4).1246-52
Article can be accessed online on: www.ijpsr.com
DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(4).1246-52
anticancer activity of *B. monnieri* saponin fractions against sarcoma-180 cells. This might be due to inhibition of DNA replication in the cancerous cell line 11.

Chronic inflammatory diseases remain one of the world’s major health problems 12. Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair 13. Non-steroidal anti-inflammatory drugs (NSAID) are among the most commonly prescribed drugs due to their consistent effectiveness in the treatment of pain, fever, inflammation and rheumatic disorders. However, their use is associated with adverse effects at the level of digestive tract, ranging from dyspeptic symptoms, gastrointestinal erosions and peptic ulcers to more serious complications, such as over bleeding or perforation 14.

Now-a-days attention is being focused on the investigation of the efficacy of plant-based drugs used in the traditional medicine because they are cheap, have little side effects and according to WHO, about 80% of the world population still rely mainly on herbal remedies 15. Therefore to overcome the toxicity of NSAID, the development of new anti-inflammatory drugs is still necessary and the natural product such as medicinal plants could lead in discovering new anti-inflammatory drugs with less undesirable effects.

Since no literature is currently available to substantiate anti-inflammatory activity, and total tannins content of the leaf extract of *B. monnieri*, the present study was designed to provide scientific evidence for its use as a traditional folk remedy by investigating the above pharmacological and tannins contents of the methanolic extract that also confirm its use in folk remedy for inflammation, pain.

**MATERIALS AND METHODS:**

**Collection and identification of plant materials:** *B. monnieri* (leaves) were collected from Karamjal, Sundarban Khulna, Bangladesh in August, 2012. The collected samples were then identified by Sarder Nasir Uddin, Senior Scientific Officer, Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. A specimen copy was deposited to Bangladesh National Herbarium for identification & the accession number was DACB-32605.

**Preparation of methanolic extract:** The plant materials (leaves) of *B. monnieri* were freed from any of the foreign materials. Then the plants were air-dried under shed temperature followed by drying in an electric oven at 40ºC. The dried plant materials were then ground into powder. About 400g of powdered material was taken in a clean, flat-bottomed glass container and soaked in 1300 ml of 95% methanol. The container with its contents was sealed and kept for a period of 14 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper (Bibby RE200, Sterilin Ltd., UK) which was concentrated with rotary evaporator at bath temperature not exceeding 40ºC to have gummy concentrate of extract (yield approx. 2.77%).

**Test Animals:** For the screening of acute toxicity activity male rats of Wister strain weighing 175-202 g were used. The animals were housed under standard Laboratory (at Pharmacology Laboratory of BCSIR, Chittagong) conditions maintained at 25±1°C and under 12/12 h light/dark cycle and feed with Balanced Trusty Chunts and water *ad libitum*. All experimental protocols were in compliance with BCSIR Ethics Committee on Research in Animals as well as internationally accepted principles for the use and care of experimental animals.

**Chemicals & drugs:** Carrageenan, histamine phosphate, indomethacin, tannic acid, and folinic-cioalteu phenol reagent were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Methanol, tween 80, sodium carbonate were of analytical grade and bought from E. Merck, India Ltd.

**Phytochemical screening:** The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents, by using the following reagents and chemicals, for example, alkaloids were identified by the Dragendorff’s reagent, flavonoids with the use of Mg and HCl, tannins with ferric chloride and potassium dichromate solutions, and steroids with Libermann-Burchard reagent and reducing sugars with Benedict’s reagent 16, 17, 18.
Acute toxicity test: The acute toxicity of *B. monnieri* methanolic extract was determined in male rats of Wister strain according to the method of Hilaly et al.\(^1\) with slight modifications. Rats fasted for 16 h were randomly divided into groups of five rats per group. Graded doses of the extract (200, 400, 800, 1600 and 3200 mg/kg p.o.) were separately administered (dissolved in 1% tween 80 in normal saline) to the rats in each of the groups by means of bulbed steel needle. All the animals were then allowed free access to food and water and observed over a period of 72 h for signs of acute toxicity. The number of deaths within this period was recorded.

