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RESEARCH ARTICLE

Anti-inflammatory and antioxidant activities of ethanolic leaf extract of *Brownlowia tersa* (L.) Kosterm

Hemayet Hossain · Ismet Ara Jahan · Sariful Islam Howlader · Jamil Ahmed Shilpi · Shubhra Kanti Dey · Arpona Hira · Arif Ahmed

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Abstract The present study was designed to investigate the inflammatory and antioxidant activities of ethanolic leaf extract of *Brownlowia tersa* (L.) Kosterm. The anti-inflammatory activity of leaf extract was studied using carrageenan and histamine-induced rat paw edema test at different doses (200 and 400 mg/kg body weight). DPPH free radical scavenging, nitric oxide scavenging, reducing power, Fe^{++} ion chelating ability and total phenolic content were used for determining antioxidant activities. The extract, at the dose of 400 mg/kg, showed a significant anti-inflammatory activity ($P<0.01$) both in the carrageenan and histamine-induced edema test models in rats showing 54.76 % and 56.96 % reduction in the paw volume comparable to that produced by the standard drug indomethacin (64.88 % and 67.09 %) at 4 h respectively. In DPPH free radical scavenging test, IC_{50} value for ethanolic extract was found fairly significant 39.33 μg/ml when compared to the IC_{50} value of the reference standards ascorbic acid and Butylated Hydroxy Anisole (BHA) (3.16 and 5.81 μg/ml) respectively. The IC_{50} values of the extract and ascorbic acid were 99.06 and 39.35 μg/ml, respectively in nitric oxide scavenging assay. The maximum absorbance for reducing power assay was found to be 1.276 at 100 μg/ml when compared to 2.821 and 1.231 for standard ascorbic acid and BHA respectively. The IC_{50} value of the extract as percentage of Fe^{++} ion chelating ability was also found significant compared to that of EDTA. The total phenolic content was 211.82 mg/g of gallic acid equivalent. Acute toxicity test showed that the plant might be safe for pharmacological uses up to a dose level of 3,200 mg/kg of body weight in rats. Therefore, the obtained results suggest the acute anti-inflammatory and antioxidant activities of the ethanolic extract of *Brownlowia tersa* leaves and thus provide the scientific basis for the traditional uses of this plant part as a remedy for pain and inflammations.

Keywords *Brownlowia tersa* · Anti-inflammatory · DPPH free-radical scavenging · Nitric oxide radical scavenging · Reducing power · Total phenolic

Introduction

Brownlowia tersa (*B. tersa*) (L.) Kosterm (Family: Tiliaceae) is a shrub about 1.5–2 m tall distributed widely throughout India (Orissa) to Southeast Asia, Myanmar, Cambodia, Thailand, Malaysia, Brunei, Indonesia, Coastal forests of Bay of Bengal. The volatile phenolic compound 2′-hydroxyacetophenone and the lignan carinol both have been isolated for the first time from *Brownlowia tersa* (Hettiarachchi et al. 2009). (4-nitrophenyl) propandiamide and (4-methylphenyl) propandiamide have also been reported from the aerial parts of *Brownlowia tersa* (Shehata et al. 2003). *B. tersa* has long been used as a traditional folk remedy for diarrhea, dysentery, wounds and boils. *B. tersa* roots have been found to possess significant antibacterial activity (Hettiarachchi et al. 2009). The leaves of *B. tersa* possess antinociceptive and antidiarrhoeal activities (Sariful et al. 2012).

Since no literature is currently available to substantiate anti-inflammatory and antioxidant properties of the leaves

H. Hossain (✉) · I. A. Jahan
Chemical Research Division, BCSIR Laboratories,
Dhaka, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka 1205, Bangladesh
e-mail: hemayethossainbd@yahoo.com

S. I. Howlader
Department of Pharmacy, World University of Bangladesh,
Dhaka 1205, Bangladesh

J. A. Shilpi · S. K. Dey · A. Hira · A. Ahmed
Pharmacy Discipline, Khulna University,
Khulna 9208, Bangladesh

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of *B. tersa*, the present study was designed to provide scientific evidence for its use as a traditional folk remedy by investigating the above pharmacological and antioxidant potential of the ethanolic extract that also confirm its use in folk remedy for inflammation, pain and other pathological conditions where free radicals are implicated.

