Bangladesh Pharmaceutical Journal ***19****(2): 185-189, 2016*

In vitro Membrane Stabilizing and *In vivo* Analgesic Activities of *Boehmeria glomerulifera* Miq. in Swiss-Albino Mice Model

Sabikunnahar Papia[1], Muhammed Mahfuzur Rahman[1], Md. Mustafezur Rahman[2], Mohaiminul Adib[3] and Mohammad Firoz Khan[1]

[1]Department of Pharmacy, State University of Bangladesh, Dhaka- 1205, Bangladesh
[2]Department of Pharmacy, Daffodil International University, Dhaka, Bangladesh
[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

Received: June 01, 2016; Accepted: July 12, 2016; Published (Web): July 31, 2016

Abstract

Boehmeria glomerulifera Miq. is an indigenous plant of Bangladesh. Traditional healers use this plant to treat various diseases. The crude methanol extract of whole plant of *B. glomerulifera* and its Kupchan fractions were screened for *in vitro* membrane stabilizing and only the crude extract at doses of 200- and 400- mg/kg body weight were screened for analgesic activity in mice model in order to establish the medicinal values of this plant. The membrane stabilizing activity of the extractives was evaluated by the ability of test materials to inhibit hypotonic solution- and heat-induced haemolysis of human erythrocytes. Moreover, the analgesic activity of methanol extract was evaluated by acetic acid induced writhing method and tail immersion method in mice. In hypotonic solution- and heat-induced conditions, the crude methanol extract showed inhibition of haemolysis by 64.80±0.34% and 21.63±0.76%, respectively as compared to 77.74±0.67% and 40.41±0.69% demonstrated by the standard, acetyl salicylic acid. On the other hand, the analgesic activity was determined for its peripheral and central pharmacological responses using acetic acid-induced writhing test and tail immersion method at doses of 200- and 400- mg/kg body weight. The extract reduced the acetic acid-induced writhings significantly ($p < 0.05$) in a dose dependent manner with the highest activity observed at 400 mg/kg (46.0%) body weight when compared to that of Diclofenac sodium (64.0%), as the standard drug. A significant increase ($p < 0.01$) of latency period was also found in tail immersion method. Therefore, the present study established that the methanol extract of *B. glomerulifera* possesses moderate membrane stabilizing and significant analgesic activities.

Key words: *Boehmeria glomerulifera*, membrane stabilizing, anti-inflammatory, writhing, analgesic.

Introduction

Folk medicines are used in Bangladesh from the early days of human civilization. Traditional medicines are playing significant roles to the national health sector of Bangladesh in parallel to the modern medicines. The plants are the important sources for screening out new drug candidates. As Bangladesh is a country with numerous plants, adequate scientific investigations are required to discover the potential of these plants for treating various diseases (Banglapedia, 2012; Ashraf *et al*., 2014; Faruk *et al*., 2015).

Pain is a part of defensive response against dysfunction of an organ. Pain management has drawn the focus of global scientific research because of its implication in all human and animal diseases (Sarker *et al*., 2012). Although a large number of drugs are used to alleviate pain, most of them are not devoid of side-effects (Devraj and Karpagam, 2011). In order to provide patient's safety and comfort regarding pain, plants might serve important sources of medicinal agents. As a result, it is a rational strategy to search for new analgesic drugs from traditionally used plant species which are used as painkillers.

The plant *Boehmeria glomerulifera* Miq. is a deciduous shrub or small trees (Jiarui *et al*., 2003) with spreading branches, belongs to the family Urticaceae. The plant is widely distributed in Bangladesh, Bhutan, India, Indonesia, Laos, Myanmar, Sikkim, Sri Lanka, Thailand, and Vietnam. Traditionally, the fresh leaves of this plant in combination of *Amomum aromaticum*

Correspondence to: Mohammad Firoz Khan; E-mail: firoz@sub.edu.bd

Roxb. (Zingiberaceae) is used to treat anaemia (Rahman *et al*., 2006). The bath with boiled leaves-water is prescribed in case of fever of babies (Medicinal Plants of Bangladesh).

As part our ongoing research program (Khan *et al.*, 2014, 2015) the present study has been undertaken and we, herein, report the membrane stabilizing and analgesic activities of the whole plant of *B. glomerulifera* for the first time.

Materials and Methods

Plant materials: The leaves of *Boehmeria glomerulifera* were collected in June, 2014 from Chittagong, Bangladesh and a voucher specimen (DACB accession no. 39726) has been deposited in Bangladesh National Herbarium, Mirpur, Dhaka for future reference.

