## **Research Article**



# Evaluation of the Anti-Nociceptive and Anti-Inflammatory Activities of the Ethanolic Extract of *Barringtonia Acutangula* Linn. (lecythidaceae) Roots

Syeda Hurmatul Quader\*, Shoaib Ul Islam, ARM Saifullah, Fakhar Uddin Majumder, JMA Hannan

Department of Pharmacy, School of Life Sciences, North South University, Bashundhara, Dhaka-1229, Bangladesh. \*Corresponding author's E-mail: shquader@yahoo.com

#### Accepted on: 22-03-2013; Finalized on: 31-05-2013.

#### ABSTRACT

*Barringtonia acutangula* (L.) Gaertn. (Family: Lecythidaceae) has been used in different formulations in *Ayurveda* for the treatment of various ailments. Among many, it has been indicated for joint pain. The objective of the present study therefore was to assess the anti-nociceptive and anti-inflammatory activity of the crude ethanolic root extract of the plant. Two doses of 250 mg/kg and 500 mg/kg body weight were orally administered to mice and rats to evaluate the anti-nociceptive activity using the Hot Plate and Acetic Acid Writhing tests. In both the tests the experimental animals exhibited analgesia in a dose dependent fashion. Maximum percentage inhibitions of analgesia induced by the root extract were 91.33% and 13.80% for the Hot Plate and Acetic Acid Writhing test, respectively. Carrageenan Induced Rat Paw Edema (acute model) and Cotton Pellet Induced Granuloma (chronic model) tests were used to evaluate the anti-inflammatory properties of the root extract using the same two doses. Maximum percentage inhibitions of inflammation of 26.82% and 24.56% were recorded for the acute and chronic models, respectively. In both the tests inhibitions were induced dose-dependently. Our findings thereby confirm that *Barringtonia acutangula* roots possess significant central and peripheral anti-nociceptive as well as anti-inflammatory activity.

Keywords: Barringtonia acutangula, Anti-nociceptive, Anti-inflammatory, Root extract.

#### **INTRODUCTION**

he practice of the medical application of herbs can be traced back to the earliest chronicled times in the history of Man as plants had been used for treating and preventing diseases in almost all ancient civilizations until the advent of iatrochemistry in the 16<sup>th</sup> century. Even in the passage of the recent 'synthetic' age, people have tried to revive the traditional practice of usage of natural drugs on account of the decreasing efficacy and increasing side effects of such synthetic drugs. At present, plant drugs with substantiated medicinal value are prescribed in almost all pharmacopoeias in the world. Separate herbal pharmacopoeias are maintained by countries such as the United Kingdom, Russia and Germany. The basis of their usage is grounded on the knowledge of the experiences of popular medicine (traditional or popular medicine) or on the results of new evidential experimental data (conventional medicine). The medical usage of only few plant species have been scientifically evaluated and even more limited are the safety and efficacy data for such plants. More vigorous and extended research is needed to substantiate the traditional practice of these medicinal plants.1-8

*Barringtonia acutangula* (L.) Gaertn. (Family-Lecythidaceae) is an evergreen tree which ranges from 5 to 8 metres. Structurally it consists of leaves which have an obovate shape, a grey bark which is full of rough fissures, red flowers housed in pendulous racemes and four-sided fruits which are usually 20 cm long. Known locally as Hijjol in Bangladesh, it thrives in the tropics especially in the regions of Southeast Asia, Australia and Africa. Its abundance is particularly noticeable on river banks, ponds, lakes and other low lying areas.<sup>9,10</sup>

*Barringtonia asiatica* and *Barringtonia racemosa* are other species of *Barringtonia*. *B. asiatica* is used to treat rheumatism and *B. racemosa* has been prescribed in the *Ayurvedic* literature in the treatment of pain, inflammation and rheumatism. *B. acutangula* has been used in *Ayurvedic* medicine formulations like *Nichuladi lepa*, *Apachihara lepa*, *Lakshmivilasa rasa*, *Taptaraja taila* based on folklore in the remedy of joint pain, intermittent fever, eye diseases, stomach disorders, diarrhoea, cough, dyspnoea, anthelmintic, leprosy, hemiplegia, spleenic disorders and poisoning. Traditional *Ayurvedic* uses of the plant also include inflammation, diseases of the skin, leprosy, dysmenorrhea, hemorrhoids, flatulence, and as anthelmintic.<sup>11-14</sup>

Properties of *B. acutangula* such as antibacterial activity of stem bark and twigs have been documented. Anticancer, antidiarrheal, antioxidant and hepatoprotective activities of the leaves, hypoglycemic activities in fruits, anti-diabetic, hypolipidemic and antioxidant activities of the root, and anti-scorpion venom activity of the plant have also been reported. Antinociceptive activity has been experimentally evaluated of the leaves although no experimental studies on the antinociceptive and anti-inflammatory properties of the root of the plant have been recorded. The objective of the present study therefore is to ascertain the antinociceptive and anti-inflammatory properties employing in-vivo studies of the crude ethanolic root extract of B. acutangula and justify the medical usage of this plant in traditional folklore medicine.<sup>15-26</sup>



#### **MATERIALS AND METHODS**

#### **Plant Collection and Identification**

The root of *B. acutangula* was obtained from Jahangirnagar University campus, Savar, Dhaka, Bangladesh. The identification of the plant was done by Shah Md. Ahsan Habib, Senior Herbarium Technician at the Bangladesh National Herbarium, Dhaka, Bangladesh. A voucher specimen (Accession no. DACB 38202) was deposited in the Herbarium for future reference.