Materials and methods

Collection and identification of plant material

The plant *B. tersa* was collected from Karamjal area of Sundarban forest, Bangladesh in June 2011 and was identified by Bangladesh National Herbarium, Mirpur, Dhaka (Accession number-DACB-39753).

Preparation of ethanolic extract

The collected plant parts were separated from undesirable materials and then were washed with water and sun-dried for 1 week. The dried plant materials were ground into a coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powdered sample was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. About 500 g of powered material was taken in a clean, flat-bottomed glass container and soaked in 1,200 ml of ethanol. The container along with its contents was sealed and kept for a period of 7 days with occasional shaking or stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. It was then filtered through whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate was concentrated by using rotary vacuum evaporator (R-210, Buchi, Switzerland) and dried. It rendered a 47 g of gummy concentrate (9.40 %) and was designated as crude ethanol extract.

Test for different chemical groups

The ethanolic extract was tested for its different chemical groups as alkaloids, flavonoids, gums, reducing sugars, saponins, steroids and tannins (Evans 1989). 10 % (w/v) solution of the extract in ethanol was used for each of the above test.

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; saponins with foam test; glycosides with Lieberman's test and reducing sugars with Benedict's reagent.

Test for anti-inflammatory activity

Test animals & drugs

For the screening of in vivo anti-inflammatory 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 Bangladesh Council of Scientific and Industrial Research (BCSIR) ethics committee on Research in animals as well as internationally accepted principles for laboratory animal use and care.

The standard drug Indomethacin was used for this study and purchased from Square Pharmaceuticals Ltd, Bangladesh.

Acute toxicity test

The acute toxicity of ethanolic extract was determined in rats according to the method of Hilaly (Hilaly et al. 2004) 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, 1,600 and 3,200 mg/kg p.o.) were separately administered 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

Carrageenan-induced edema test

Carrageenan induced rat hind paw edema was used as the animal model of acute inflammation according to the method of Lanhers (Lanhers et al. 1991). In this experiment, the rats were divided into four groups of five animals each. Group I (control) received 2 % Tween 80 in normal saline (2 ml/kg). Group II (Positive control) received 10 mg/kg body wt. of Indomethacin orally. Group III and IV received 200 and 400 mg/kg body wt. of the extract orally respectively. Acute inflammation was induced in all the four groups by sub plantar injection of 0.05 ml of its suspension of Carrageenan with 2 % Tween 80 in normal saline in the right Paw of the rats 30 min after the oral administration of the tested materials. The paw volume was measured with a

micrometer screw gause at 1, 2, 3 and 4 h after the administration of the drug and the extract. The percentage inhibition of inflammatory effect of the extract was calculated using the following expression:

Percentage inhibition of inflammation

$$= [(Vc - Vt)/Vc] \times 100$$

Where Vc is the average degree of inflammation by the control group and Vt is the average degree of inflammation by the test group.

Histamine-induced edema test

Using the method of Perianayagam (Perianayagam et al. 2006) with slight modification, the paw edema was produced by sub-plantar administration of 0.1 % freshly prepared solution of histamine into the right hind paw of the rats. In this experiment, twenty rats were divided into four groups of five animals each. Group I (control) received 2 % Tween 80 in normal saline (2 ml/kg). Group II (Positive control) received 10 mg/kg body wt. of Indomethacin orally. Group III and IV received 200 and 400 mg/kg body wt. of the extract orally respectively. Acute inflammation was induced in all the four groups by sub plantar injection of 0.1 ml of Histamine with 2 % Tween 80 in normal saline in the right hind paw of the rats 1 h after the oral administration of the tested materials. The paw volume was measured with a micrometer screw gause at 1, 2, 3 and 4 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 edema.