Extraction and fractionation: The aerial parts of *B. glomerulifera* was collected and sun dried for several days and then oven dried for 24 hours at 40 °C to facilitate grinding. The powdered material (600 gm) was extracted with about 2.0 L methanol for 7 days at room temperature. Then it was filtered through a cotton plug followed by Whatman filter paper number 1. The filtered extract was then concentrated by using a rotary evaporator at reduced temperature (40-45 °C) and pressure. Later, the concentrated methanol extract (ME) was fractionated by the modified Kupchan partitioning protocol (Van Wagenen *et al.*, 1993) and the resultant partitionates i.e., methanol extract (ME), petroleum ether (PE), carbon tetrachloride (CT) and chloroform (CL) soluble materials were used for the pharmacological screenings.

Animals: Swiss albino mice (23-25 g) of both sexes bred in the animal house of Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh were utilized for the studies. All the animals were acclimatized to the laboratory condition. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0 ± 20 °C, and 12 h light dark cycle) and nourished with standard diet (ICDDRB, B formulated) and had free access to tap water but were fasted 12 h prior to each test. One week prior to the experiments all protocols for animal experiment were approved by the institutional animal ethics committee.

Drugs: Drugs and chemicals used in this study include acetic acid solution (1%), acetylsalicylic acid, Diclofenac sodium (Square Pharmaceuticals Ltd., Bangladesh) and Tramadol (Beximco Pharmaceuticals Ltd., Bangladesh).

Membrane stabilizing activity: The ability of the extractives on the stabilization of erythrocytes membrane was evaluated by following the method described by Omale *et al*. (2008) and Khan *et al*. (2014).

Acetic acid-induced writhing: The peripheral analgesic activity of the samples was assesed in mice using acetic acid-induced writhing method (Amabeoku and Kabatende, 2012; Chen *et al*., 2012; Faruk *et al*., 2015). Mice were divided into 4 groups of 4 mice each. The normal saline solution mixed with 1% Tween 80 (10 ml/kg body weight) was administered to the negative control group, diclofenac sodium (50 mg/kg body weight) was given to the positive control group and the crude extract at 200- and 400- mg/kg body weight were administered to the mice of experimental groups. Forty minutes later each mouse was injected with 1% acetic acid at 10 ml/kg body weight. For each animal, the number of writhing responses was recorded during the subsequent 5 min period after 10 min of intraperitoneal administration of acetic acid and the mean writhings for each group was obtained. Then the percentage inhibition was calculated using the formula –

$$\%\text{Inhibition} = \frac{\text{mean number of writhing (negative control)} - \text{mean number of writhing}\left(\text{positive}\frac{\text{control}}{\text{test}}\right)}{\text{mean number of writhing (negative control)}} \times 100$$

Tail immersion test: The tail immersion method is an alternative method to evaluate central analgesic activity (Aydin *et al*., 1999; Kaushik *et al*., 2012; Faruk *et al*., 2015). Again the mice were equally divided into 4 groups, each group containing 4 mice. Then normal saline solution with 1% Tween 80 (10 ml/kg body

weight) was administered to the negative control group and Tramadol (10 mg/kg body weight) was given to the positive control group whereas the crude extract at 200- and 400- mg/kg body weight were administered to experimental groups. The lower portion (5 cm) of the tail was marked and inserted in warm water (55 ± 2 °C). Within a few seconds the mice were seen to react by withdrawing the tail and the reaction time was recorded by a stopwatch. The experimental reaction time was recorded at a regular interval like 0, 30, 60 and 90 minutes after the oral administration of test materials. The cut-off time for tail immersion latency was set at 15 seconds. The reaction time was also determined before the administration of any substances.

Statistical analysis: The values are expressed as mean ± standard error of mean (SEM) and one way ANOVA analysis was conducted to determine the significance in differences between the control and experimental groups, the p values < 0.05 were considered to be statistically significant.

Results

The methanol extract of *B. glomerulifera* as well as the Kupchan partitionates derived from it were subjected to assay for membrane stabilizing activity while the methanolic crude extract was assessed for analgesic activity.

The different partitionate fractions of *B. glomerulifera* protected the haemolysis of RBC induced by hypotonic solution and heat which were comparable to the standard acetyl salicylic acid. The crude methanol extract inhibited 64.80 ± 0.34% and 21.63 ± 0.76% of haemolysis induced by hypotonic solution and heat as compared to 77.74 ± 0.67% and 40.41 ± 0.69% by acetyl salicylic acid, respectively (Table 1).

Table 1. Percentage (%) inhibition of heat- and hypotonic solution- induced haemolysis of erythrocyte membrane by standard and *B. glomerulifera*.

Samples	% Inhibition of haemolysis	
	Heat induced	Hypotonic solution induced
Hypotonic medium (Negative control)	-	-
ME	21.63 ± 0.76	64.80 ± 0.34
PE	18.57 ± 0.83	57.24 ± 0.88
CT	20.04 ± 0.62	59.57 ± 0.61
CL	26.17 ± 0.48	54.30 ± 0.71
ASA (Positive control)	40.41 ± 0.69	77.74 ± 0.67

ME= Methanol extract, PE= Petroleum ether soluble fraction, CT= Carbon tetrachloride soluble fraction, CL= Chloroform soluble fraction, ASA= Acetyl salicylic acid.