#### **Plant material Preparation and Extraction**

After collection, the root of *B. acutangula* was cleaned, cut into smaller pieces and dried in shade. The dried root was powdered using mixer grinder and a weighed amount of 1000g of the powder was passed through the sieve number 20 to collect the fine uniform powder. Cold solvent extraction was employed to extract the powder with ethanol (95% v/v, 3L) for two times in seven days. After collection, the filtrate was concentrated in a rotary vacuum evaporator (Bibby RE-200, Sterilin Ltd., UK) under reduced pressure at a temperature of 50°C to give a blackish brown dried extract (68.8 g, yield ratio 6.9% w/w) which was refrigerated for experimental use.<sup>27</sup>

#### Animals

Anti-nociceptive and anti-inflammatory activities were evaluated using Swiss albino mice (Mus musculus) of either sex weighing between 20g and 25g and aged between 4 to 5 weeks, and male Wistar albino rats (Rattus norvegicus) weighing between 150g and 180g, respectively. The animals were procured from the Animal House of the Pharmacy Department of Jahangirnagar University. They were kept in a cross ventilated laboratory and were acclimatized to standard laboratory conditions maintained at temperature of 25±2°C, relative humidity of 55 ±5%, and light and dark cycles of 12:12 hours for one week before the experiment. They were housed in standard cages which were cleaned on a routine basis and had free access to standard nutritional diet of rodent pellets and water ad libitum. The experiments were conducted in accordance with the UK Home Office regulations (UK Animals Scientific Procedures Act 1986) and the 'Principles of Laboratory Animal Care' (National Institutes of Health publication no. 86-23, revised 1985) and were approved by the Ethics Committee on Animal Research, North South University.

## **Phytochemical Screening**

Crude ethanolic extract of *B. acutangula* root was subjected to phytochemical screening to qualitatively detect the presence of tannins, flavonoids, saponins, gums, steroids, alkaloids, reducing sugars, and terpenoids following the phytochemical procedures as described.<sup>28,29</sup>

## Chemicals

Acetic acid was the product of Merck (Germany), and Carrageenan was of Sigma Aldrich (USA). Diclofenac sodium and Tramadol were purchased from Novartis (Bangladesh) Ltd. Sterile normal saline was the control. All the other chemicals used in the experiments were of analytical grade.

## **Evaluation of In Vivo Anti-nociceptive activity**

## Acetic acid induced writhing test<sup>30,31</sup>

Acetic acid induced writhing test was conducted on mice fasted with free access to water for 12 hours prior to the experiment. The four groups of mice (n=6) were divided as follows: $^{32,33}$ 

Group I- Control group received 0.9% saline solution (0.25 ml, *p.o.*)

Group II- Standard group received Diclofenac sodium (positive control, 20 mg/kg, *p.o.*)

Group III-Test group received the root extract (250 mg/kg, *p.o.*)

Group IV- Test group received the root extract (500 mg/kg, *p.o.*)

An hour after administration, the mice were injected intraperitoneally(i.p.)with 0.1mL of0.6% (v/v solution) acetic acid. The mice were placed in different boxes and two to three minutes after the injection they started experiencing writhing. The number of writhes were counted for the next ten minutes. The experiment was repeated twice. Comparison of the values obtained from the positive control group and the test groups were compared with those of the control group. Percent writhing response was calculated as:

 $PWR = (W_C - W_T)/W_C \times 100$ 

Where,  $W_T$  and  $W_c$  represent the mean number of writhes of treatment and control groups, respectively

## Hot plate method <sup>36-37</sup>

The assessment of analgesia was conducted by a slight modification of the hot plate test on mice (n=6) in four groups as described below:

Group I- Control group received 0.9% saline solution (0.25 ml, p.o.)

Group II- Standard group received Tramadol (positive control, 10 mg/kg, *p.o.*)

Group III-Test group received the root extract (250 mg/kg, p.o.)

Group IV- Test group received the root extract (500 mg/kg, p.o.)

The mice had free access to only water for 12 hours before the experiment. They were subjected to a screening test, to ensure behavioral consistency, prior to the experiment on the hot plate apparatus (UGO- BASIL, Model-35100-001, Italy) set at a temperature of 55  $\pm 0.1^{\circ}$ C. Nociceptive response was elicited by the mice during sensation of thermal pain (licking the fore or hind paws, or jumping).Mice with reaction time (the time the mouse stayed on the hot plate without licking or jumping) beyond a maximum cut-off time of 20 seconds were rejected to prevent possible tissue damage or blister



formation. Record of the reaction time (i.e. latency time) was carried out before as well as after receiving treatment. The reading times were scheduled as 0h, ½h, 1h, 2h, 3h and 4h after the treatment. Percent analgesia was calculated using the following formula:

$$\mathsf{PA} = (\mathsf{L}_{\mathsf{A}} - \mathsf{L}_{\mathsf{C}}) / \mathsf{L}_{\mathsf{A}} \times 100$$

Where,  $L_{A}$  and  $L_{C}$  represent mean latency time of treatment and control groups, respectively

## Evaluation of In Vivo Anti-inflammatory activity

#### Carrageenan induced rat paw edema method

In carrageenan induced rat paw edema experiment the rats (n=5) were grouped as follows:  $^{38}$ 

Group I- Control group received 0.9% saline solution (0.5 ml, p.o.)

Group II- Standard group received Diclofenac sodium (positive control, 20 mg/kg, *p.o.*)

Group III-Test group received the root extract (250 mg/kg, p.o.)

Group IV- Test group received the root extract (500 mg/kg, p.o.)