Antioxidant activities

Chemicals

1,1-Diphenyl-2-picryl hydrazyl (DPPH), L-ascorbic acid, BHA (Butylated Hydroxy Anisole), Gallic acid, Folinciocalteu phenol reagent, Ferrozine and Griess reagent were obtained from Sigma Chemical Co.[(St. Louis, MO, USA)]. Trichloroacetic acid (TCA), Phosphate buffer (pH 6.6), Potassium ferricyanide [$K_3Fe(CN)_6$], $FeCl_2$, $FeCl_3$, Sodium nitroprusside, Sodium phosphate, EDTA, Tween 80, Ammonium molybdate and Sodium carbonate were of analytical grade and purchased from Merck (Darmstadt, Germany).

DPPH free radical scavenging activity

The method of Chang (Chang et al. 2001; Hemayet et al. 2011) was used for performing the DPPH radical scavenging activity. A stock solution (5 mg/ml) of ethanolic extract of *B. tersa* (5 mg/ml) was prepared in respective solvent systems. A serial dilutions were the carried out to obtain concentrations of 5, 10, 20, 40, 60, 80, 100 μg/ml. An equal amount of sample solution was mixed with an equal amount of 0.1 mM methanolic solution of DPPH, The mixture was vortex and allowed to stand at the dark at 25 °C for 30 min. After 30 min incubation, the absorbance of the mixture was read against a blank at 517 nm using a double beam Analykjena UV/Visible spectrophotometer (Model 205, Jena, Germany). The radical scavenging activity was expressed as the inhibition percentage (I%) and calculated as per the equation:

$$I(\%) = \left(A_{blank} - A_{sample}/A_{blank}\right) \times 100$$

Where Ablank is the absorbance of the control (containing all reagents except the test compound), and A_{sample} is the absorbance of the experimental sample with all reagents. IC_{50} value (the concentration of sample required to scavenge 50 % DPPH free radical) was calculated from the plot of inhibition (%) against the concentration of the extract. All determination was carried out in triplicate and average of the results was noted. Ascorbic acid and BHA was used as standard for this study.

Nitric oxide (NO) scavenging activity

Nitric oxide scavenging activity was measured spectrophotometrically (Govindarajan et al. 2003). Sodium nitroprusside (5 mmol) in phosphate buffered saline was mixed with different concentrations of the extract (5–100 μg/ml) dissolved in methanol and incubated at 25 °C for 30 min. A control without the test compound but with an equivalent amount of methanol was taken. After 30 min, 1.5 ml of the incubation solution was removed and diluted with 1.5 ml of Griess reagent (1 % sulphanilamide, 2 % phosphoric acid, and 0.1 % naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine dihydrochloride was measured at 546 nm. The nitric oxide (NO) radical scavenging activity was expressed as the inhibition percentage (I%) and calculated as per the equation:

$$I(\%) = \left(A_{blank} - A_{sample}/A_{blank}\right) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound with all reagents. IC_{50} value is the concentration of sample required to scavenge 50 % nitric oxide free radical and was calculated from the plot of inhibition (%) against extract concentration. All the tests were carried out in triplicate and average

of the absorptions was noted. Ascorbic acid was used as positive control standard for this study.

Reducing power assay

The reducing power of *B. tersa* was determined according to method followed by Dehpour (Dehpour et al. 2009). Different concentrations of *B. tersa* extract (5–100 μg/ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1 %). The mixture was incubated at 50 °C for 20 min. A 10 % solution of trichloroacetic acid (2.5 ml) was added to the mixture, which was then centrifuged at 3,000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1 %) and the absorbance of the mixture was measured at 700 nm with spectrophotometer. Increased absorbance of the reaction mixture indicated increased reducing power. All the tests were carried out in triplicate and average of the absorptions was recorded. Ascorbic acid and BHA were used as the standard reference compounds.