Anti-inflammatory activity:

1. **Carrageenan-induced oedema test:** Carrageenan induced rat hind paw oedema was used as the animal model of acute inflammation. In this experiment, the rats were divided into five groups of five animals each. The different groups of animals were administered with 1% Tween 80 in normal saline (10 ml/kg, p.o.); *B. monnieri* (200 and 400 mg/kg, p.o.) and indomethacin (10 mg/kg, p.o.). Acute inflammation was induced in all the five groups by sub plantar injection of 0.1 ml of carrageenan with (1% suspension in Tween 80) in the right hind paw of the rats 30 min after the oral administration of the tested materials. The paw volume was measured with micrometer screw gauze at 1, 2, 3, 4 and 5 h after the administration of the drug and the extract. The percentage inhibition of inflammatory effect of the extract was calculated using the same formula for carrageenan-induced paw oedema.

2. **Histamine-induced oedema test:** This test was studied using the paw oedema model induced by 0.1% histamine into the sub plantar region of the right hind paw of the rats.\(^13, 22\) The different groups of animals were administered with 1% Tween 80 in normal saline (10 ml/kg, p.o.); *B. monnieri* (200 and 400 mg/kg, p.o.) and indomethacin (10 mg/kg, p.o.). Acute inflammation was induced in all the five groups by sub plantar injection of 0.1 ml of histamine with (1% suspension in Tween 80) in the right hind paw of the rats 30 min after the oral administration of the tested materials. The paw volume was measured with micrometer screw gauze at 1, 2, 3, 4 and 5 h after the administration of the drug and the extract. The percentage inhibition of inflammatory effect of the extract was calculated using the same formula for histamine-induced paw oedema.

**Determination of total tannins content:** The tannins were determined using the Folin-Ciocalteu Phenol reagent as reported by Amorim et al.\(^23, 24\). Briefly, 1.0 ml of the sample extract was added with 7.5 ml of distilled water and added 0.5 ml of Folin-Ciocalteu Phenol reagent, 1 ml of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well, kept at room temperature for 30 min and absorbance was measured at 725 nm. Blank was prepared with water instead of the sample. A set of standard solutions of tannic acid is read against a blank. The results of tannins were expressed in terms of tannic acid in mg/g of dry extract.

Total tannin content was determined as mg of tannic acid equivalent per gram using the equation obtained from a standard tannic acid calibration curve \(y=4.5692x-0.2538\), \(R^2=0.9953\).

**Statistical Analysis:** Data were presented as mean ± Standard deviation (S.D). Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnett’s multiple comparisons using SPSS Data Editor for Windows, Version 11.5.0 (SPSS Inc., U.S.A.).

The results obtained were compared with the control group. \(P\) values < 0.05 were considered to be statistically significant.

**RESULTS:**

**Chemical group test:** Results of different chemical tests on the methanolic extract of *B. monnieri* leaves showed the presence of alkaloid, reducing sugars, saponins, tannins, and flavonoids (Table 1).
1. **Carrageenan-induced paw oedema**: The anti-inflammatory effect of the methanolic extract of the leaves of *B. monnieri* using carrageenan induced oedema tests is expressed in (Table 2). In this test, the positive control (Indomethacin) significantly (p<0.05; p<0.01) decreased the paw edema at 1h to 5 h after carrageenan injection compared to saline with inhibition 52.43% to 67.08%. A maximum oedema paw volume of 1.66 ± 0.09 mm was observed in the control rats, 4 h after the carrageenan injection.

Rats with the extract at 400 mg/kg body weight significantly decreased (p<0.05; p<0.01) the carrageenan-induced oedema paw volume from 1 h to 5 h compared to the standard drug indomethacin at a dose of 10 mg/kg body weight. The inhibition percentage of the oedema paw volume by the 400 mg/kg body weight of the extract was also found statistically significant compared to the indomethacin treated animals at 1, 2, 3, 4 and 5 h. The highest reduction in the paw volume by the 400 mg/kg body weight was 62.73% was comparable to that of the indomethacin (67.08%) at 5 h.