The animals were fasted overnight with water given *ad libitum*. Half an hour after receiving treatment, edema was induced by subcutaneous injection of carrageenan (1%, 0.05ml) in the sub plantar tissue of the right hind paw of each experimental animal. Using plethysmometer (Model 7141, UGO Basile, Italy)the paw volume (i.e. inflammation) was measured before (0h) and at 1h,2h,3h,4h,9h after carrageenan injection. The left paw served as the reference paw which did not undergo inflammation. The average paw volumes of the treatment groups were compared to the control group. The percentage inhibition of the paw edema was calculated using the formula:<sup>39</sup>

 $\mathsf{PPE} = (\mathsf{V}_{\mathsf{C}} - \mathsf{V}_{\mathsf{T}}/\mathsf{V}_{\mathsf{C}}) \times 100$ 

Where,  $V_{\text{C}}$  and  $V_{\text{T}}$  represent mean paw volume of control and treatment groups, respectively.

## Cotton pellet induced granuloma method<sup>40</sup>

The groups of mice (n=5) for the cotton pellet induced granuloma experiment were as follows:

Group I- Control group received 0.9% saline solution (0.5 ml, p.o.)

Group II- Standard group received Ibuprofen (positive control, 20 mg/kg, *p.o.*)

Group III-Test group received the root extract (250 mg/kg, p.o.)

Group IV- Test group received the root extract (500 mg/kg, p.o.)

Sterile cotton pellets of 40mg weight were used to induce granuloma in the Wistar albino rats. After anesthetization of the rats with ketamine (60mg/kg body weight), the fur was shaved and one cotton pellet in each axilla was inserted. The incisions were then stitched. The control vehicle, extract and standard drug were administered to the rats orally every day for seven days. During this time, standard pellet diet was continued and the rats had free access to water *ad libitum*. On the eight day, the animals were anaesthetized and the cotton pellets, after being removed surgically, were cleaned to separate extraneous tissues. The moist cotton pellets were weighed and dried at 60°C for 18 hours after which they were reweighed. Granuloma formation was measured by the increment in the weight of the dried pellets. Percentage inhibition of granuloma formation was calculated using the formula:<sup>40,41</sup>

 $PGF=(DW_{C} - DW_{T}/DW_{C}) \times 100$ 

Where,  $DW_{\text{C}}$  and  $DW_{\text{T}}$  represent the mean weight of the dried cotton pellets of the control and treatment groups, respectively

#### Statistical analysis

The obtained data was statistically analysed and expressed as the mean  $\pm$  SEM by one-way analysis of variance (ANOVA) and Dunnett's *t*-test as the test of significance. The minimum level of significance was considered to be p value <0.05.The SPSS 17.0 statistical software was used to carry out all the statistical tests.

#### **RESULTS AND DISCUSSION**

#### Anti-nociceptive Activity

#### Hot plate method

The results of the hot plate test are presented in Table 2 for the crude ethanolic root extract of *B. acutangula*. Significant dose dependent increase (p=0.05) of latency time of *B. acutangula* treated groups from 1h to 3h was noted when compared to the control group. Tramadol produced the second highest anti-nociception of 89.79% at 3h after treatment. For the *B. acutangula* 250mg/kg and 500mg/kg, the maximum analgesia of 69.37% (P<0.001) and 91.33% (P<0.001) were recorded at 3h, respectively. The latency time decreased for all the treatment groups at the 4h.

## Acetic acid-induced writhing test

Table 3 illustrates the mean number of writhes along with the corresponding percentage inhibition in mice during the acetic acid induced writhing test. The percentage inhibition of writhes by *B. Acutangula* was 10.34% (p<0.05,) and 13.80% (p<0.01) at 250 and 500 mg/kg doses, respectively. The data shows that 250mg/kg and 500mg/kg of the extract induced significant reduction of the mean number of writhes, compared to the control group, in a dose-dependent manner. The standard drug Diclofenac sodium exhibited the maximum reduction of 44.33 % (p<0.001) which is higher than the percentage inhibition observed for the other test group animals.

## Anti-inflammatory activity

#### Carrageenan induced rat paw edema

Table 4 represents the reduction in the carrageenan induced rat paw edema after treatment of the extract and standard drug. In the control group animals, the injection of carrageenan in the rat paw induced a local



edema that increased progressively and reached a maximum paw volume at 3h after injection. At 3h the highest percentage inhibition was recorded in both the extract treated groups with 6.70% for *B. acutangula* 250mg/kg (p<0.05) and the 26.82% for *B. acutangula* 500mg/kg (p<0.001). Diclofenac sodium produced highly statistically significant results (p<0.001) from 1h to 4h with the highest anti-edematous responses of 27.27% and

27.22% drawn at 1 h and at 4 h, respectively. The inhibitory response of *B. acutangula* 500mg/kg was statistically significant from 1h to 4h and the anti-inflammatory effect was comparable to the standard. Both the extract treated groups exhibited responses dose-dependently. After the 4h the inhibitory response declined for all the treatment groups.

Table	1: Phytochemical	screening of B.	. acutangula ro	oot extract
	<b>J</b> · · · · · · · ·		J	

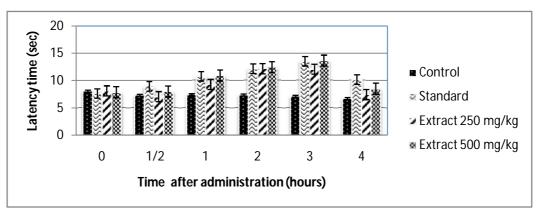
Extract	Tannin	Flavonoid	Saponin	Gum	Steroids	Alkaloid	Reducing Sugars	Terpenoid
Root Extract of <i>B. acutangula</i>	+++	++	+++	+++	++	+++	+++	++

Symbols '+++' indicates presence of the phytochemical in high concentration; '++' indicates presence in moderate concentration; and '+' indicates presence in trace concentration and '-' indicates absence of the stated phytochemical.