Ferrous ion chelating ability

The ferrous ions chelating activity of ethanol extract and standards were investigated according to the method of Dinis (Dinis et al. 1994). Briefly, different concentrations of the extract (5–100 μg/ml) were added to 0.1 ml solution of 2 mM ferrous chloride ($FeCl_2$). Then, the reaction was initiated by the addition of 0.2 ml of 5 mM Ferrozine and mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured at 562 nm in spectrophotometer, wherein the Fe^{+2} chelating ability of extracts was monitored by measuring the ferrous ion-Ferrozine complex. The percentage of inhibition of ferrozine-Fe^{+2} complex formation was given in the below formula:

$$\text{Ferrous ions chelating ability}(\%) = [(A_0 - A)/A_0] \times 100$$

Where A_0 is the absorbance of the control solution (containing all reagents except extract); A is the absorbance in the presence of the sample of plant extracts. All the tests were carried out in triplicate and EDTA was used as standard.

Total phenolic content determination

The total phenolic content of the extract was determined by the modified Folin-Ciocaltu method (Wolfe et al. 2003). Briefly, 0.5 ml of each extract (1 mg/ml) was mixed with 5 ml Folin-Ciocaltu reagent (1:10 v/v distilled water) and 4 ml (75 g/l) of sodium carbonate. The mixture was vortexed for 15 s and allowed to stand for 30 min at 40 °C for color development. The absorbance was read at 765 nm with a spectrophotometer. Total phenolic content was determined as mg of gallic acid equivalent per gram using the equation obtained from a standard gallic acid calibration curve $y=6.2548x - 0.0925$, $R2=0.9962$.

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 Dunnet'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 ethanolic extract of *B. tersa* leaves showed the presence of reducing sugars, saponins, glycosides, flavonoids and tannins (Table 1).

Acute toxicity test In acute toxicity study, oral administration of graded doses (200, 400, 800, 1,600 and 3,200 mg/kg p.o.) of the ethanol extract of *B. tersa* to rats did not produce any significant changes in behaviour, breathing, cutaneous effects, sensory nervous system responses or gastrointestinal effects during the observation period. No mortality or any toxic reaction was recorded in any group after 72 h of administering the extract to the animals. *B. tersa* leaf extract was safe up to a dose level of 3,200 mg/kg of body weight in rats.

Table 1 Results of different chemical group tests of the ethanolic extract of *B. tersa* leaves

Plant Extract	Alkaloid	Saponins	Reducing sugar	Glycosides	Tannins	Flavonoids
EE	−	+	+	+	+	+

EE Ethanolic extract of *B. tersa*; +: Positive result; −: Negative result

Anti-inflammatory activity

Carrageenan-induced paw edema The anti inflammation effect of the ethanolic extract of the leaves of *B. tersa* using carrageenan induced edema 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 1 h to 4 h after carrageenan injection compared to saline with inhibition 50.98 % to 64.88 %. A maximum edema paw volume of 1.68±0.05 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 edema paw volume from 1 h to 4 h compared to the standard drug indomethacin at a dose of 10 mg/kg body weight. The inhibition percentage of the edema paw volume by the 400 mg/kg body weight of the extract was also found statistically significant when it was compared with the indomethacin treated animals at 1, 2, 3 and 4 h. The highest reduction in the paw volume by the 400 mg/kg body weight was 54.76 % was comparable to that of the indomethacin (64.88 %) at 4 h.

Histamine-induced paw edema Table 3 showed the anti-inflammation effect of the ethanolic extract of *B. tersa* leaves using histamine-induced paw edema tests. In the histamine-induced edema test, a maximum edema paw volume of 1.58± 0.09 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 edema paw volume from 1 h to 4 h compared to the standard drug indomethacin at a dose of 10 mg/kg body weight. The percentage inhibition of the edema 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 and 4 h. The maximum reduction in the paw volume by the 400 mg/kg body weight was 56.96 % compared to the indomethacin (67.09 %) at 4 h.