### Histamine-induced paw oedema: Table 3 showed the anti-inflammation effect of the methanolic extract of *B. monnieri* leaves using histamine-induced paw oedema tests. In the histamine-induced oedema test, a maximum oedema paw volume of 1.75 ± 0.07 mm was observed in the control rats, 4 h after the histamine injection. Rats pre-treated with the extract at 400 mg/kg body weight significantly decreased (p<0.05; p<0.01) the histamine-induced oedema paw volume from 1h to 4 h compared to the standard drug indomethacin at a dose of 10 mg/kg body weight.

The percentage inhibition of the oedema paw volume by the 400 mg/kg body weight of the extract was also statistically significant (p<0.05; p<0.01) compared favorably with the indomethacin treated animals at 1, 2, 3, 4 and 5 h. The maximum reduction in the paw volume by the 400 mg/kg body weight was 61.99% compared to the indomethacin (70.76%) at 5 h.

### Total tannins content: The amount of total tannins content was calculated as significant in the methanolic crude extract of *B. monnieri* (41.07 ± 2.87 mg/g of gallic acid equivalent) (Table 4).
Histamine is an important inflammation mediator, potent vasodilatory substance and also increases the vascular permeability. Since the extract effectively suppressed the oedema produced by histamine, it showed that the extract exhibited anti-inflammatory actions by inhibiting the synthesis, release or action of inflammatory mediators such as histamine, serotonin and prostaglandins. This study has shown that the methanol extract of the leaves of *B. monnieri* possessed a significant anti-oedematogenic effect (P<0.01) on paw oedema induced by carrageenan and histamine compared favorably with the standard drug (indomethacin) in treated rats.

Non-steroidal anti-inflammatory drugs (NSAID) such as indomethacin used in this study are known to inhibit cyclooxygenase enzymes I and II which are implicated in the production of inflammation-mediating agent prostaglandin E2 (PGE2) from arachidonic acid. Therefore, the pattern of anti-inflammatory activity exhibited by this extract was similar to that of indomethacin.

Tannins and Flavonoids, commonly found in plants have been reported to have significant antioxidant activity. Polyphenolic compounds, like flavonoids, tannins and phenolic acids, commonly found in plants have been reported to have multiple biological effects, including antioxidant activity. Phytochemical components, especially phenolic compounds (such as flavonoids, phenylen propanoids, phenolic acids, tannins etc.) are very important components for the free radical.
scavenging and antioxidant activities of plants. Polyphenols are generally of the chemical patterns; phenolic groups react as hydrogen donors and neutralize the free radicals\(^39\). In the present study the total amount of tannin compounds was calculated as quite high in the methanol extract of \textit{B. monnieri} leaves. The result of present study revealed that the presence of high concentration of tannin components in the extract might cause the high inhibition value of the extract.

CONCLUSION: Since the plant extract reduced significantly the formation of oedema induced by carrageenan and histamine, the leaves of \textit{B. monnieri} exhibited acute anti-inflammatory activity. The potential of the extract as acute anti-inflammatory activity, free radical scavenging agents may be due to the presence of phytoconstituents like saponins, tannins, and flavonoids and might be responsible for its activity. Again, no mortality was recorded in the acute toxicity test; it showed that the plant might be safe for use. Therefore, it can be revealed that the methanolic extract of \textit{B. monnieri} leaves possess acute anti-inflammatory activity as well as tannin contents and justify its use as a traditional folk remedy for inflammation, pain. However, a more extensive study is necessary to determine the exact mechanism(s) of action of the extract and its active compound(s).

ACKNOWLEDGEMENT: We are grateful to Dr. Md. Tofazzal Hossain, Member Science & Technology, BCSIR & Ex. Director BCSIR Laboratories Dhaka, for his inspiration during this research work.

REFERENCES:


How to cite this article:

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.