Table 2. The effect of <i>D. deatangula</i> of faterby time by the not plate method								
Group No.	Treatment Group	Latency time (seconds)						
Group No.		0h	½h	1h	2h	3h	4h	
I	Control	8.08±0.56	7.33±0.35	7.42±0.33	7.40±0.38	7.15±0.27	6.73±0.27	
Ш	Standard	7.65±0.33	8.97±0.38 (22.37%)	10.82±0.39 <sup>***</sup> (45.82%)	12.18±0.44 <sup>****</sup> (64.59%)	13.57±0.29 <sup>****</sup> (89.79%)	10.22±0.33 <sup>****</sup> (51.86%)	
ш	Extract 250mg/kg	8.13±0.10	7.07±0.57 (Nil)	9.32±0.37 <sup>*</sup> (25.61%)	(64.86%)	12.11±0.61 <sup>***</sup> (69.37%)	7.47±0.67 (11.0%)	
IV	Extract 500mg/kg	7.90±0.19	8.02±0.51 (9.41%)	10.95±0.54 <sup>****</sup> (47.57%)	12.48±0.43 <sup>****</sup> (68.65%)	13.68±0.31 <sup>****</sup> (91.33%)	8.53±0.54 <sup>*</sup> (26.75%)	

Table 2: The effect of *B. acutangula* on latency time by the hot plate method

Each value represents the mean±SEM (n=6); \* p<0.05, \*\* p<0.01, \*\*\* p<0.001. Values in parentheses indicate percentage increase of latency time. Groups II, III and IV are compared with group I (control group).





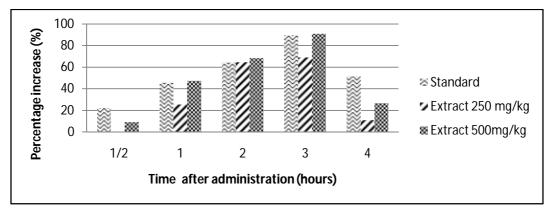






Table 3: The effect of *B. acutangula* on writhing in the acetic acid writhing test

Group No.	Treatment Group	Number of Writhes	Percentage Inhibition (%)
I	Control	40.60±1.81	
II	Standard	22.60±0.25****	44.33
III	Extract 250mg/kg	36.40±0.51 <sup>*</sup>	10.34
IV	Extract 500mg/kg	35.00±0.45 <sup>**</sup>	13.80

Each value represents the mean $\pm$ SEM (n=6); \* p<0.05, \*\* p<0.01, \*\*\* p<0.001. Values in parentheses indicate percentage increase of latency time. Groups II, III and IV are compared with group I (control group).

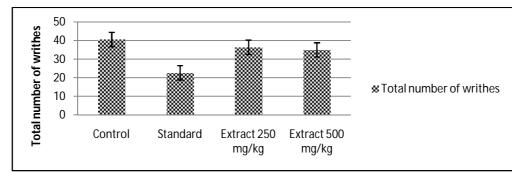


Figure 3: Comparative study of the number of writhes induced by *B. acutangula* in the acetic acid writhing test

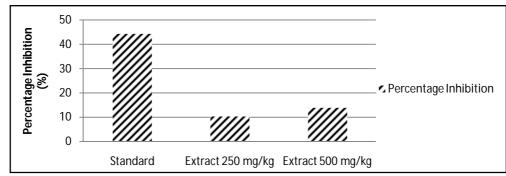


Figure 4: Comparative study of the percentage inhibition of writhes by B. acutangula in the acetic acid writhing test

Table 4: The effect of B. acutangula on paw edema volume in the carrageenan induced rat paw edema test

Group	Treatment	Decrease in carrageenan induced paw edema volume (mL)						
No.	o. Group	0h	1h	2h	3h	4h	9h	
1	Control	0.73±0.04	1.32±0.02	1.58±0.02	1.79±.03	1.69±0.02	1.58±0.01	
П	Standard	0.69±0.03	0.96±0.05 <sup>****</sup> (27.27%)	1.22±0.05 <sup>****</sup> (22.78%)	1.37±0.05 <sup>****</sup> (23.46%)	1.23±0.06 <sup>***</sup> (27.22%)	1.52±0.03 (3.8%)	
ш	Extract 250mg/kg	0.80±0.02	1.30±0.02 (1.52%)	1.50±0.02 (5.06%)	1.67±0.01 <sup>*</sup> (6.70%)	1.62±0.02 (4.14%)	1.55±0.02 (1.9%)	
IV	Extract 500mg/kg	0.68±0.02	1.20±0.02 <sup>*</sup> (9.09%)	1.23±0.02 <sup>***</sup> (22.15%)	1.31±0.02 <sup>***</sup> (26.82%)	1.24±0.02 <sup>***</sup> (26.63%)	1.52±0.16 (3.80%)	

Each value represents the mean $\pm$ SEM (n=5); \* p<0.05, \*\* p<0.01, \*\*\* p<0.001. Values in parentheses indicate percentage increase of latency time. Groups II, III and IV are compared with group I (control group).