Antioxidant activities

Ethanol extract of *B. tersa* was screened for evaluation of its possible antioxidant activities. Five complementary test systems, namely DPPH free radical scavenging, nitric oxide scavenging activity, reducing power, ferrous ion chelating ability and total phenolic contents determination were followed for this analysis.

DPPH free radical scavenging activity DPPH free radical scavenging activity of the *B. tersa* was found to be increased with the increase of concentration of the extract (Fig. 1). The extract exhibited 81.91 % radical inhibitions at 100 μg/ml whereas at the same concentration the standards ascorbic acid and BHA exhibited 95.86 and 93.09 % inhibitions respectively. IC_{50} value of the extract was found to be very fairly significant (39.33±0.53 μg/ml) compared to the IC_{50} value of the reference compounds ascorbic acid and BHA (3.16±0.09 and 5.81±0.07 μg/ml) respectively.

Nitric oxide (NO) scavenging assay The scavenging of NO by the ethanol extract of *B. tersa* was also increased in dose dependent manner. A significant decrease in the NO radical due to the scavenging ability of the extract and ascorbic acid. The ethanol extract showed maximum scavenging activity of 52.66 % at 100 μg/ml, where as ascorbic acid at the same concentration exhibited 79.18 % inhibition (Fig. 2). The IC_{50} value for ethanolic extract was found fairly significant (99.06 μg/ml) while compared to the IC_{50} value of the reference standard ascorbic acid (39.35 μg/ml).

Reducing power assay The reducing power of ethanolic crude extract of *B. tersa* was also determined using ascorbic acid and BHA as positive control (table 4). The maximum absorbance for ethanolic crude extract was 1.276±0.06 at 100 μg/ml while compared to 2.821±0.09 and 1.231±0.07 for standard ascorbic acid and BHA respectively, at the same concentration.

Table 2 Effect of ethanol extract of *B. tersa* leaves and indomethacin on carrageenan-induced edema paw volume in male wistar rats

Treatment Groups	Doses (mg/kg body weight)	Right hind paw volume (mm)			
		1 h	2 h	3 h	4 h
Control	2 ml/kg	1.02±0.05	1.29±0.08	1.48±0.07	1.68±0.05
Positive Control (Indomethacin)	10	0.50±0.06** (50.98)	0.62±0.07* (51.93)	0.69±0.08** (53.37)	0.59±0.07** (64.88)
Extract	200	0.94±0.09* (7.84)	1.08±0.06* (16.27)	1.14±0.04* (22.97)	1.17±0.05* (30.35)
Extract	400	0.58±0.08* (43.13)	0.69±0.03** (46.41)	0.75±0.06* (49.32)	0.73±0.04** (56.54)

Values in brackets denote percentage inhibition of the edema paw volume

Values are expressed as mean±SD; Values are calculated as compared to control using one way-ANOVA followed by Dunnet's Test; * indicates $P<0.05$; ** indicates $P<0.01$ vs. control; $n=5$

Table 3 Effect of ethanol extract of *B. tersa* leaves and indomethacin (standard drug) on histamine-induced edema paw volume in male wistar rats

Treatment Groups	Doses (mg/kg body weight)	Right hind paw volume (mm)			
		1 h	2 h	3 h	4 h
Control	2 ml/kg	1.08±0.05	1.28±0.03	1.39±0.07	1.58±0.09
Positive Control (Indomethacin)	10	0.47±0.08** (56.48)	0.55±0.09* (57.03)	0.52±0.05* (62.58)	0.54±0.06** (65.82)
Extract	200	0.82±0.07* (24.07)	0.85±0.04* (33.59)	0.91±0.08** (34.53)	0.98±0.09* (37.97)
Extract	400	0.59±0.05* (45.37)	0.66±0.06** (48.43)	0.70±0.04* (49.64)	0.68±0.03** (56.96)

Values in brackets denote percentage inhibition of the edema paw volume

Values are expressed as mean ± SD; Values are calculated as compared to control using one way-ANOVA followed by Dunnet's Test; * indicates $P<0.05$; ** indicates $P<0.01$ vs. control; $n=5$

Fe^{+2} ion chelating ability Fe^{+2} ion chelating ability of ethanol extract is shown in table 5. The extract showed 62.41±0.44 % Fe^{+2} ion chelating ability at 100 µg/ml where as the standard EDTA showed 99.75±0.67 % at the same concentration. The IC_{50} value of the extract was also found significant (76.28±0.31 µg/ml) while compared to the IC_{50} value of the reference standard EDTA (8.87±0.36 µg/ml).