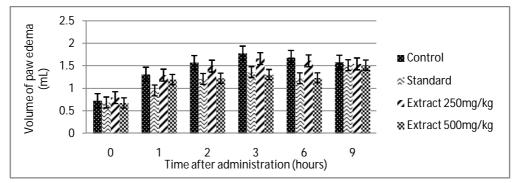


Figure 5: Comparative study of the effect of *B. acutangula* on paw edema volume in the carrageenan induced rat paw edema test



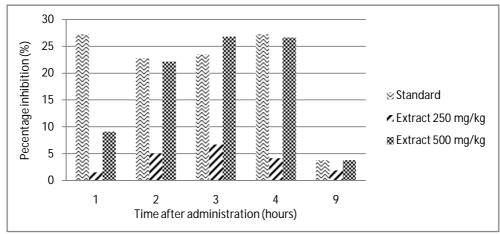
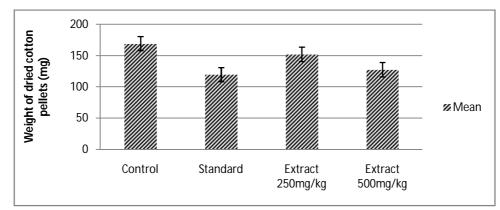


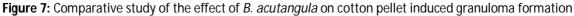
Figure 6: Comparative study of the effect of *B. acutangula* on the percentage inhibition of carrageenan induced rat paw edema

Table 5: The effect of *B. acutangula* on granuloma formation in the cotton granuloma test

Group No.	Treatment Group	Weight of dried Cotton Pellets (mg)	Percentage Inhibition
I	Control	169.40±1.33	
II	Standard	120.00±1.30 <sup>***</sup>	29.16
Ш	Extract 250mg/kg	152.40±2.11 <sup>**</sup>	10.04
IV	Extract 500mg/kg	127.80±4.87***	24.56

Each value represents the mean±SEM (n=5); \* p<0.05, \*\* p<0.01, \*\*\* p<0.001. Values in parentheses indicate percentage increase of latency time. Groups II, III and IV are compared with group I (control group).





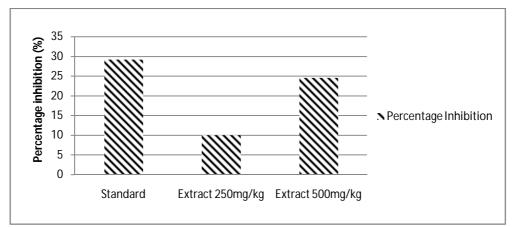


Figure 8: Comparative study of the effect of *B. acutangula* on the percentage inhibition of cotton pellet induced granuloma formation



#### Cotton pellet induced granuloma

The results of the cotton pellet induced granuloma experiment conducted in rats are displayed in Table 5. The mean dry weight of the sterile cotton pellets excised from the control group after the seven days were compared to that of the test animals. Ibuprofen induced the highest percentage reduction in weight of 29.16% (p<0.001), closely led by *B. acutangula* 500mg/kg with 24.56% (p< 0.001) followed by *B. acutangula* 250mg/kg with 10.04% (p<0.01). This clearly demonstrates a dose dependent pattern of anti-inflammatory activity.

## DISCUSSION

To investigate the therapeutic usefulness of *B. acutangula* in pain and inflammation the findings of the four analgesic and anti-inflammatory models provided valuable insight. As a visceral pain model, the pain experienced in the acetic acid writhing test is induced at the peripheral nociceptive neurons when the amount of endogenous mediators of pain, such as prostaglandin E2, prostaglandin  $E2\alpha$ , kinins, serotonin and histamine increases in the peritoneal fluids. The spike in prostaglandin levels then enhances the inflammatory pain by increasing capillary permeability in the peritoneal cavity. Drugs elicit peripherally mediated analgesia in this test preferably by inhibiting prostaglandin synthesis. Since B. acutangula produced significant dose dependent results at both doses, it might be acting along the same pathways of prostaglandin and inhibiting its secretion. The hot plate method, measures phasic, noninflammatory pain and thus modeling to selectively discern central nociceptive activity. Any drug which extends the latency time in the hot plate test must be acting centrally through the inhibition of central pain receptors. The paw-licking or jumping responses in hot plate are complex supraspinally organized behavior of mice. B. acutangula root extract was found to have significant dose-dependent suppression of centrally mediated pain at the opioid receptors.<sup>42-49</sup>

The most commonly used experimental animal model for studying acute inflammation is the Carrageenan-induced paw edema and can probably be depicted as biphasic. The model is marked by the early phase (1-2 h) mediated mainly by serotonin, histamine and increased prostaglandins synthesis in the damaged tissue region. This induces the edema and swelling as observed in the rat paws. This is followed by the late phase (2-4 h) which is sustained by prostaglandin release and mediated by mediators as leukotrienes, polymorphonuclear cells, bradykinin, lysosomes and prostaglandins produced by macrophages present in the tissue. Most antiinflammatory drugs therapeutically mount effective responses in the second phase. The release of the endogenous mediators peaks in the 3h and thus it can be extrapolated that since B. acutangula most effectively inhibited the carrageenan induced edema in the 3h of the experiment there is an inhibitory effect on the synthesis release and/or of the endogenous mediators (prostaglandin, lysosomes, bradykinins, leukotrienes) which induce the anti-edematous effect. NSAIDs elicit peripheral analgesic and anti-inflammatory response by inhibiting the prostaglandin synthesis. The same properties can be also possibly be attributed to our root extract which was substantiated by significant dose-dependent response in both the acetic acid writhing test and carrageenan induced paw edema model.<sup>50-53</sup>

Cotton pellet induced granuloma is an established chronic inflammation model which evaluated the components in the transudative, exudative and proliferative phases. The drug's efficacy to significantly inhibit the granulomatous tissue formation in the proliferative stage after the cotton pellet implantation correlates with the decrease in the dried weight of the pellets in comparison to the control group. Reduction of the granulomatous tissue indicates the drug's anti-proliferative effect. This may be due to the effectiveness of B. acutangula in decreasing the number fibroblasts and synthesis of collagen of and mucopolysaccharide, which are natural proliferative agents of the tissue formation in the granuloma model. Both doses inhibited inflammation albeit the higher dose drew a more prominent response. A highly significant percentage inhibition (24.56 %) of 500mg/kg of the root extract was evidential to confirm the efficacy of the root extract to inhibit chronic inflammation.<sup>54,55</sup>

Phytoconstituents with documented analgesic and antiinflammatory properties in the extract of many plants are possible evidence of the therapeutic claims being made of them. To illustrate, effective anti-inflammatory and analgesic activities are exhibited by tannins, saponins and flavonoids. Terpenoids are also known to cease prostaglandin synthesis by inhibiting phospholipase A2 and thus confer both analgesic and anti-inflammatory properties, while alkaloids inhibit the arachidonic acid metabolism and play role in anti-inflammation. The presence of these mentioned phytoconstituents in moderate to high concentration in the root extract of *B. acutangula* possibly confer the anti-nociceptive and antiinflammatory activities during the indicated use of the plant.<sup>56-67</sup>

#### CONCLUSION

The non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most widely used over the counter (OTC) medications today, however these drugs cause serious side effects like gastric ulcer and even hepatotoxicity. At present, an overwhelming fraction of the global plant population still remains unexplored, and it might be suggested that they hold the promise of the discovery of a safer and more effective new drug. In this experiment, our findings are in support of the traditional practices of *B. acutangula* in inflammation and joint pain. Thus, we conclude that the centrally and peripherally mediated anti-nociceptive and anti-inflammatory activities of the root of the plant can be possibly attributed to the pharmacological actions of the tannins, flavonoids, saponins, alkaloids and terpenoids. However extended



research studies are required to isolate and identify the specific compounds which confer such properties, and shed light on these phytochemicals' possible mechanisms of action.

#### REFERENCES

- 1 Bakhru HK, Herbs that Heal: Natural Remedies for Good Health, 1<sup>st</sup> Ed, Vision Books Ltd, Delhi, 1995, 17.
- 2 Kelly K, History of Medicine, New York: Facts on file, New York, 2009, 29–50
- 3 Petrovska BB, Historical review of medicinal plants' usage, Pharmacogn. Rev., 6(11), 2012, 1–5
- 4 European Pharmacopoeia, 6<sup>th</sup> Ed, Council of Europe, Strasburg, 2008.
- 5 United States Pharmacopoeia, 31<sup>st</sup> Ed, The United States Pharmacopoeial Convention, Washington, 2008.
- 6 British Pharmacopoeia, British Pharmacopoeial Commission, London, 2007.
- 7 Blumenthal M, The Complete German Commission E Monographs, Special Expert Committee of the German Federal Institute for Drugs and Medical Devices, Austin, 1998.
- 8 WHO Monographs on Selected Medicinal Plants, Volume 1, World Health Organization Geneva, 1999, 1.
- 9 Agunu A, Yusuf S, Andrew GO, Zezi AU, Abdurahman EM, Evaluation of five medicinal plants used in diarrhoea treatment in Nigeria, J Ethnopharmacol, 101, 2005, 27–30.
- 10 Kapoor LD, Handbook of Ayuvedic Medicinal Plants, CRC Press, Boca Raton, Florida, USA, 1990.
- 11 Jain SK, Dictionary of Indian Folk Medicine and Ethnobotany, National Botanical Research Institute, Lucknow, 1991, 33.
- 12 Padmavathi D, Susheela L, Bharathi RV, Pharmacognostical evaluation of *Barringtonia acutangula* leaf, International Journal of Ayurveda Research, 2(1), 2011, 37-41.
- 13 Satapathy KB, Brahmam M, Ethnobotanical survey on tribal area plant Barringtonia acutangula, Fourth Int. Cong. Ethnobiol., Lucknow, 1994.
- 14 Sahoo S, Panda PK, Mishra SR, Parida RK, Ellaiah P, Dash SK, Antibacterial activity of Barringtonia acutangula against selected urinary tract pathogens, Indian J Pharm Sci., 70, 2008, 677–679.
- 15 Bharathi RV, Suresh AJ, Thirumal M, Sriram L, Lakshmi SG, Kumudhaveni B, Antibacterial and antifungal screening on various leaf extracts of *Barringtonia acutangula*, International Journal of Research in Pharmaceutical Sciences, 1(4), 2010, 407-410.
- 16 Rahman, MM, Polfreman D, MacGeachan J, Gray A, Antimicrobial activities of *Barringtonia acutangula*, Phytother. Res., 19, 2005, 543-545.
- 17 Lakshmi PJ, Selvi KV, Anticancer potentials of secondary metabolites from endophytes of *Barringtonia acutangula* and its molecular characterization, Int. J. Curr. Microbiol. App. Sci., 2(2), 2013, 44-45.
- 18 Padmavathi D, Antidiarrheal activity of ethanolic extract of leaves of *Barringtonia acutangula* (L.), Gaertn- Inventi Impact: Ethnopharmacology, 1(3), 2011, 177-178.
- 19 Rahman A, Sikder MA, Kaisar A, Rahman S, Hasan CM, Rashid MA, In vitro antioxidant activity of *Barringtonia Acutangula* (L), Bangladesh Pharmaceutical Journal, 13(1), 2010, 38-41.
- 20 Rashmi K, Shenoy K, Bhasker, Hegdec, Karunakar, Shabarayac AR, Hepatoprotective effect of *Barringtonia acutangula* (L.) Gaertn leaf extracts against CCl4 induced hepatic damage, Journal of Pharmacy Research, 4, 2011, 540.