Total phenolic content The amount of total phenolic content was calculated as quite high in the ethanolic crude extract of *B. tersa* (211.82±7.86 mg/g of gallic acid equivalent) (Table 6).

Discussion

The anti-inflammatory effect of ethanolic extract of *B. tersa* was evaluated in carrageenan and histamine induced paw edema, which are widely used for the screening of anti-inflammatory compounds and have frequently been used to assess the antiedematogenic effect of medicinal plants. The experimental model exhibits a high degree of reproducibility. In rats, the inflammatory response induced by carrageenan is characterized by a biphasic response with marked edema formation resulting from the rapid production of several inflammatory mediators such as histamine, serotonin, and bradykinin (first-phase), which is subsequently sustained by the release of prostaglandins and nitric oxide (second-phase) with peak at 3 h, produced by inducible isoform of cyclooxygenase (COX-2) and nitric oxide synthase (iNOS), respectively (Seibert et al. 1994). In the present work, pre-treated oral administration of extract was effective in reducing the edematogenic response evoked by carrageenan in rats between the second and the fourth hours after the injection. This evidence allows us to suggest that anti-inflammatory actions of the extract are related to the inhibition of one or more intracellular signaling pathways involved in the effects of several inflammatory mediators.

Again, histamine is an important inflammation mediator, potent vasodilator substance and also increases the vascular permeability (Cuman et al. 2001). When histamine is subcutaneously injected into a rat, it forms a wheal around the

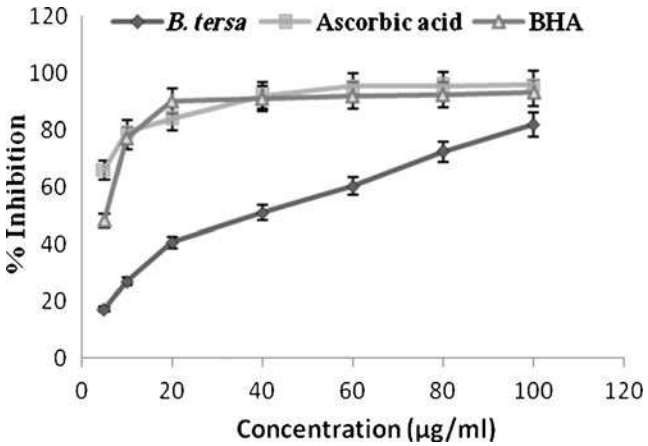

Fig. 1 DPPH free radical scavenging activity of ethanolic leaf extract of *B. tersa* and standards. The values are expressed as mean ± standard deviation ($n=3$)

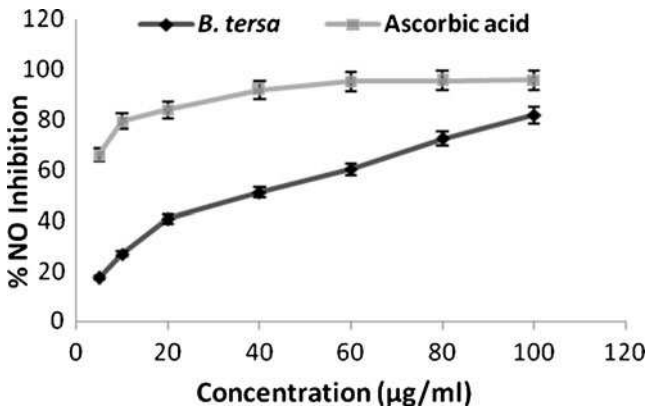

Fig. 2 Nitric oxide radical scavenging activity of ethanolic leaf extract of *B. tersa* and standard. The values are expressed as mean ± standard deviation ($n=3$)