- 21 Khatib NA, Patil PA, Evaluation *of* hypoglycemic activity *of* Barringtonia acutangula fruit extracts in streptozotocin induced hyperglycemic Wistar rats, Journal of Cell and Tissue Research, 11(1), 2011 2573-2578.
- 22 Babre NP, Debnath S, Manjunath SY, Reddy VM, Murlidharan P, Manoj G, Antidiabetic effect of hydroalcoholic extract of *Barringtonia acutangula* Linn. root on streptozotocin-induced diabetic rats, International Journal of Pharmaceutical Sciences and Nanotechnology, 3(3), 2010, 1158-1164.
- 23 Babre NP, Debnath S, Manjunath YS, Deshmaukh G, Hariprasath K, Sharon K, Hypolipidemic effect of hydro-alcoholic extract of *Barringtonia acutangula* Linn root extract on streptozotocin induced diabetic rats, J. Pharm. Sci. Tech., 2, 2010a, 368–371.
- 24 Babre NP, Debnath S, Manjunath YS, Parameshwar P, Wankhede SV, Hariprasath K, Antioxidant potential of hydroalcoholic extract of *Barringtonia acutangula* Linn roots on streptozotocin induced diabetic rats, Int. J. Pharm. Pharm. Sci., 4, 2010b, 201–203.
- 25 Uawonggul N, Chaveerach A, Thammasirirak S, Arkaravichien T, Chuachan C, Daduang S, Screening of plants acting against Heterometruslaoticus scorpion venom activity on fibroblast cell lysis, J. Ethnopharmacol., 103, 2006, 201–207.
- 26 Imam MZ, Sultana S, Akter S, Antinociceptive, antidiarrheal, and neuropharmacological activities of *Barringtonia acutangula*, Pharmaceutical Biology, 50(9), 2012, 1078–1084.
- 27 Selvan VT, Manikandan L, Kumar KGP, Suresh R, Kumar DA, Mazumdar UK, Antidiabetic and antioxidant effect of methanol extract of *Artanema sesamoides* in streptozotocin induced diabetic rats, International journal of applied research in natural products, 1(1), 2008, 25-33.
- 28 Harborne JB, Phytochemical Methods: A guide to modern techniques of plant analysis, 3<sup>rd</sup> Ed, Chapman and Hall, London, 1998, 302.
- 29 Siddiqui S, Verma A, Rather AA, Jabeen F, Meghvansi MK, Preliminary phytochemicals analysis of some important medicinal and aromatic plants, Adv. Biol. Res., 3, 2009, 188-195.
- 30 Parmer NS, Prakash S, Screening methods in Pharmacology, 1st Ed, Narosa Publishing House Pvt. Ltd, New Delhi, 2011.
- 31 Koester K, Anderson M, Beer EJ, Acetic acid for analgesic screening, Federal Proceedings, 18, 1959, 412.
- 32 Williamson EM, Okpako DT, Evans FJ, Pharmacological methods in Phytotherapy Research in Selection, Preparation and Pharmacological Evaluation of Plant Materials, Vol 1, John Wiley & Sons, Chichester, 1996, 184–186
- 33 Chou SC, Chiu YJ, Chen CJ, Lin YC, Wu CH, Chao CT, Chang CW, Peng WH, Analgesic and anti-inflammatory activities of the ethanolic extract of *Artemisia morrisonensis* Hayata in mice, Evid. Based Complement. Alternat. Med., Vol 2012, 2012, 1-11.
- 34 Kulkarni SK, Hand Book of Experimental Pharmacology, 3rd Ed, Vallabh Prakashan, New Delhi, 2007.
- 35 Agrahari AK, Khaliquzzama M, Panda SK, Evaluation of analgesic activity of methanolic extract of *Trapa natans* I.var. *Bispinosa* roxb. Roots, Journal of Current Pharmaceutical Research, 1, 2010, 8-11.
- 36 Turner RA, Analgesics: Screening methods in pharmacology, Academic Press, New York, 1965, 100.
- 37 Eddy NB, Leimbach D, Synthetic analgesics II. Dithienylbutenyl- and dithienylbutylamines, J. Pharmacol. Exp. Ther., 107, 1953, 385– 393.
- 38 Winter CA, Risley EA, Nuss GW, Carrageenan induced edema in hind paw of rat as an assay for anti-inflammatory drugs. Proceedings of the Society for Experimental Biology and Medicine, 111, 1962, 544–546.



- 39 Ferreira SH, A new method for measuring the variations of rat paw volume, Journal of Pharmaceutical and Pharmacology, 31, 1979, 648.
- 40 D'Arcy PF, Howard EM, Muggleton PW, Townsend SB, The antiinflammatory action of griseofulvin in experimental animals, J. Pharm. Pharmacol., 12, 1960, 659-665.
- 41 Sahaa S, Subrahmanyamb EVS, Chandrashekarc KS, Shastry SC, In vivo study for anti-inflammatory activity of *Bauhinia variegata* L. Leaves, Pharmaceutical Crops, 2, 2011, 70-73.
- 42 Deraedt R, Jouquey S, Delevallée F, Flahaut M, Release of prostaglandins E and F in an algogenic reaction and its inhibition, Eur. J. Pharmacol., 61, 1980, 17–24.
- 43 Bentley GA, Newton SH, Starr J, Studies on the anti-nociceptive action of alpha-agonist drugs and their interactions with opioid mechanisms, Br. J. Pharmacol., 79, 1983, 125–134.
- 44 daSilveira e Sa´ RA, de Oliveira LEG, Nobrega FF, Bhattacharyya J, de Almeida RN, Antinociceptive and toxicological effects of *Diocleagrandiflora* seed pod in mice, Journal of Biomedicine and Biotechnology, Volume-2010, 2010, 1-6.
- 45 Zakaria ZA, Ghani ZDFA, Nor RNSRM, Gopalan HK, Sulaiman MR, Jais AMM, Somchit MN, Kader AA, Ripin J, Antinociceptive, antiinflammatory, and antipyretic properties of an aqueous extract of *Dicranopteris linearis* Leaves in experimental animal models, J. Nat. Med., 62, 2008, 179-187.
- 46 Bars DL, Gozariu M, Cadden SW, Animal models of nociception, Pharmacol. Rev., 53, 2001, 597–652.
- 47 Sabina E, Chandel S, Rasool MK, Evaluation of analgesic, antipyretic and ulcerogenic effect of *Withaferin A.*, Int. J. Integ. Biol., 6(2), 2009, 52-56.
- 48 Ibironke GF, Ajiboye KI, Studies on the anti–inflammatory and analgesic properties of *Chenopodium ambrosioides*e Leaf extract in rats, Int. J. Pharmacol., 3, 2007, 111-115.
- 49 Chapman CR, Casey KL, Dubner R, Foley KM, Gracely RH, Reading AE, Pain measurement: An overview, Pain, 22, 1985, 1–31.
- 50 EI-Shenawy SM, Abdel-Salam OME, Baiuomy AR, EI-Batran S, Arbid MS, Studies on the anti-inflammatory and antinociceptive effects of Melatonin in the rat, Pharmacol. Res., 46, 2002, 235-243.
- 51 Gupta M, Mazumder UK, Gomathi P, Selvan VT, Anti-inflammatory evaluation of leaves of *Plumeria acuminata*, BMC Complementary and Alternative Medicine, 6(36), 2006.
- 52 Antonio MA, Brito ARMS, Oral anti-inflammatory and antiulcerogenic activities of a hydroalcoholic extract and partitioned fractions of *Turnera ulmifolia* (Turneraceae), Journal of Ethnopharmacology, 61 (3), 1998, 215–228.
- 53 Vinegar R, Schreiber W, Hugo R, Biphasic development of carrageenan edema in rats, J. Pharmacol. Exp. Ther., 166(1), 1969, 96–103.
- 54 Swingle KF, Shideman FE, Phases of the inflammatory response to subcutaneous implantation of a cotton pellet and their

modification by certain anti-inflammatory agents, J. Pharmacol. Exp. Ther., 183 (1), 1972, 226–234.

- 55 Arrigoni-Martellie E, Inflammation and Anti-inflammatory, New York: Spectrum Publications Inc., New York, 1977, 119-120.
- 56 Fawole OA, Amoo SO, Ndhlala AR, Light ME, Finnie JF, Staden JV, Anti-inflammatory, anticholinesterase, antioxidant and phytochemical properties of medicinal plants used for pain-related ailments in South Africa, J. Ethnopharm., 127, 2010, 235-241.
- 57 Ramprasath VR, Shanthi P, Sachdanandam P, Immunomodulatory and anti-inflammatory effects of *Semecarpus anacardium* Linn. nut milk extract in experimental inflammatory conditions, Biol. Pharm. Bull., 29, 2006, 693-700.
- 58 Rao MR, Rao YM, Rao AV, Prabhkar MC, Rao CS, Muralidhar N, Anti-nociceptive and anti-inflammatory activity of a flavonoid isolated from *Caralluma attenuata*, J. Ethnopharmacol.,62, 1998, 63-66.
- 59 Kim HP, Son KH, Chang HW, Kang SS, Anti-inflammatory plant flavonoids and cellular action mechanisms, J. Phamacol. Sci., 96, 2004, 229-245.
- 60 Küpeli E, Yesilada E, Flavonoids with anti-inflammatory and antinociceptive activity from *Cistus laurifolius* L. Leaves through bioassay-guided procedures, J. Ethnopharm., 112, 2007, 524-530.
- 61 Barar FSK, Essentials of Pharmacology, 3<sup>rd</sup> Ed, S. Chad and Company, New Delhi, 2000, 1171-3137.
- 62 Koganov, MM, Dues OV, Tsorin BL, Activities of plant-derived phenols in a fibroblasts cell culture model, J. Natural Products, 62, 1998, 481-3.
- 63 Calixto JB, Beirith A, Ferreira J, Santos ARS, Filho VC, Yunes RA, Naturally occurring anti-nociceptive substances from plants, Phytother. Res., 14, 2000, 401-418.
- 64 Neukirch H, D'Ambrosio M, Sosa S, Altinier G, Loggia RD, Guerriero A, Improved anti-inflammatory activity of three new terpenoids derived, by systematic chemical modifications, from the abundant triterpenes of the flowery plant *Calendula officinalis*, Chem. Biodiv., 2(5), 2005, 657-671.
- 65 Moody JO, Robert VA, Connolly JD, Houghton PJ, Antiinflammatory activities of the methanol extracts and an isolated furanoditerpene constituent of *Sphenocentrum jollyanum* Pierre (Menispermaceae), J. Ethnopharm, 104, 2006, 87-91.
- 66 Barik BR, Bhowmik T, Dey AK, Patra A, Chatterjee A, Joy S, Susan T, Alam M, Kundu AB, Premnazole and isoxazole alkaloid of *Prema integrifolia*and *Gmelina arborea* with anti-inflammatory activity, Fitoterapia, 53, 1992, 295-299.
- 67 Chao J, Lu TC, Liao JW, Huang TH, Lee MS, Cheng HY, Ho LK, Kuo CL, Peng WH, Analgesic and anti-inflammatory activities of ethanol root extract of *Mahonia oiwakensis* in mice, J. Ethnopharm., 125, 2009, 297-303.

#### Source of Support: Nil, Conflict of Interest: None.