Table 4 Reducing power assay of the ethanolic extract of *B. tersa leaves* and standards

Concentration (µg/ml)	Absorbance at 700 nm of extract and standards at different concentration		
	Ethanol Extract of *B. tersa* Leaves	Ascorbic acid (standard)	BHA (standard)
5	0.386±0.09	0.643±0.02	0.435±0.07
10	0.532±0.07	0.999±0.03	0.497±0.05
20	1.006±0.09	1.511±0.04	0.608±0.06
40	1.105±0.07	2.746±0.06	0.849±0.05
60	1.198±0.04	2.822±0.05	1.136±0.09
80	1.218±0.05	2.772±0.07	1.197±0.06
100	1.276±0.06	2.821±0.09	1.231±0.07

The values are expressed as mean ± standard deviation (n=3)

injected place, due to increase of vascular permeability of the host capillary venules in the skin. Substances that antagonize the activity of histamine receptors reduce the area of the wheal formed. This result tends to suggest that the anti-inflammatory activity of the extract is possibly backed by its anti-histamine activity. The antihistaminic effect of the extract increased with the increase in the dose of the extract. Since the extract effectively suppressed the edema 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 ethanol extract of the leaves of *B. tersa* possesses a significant antiedematogenic effect ($P<0.01$) on paw edema induced by carrageenan and histamine compared favorably with the standard drug (indomethacin) in treated rats.

Phytochemically, the leaves of *B. tersa* have been also reported to yield flavonoids and tannins. Flavonoids (or bioflavonoids) are naturally occurring compounds, considered to possess anti-inflammatory properties, both in vitro and in vivo (Vasudevan et al. 2007). Flavonoids also have anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediators of inflammation (Sawadogo et al. 2006). Tannins are important compounds known to be potent cyclooxygenase-1 inhibitors and with antiphlogistic activity (Pan et al. 2010). Again it has been found that phenolic compounds exhibited a significant anti-inflammatory action on carrageenan-induced paw edema by inhibition of leukocyte migration, reduction of serum lysozyme levels, nitric oxide and PGE_2 (Wu et al. 2006) .

The mechanisms of anti-inflammatory activity may be related to the anti-phlogistic action of the tannins. Therefore, the acute anti-inflammatory activity of the ethanolic leaf extract of *B. tersa* might be due to presence of these phenolics, flavonoids, tannins etc.

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 E_2 (PGE_2) from arachidonic acid (Dhara et al. 2000). Therefore, the pattern of anti-inflammatory activity exhibited by this extract was similar to that of indomethacin.

The ethanolic extract of *B. tersa was* subjected to screen for its possible antioxidant activities. Five complementary test systems, namely DPPH free radical scavenging, nitric oxide scavenging activity, reducing power, ferrous ion chelating ability and total phenolic contents determination were followed for this analysis.

Table 5 Fe^{2+} ion chelating ability of ethanol extract of *B. tersa* leaves and EDTA (Standard)

Concentration (µg/ml)	% Chelating ability of different solvent extract and Standard	
	Ethanol extract of *B. tersa* leaves	Na_2EDTA (Standard)
5	3.59±0.58	36.97±0.33
10	8.76±0.76	57.71±0.61
20	12.82±0.37	81.69±0.40
40	24.67±0.61	91.35±0.81
60	40.58±0.83	99.19±0.72
80	58.33±0.59	99.30±0.89
100	62.41±0.44	99.75±0.96
IC_{50} (µg/ml)	76.28±0.31	8.87±0.33

The values are expressed as mean ± standard deviation (n=3)

Table 6 Total phenolic content of the ethanol extract of *B. tersa* leaves

Extract	Avg. absorbance at 765 nm	Total phenolic content mg of gallic acid equivalent (GAE) per g of dry extract
Ethanol extract of *B. tersa*	1.2457±0.08	211.82±7.86

The values are expressed as mean ± standard deviation (n=3)

A method based on the scavenging of the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) has been used extensively to predict the antioxidant activities of extracts of plants (Kulisic et al. 2004). The high inhibition value of *B. tersa* ethanol extract may due to the presence of flavonoids and significant amount of tannins in the extract as phytochemicals.

Nitric oxide (NO) scavenging capacity of the extract may help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to the human health. Nitric oxide is also implicated for inflammation, cancer and other pathological conditions (Moncada et al. 1991). NO works as an atypical neural modulator that is involved in neurotransmitter release, neuronal excitability and learning and memory. Besides its role in physiologic processes, it also participates in pathogenic pathways underlying a large group of disorders including muscle diseases, inflammatory bowel disease, sepsis and septic shock, primary headaches and stroke. Additionally, increasing evidence shows that NO modulates neurotoxin induced cell damage and is involved in neuronal cell death in Parkinson's disease (PD) and other neurodegenerative disorders such as Alzheimer disease (Aliev et al. 2009). Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and treatment of diseases associated with oxidants or free radicals (Soares et al. 1997). The high inhibition value of *B. tersa* ethanol extract may due to the presence of flavonoids as phytochemical.

A direct correlation between antioxidant capacity and reducing power of certain plant extracts has been reported (Tanaka et al. 1988). The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Duh et al. 1999). In case of reducing power assay the higher the absorbance, the higher the antioxidant activity.

Bivalent transition metal ions (e.g. Fe^{++}) play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions via Fenton chemistry (Halliwell et al. 1992). These processes can be delayed by iron chelation. Iron can generate free radicals from peroxides and may be implicated in human cardiovascular disease (Gutteridge, 1993). Therefore, minimizing its concentration affords protection against oxidative damage. Ferrozine can quantitatively form complexes with Fe^{2+}. The absorbance of Fe^{2+}-ferrozine complex was decreased dose-dependently, that is, the activity was increased on increasing concentration from 5 to 100 μg/ml. Table 5 is exhibiting the comparative percentage Fe^{++} ion chelating ability of ethanol extract and standard compound (Na_2EDTA). The IC_{50} value of extract as percentage (%) Fe^{++}ion chelating ability was determined as 76.28±0.31 μg/ml where Na_2EDTA showed 8.87±0.36 μg/ml.

Various phytochemical components, especially polyphenols (such as flavonoids, tannins, phyenyl propanoids, phenolic acids etc.) are known to be responsible for the free radical scavenging and antioxidant activities of plants. In generally, polyphenols all share the same chemical patterns, one or more phenolic groups for which they react as hydrogen donors and in that way neutralize free radicals (Miliauskas et al. 2004; Atoui et al. 2005). The amount total phenolic compound was calculated as quite high in the ethanol extract of *B. tersa* (211.82±7.86 mg/g of gallic acid equivalent). According to the results of this study, it can be revealed that the high inhibition value in the ethanol extract might be due to the high concentration of phenolic compounds present in the extract. Phenols are important components of plants. They are reported to eliminate radicals due to their hydroxyl groups and they contribute directly to antioxidant effect of system (Duh et al. 1999).

Conclusion

Since the plant extract reduced significantly the formation of edema induced by carrageenan and histamine, the leaves of *B. tersa* exhibited acute anti-inflammatory activity. The potential of the extract of *B. tersa* as acute anti-inflammatory and antioxidant agents may be due to the presence of phytoconstituents like flavonoids, tannins, phenolics 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 in rats. Therefore, it can be revealed that the ethanolic leaf extract of *B. tersa* possess acute anti-inflammatory activity as well as antioxidant activities and justify its use as a traditional folk remedy for inflammation, pain, boils etc. However, a more extensive study is necessary to determine the exact mechanism(s) of action of the extract and its active compound(s).

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