In acetic acid induced writhing test, the methanol extract significantly attenuated the number of writhing movements induced by the intraperitoneal administration of acetic acid solution. The dose-dependent inhibition (Table 2) of writhing response by the methanol extract indicates peripheral analgesic potential of the plant.

The extract of *B. glomerulifera* when administered orally at 200- and 400- mg/kg body weight exhibited significant analgesic activity in tail immersion method as supported by the increase in latency time when compared to control. The increase in latency period was found to be dose dependant. However, maximum effect was seen at 400 mg/kg body weight and was comparable with the standard drug (Table 3).

Table 2. Effect of methanol extract of *B. glomerulifera* on acetic acid-induced writhing in mice.

Group	Doses	Number of writhing	% Inhibition
1% Tween 80 in normal saline (Negative control)	10 ml/kg body weight	12.5 ± 1.26	--
Diclofenac (Positive control)	50 mg/kg body weight	4.5 ± 0.29	64.0**
Whole plant extract of *B. glomerulifera*	200 mg/kg body weight	8.0 ± 0.41	36.0*
	400 mg/kg body weight	6.8 ± 1.11	46.0*

Number of writhing values are (mean ± SEM.); n=4, * $p < 0.05$, ** $p < 0.01$, significant compared to control.

Table 3. Analgesic activity of *B. glomerulifera* in tail immersion test.

Groups	Dose	Reaction time in seconds at time (min)			
		0	30	60	90
1% Tween 80 in normal saline (Negative control)	20 ml/kg body weight	1.93 ± 0.07	2.20 ± 0.15	3.03 ± 0.37	2.78 ± 0.20
Tramadol (Positive control)	10 mg/kg body weight	2.26 ± 0.17	3.56 ± 0.38	6.33 ± 0.40**	6.88 ± 0.54**
Whole plant extract of *B. glomerulifera*	200 mg/kg body weight	2.29 ± 0.08	4.08 ± 0.32*	5.51 ± 0.33**	7.97 ± 0.57**
	400 mg/kg body weight	2.01 ± 0.05	3.71 ± 0.13*	4.83 ± 0.05**	6.53 ± 0.55**

All values are expressed as mean ± SEM; n = 4, * $p < 0.05$, ** $p < 0.01$, significant compared to control

Discussion

The present study was conducted to investigate the membrane stabilizing and analgesic activities of the whole plant extract of *B. glomerulifera*. The erythrocyte membrane resembles to lysosomal membrane and the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane (Omale *et al*., 2008). On the other hand, substances that stabilize the membrane will interfere the release and or action of mediators like histamine, serotonin, prostaglandins, leukotrienes etc (Shinde *et al*., 1999). As different partitionates of *B. glomerulifera* demonstrated mild to moderate membrane stabilizing activity so we can conclude that the plant extract may have anti-inflammatory activity.

Acetic acid-induced abdominal constriction test is used for the evaluation of peripheral analgesic activity (Gene *et al*., 1998; Faruk *et al* 2015). The test material demonstrated significant analgesic activity in acetic acid-induced writhing test in mice. As prostaglandin is known for the production of pain in peripheral origin, so it can be assumed that this plant extract might inhibit the pain in one of the two following pathways, i.e. the biosynthesis of prostaglandin or the binding of prostanoids to their receptors. The extract might also be responsible for inhibiting the production of neuronal mediators (Duarte *et al*., 1988; Faruk *et al*., 2015).

In the skin, the thermal pain is generated due to the presence of delta and C fibres sensory neurons. Besides, ion channels present in the skin are also responsible to response to temperature. So, the tested plant materials might have the ability to modulate the action potential and signal transmission to counteract the pain produced by heat (Harris and Ryall, 1988).

Conclusion

We can conclude that the methanol extract of aerial parts of *B. glomerulifera* demonstrated moderate membrane stabilizing and remarkable dose dependent analgesic activities. However, the bioactive compounds which are responsible for these pharmacological activities need to be isolated. also further studies are required to understand the mechanism of such inflammatory and analgesic effects of these compounds.

References

Amabeoku, G.J. and Kabatende, J. 2012. Antinociceptive and anti-inflammatory activities of leaf methanol extract of *Cotyledon orbiculata* L. (Crassulaceae). *Adv. Pharmacol. Sci.* 1-6.

Ashraf, M.A., Khatun, A., Sharmin, T., Mobin, F., Tanu, A.R., Morshed, T., Fakir, T.A., Begum, R.A. and Nabi, A.N. 2014. MPDB 1.0: a medicinal plant database of Bangladesh. *Bioinformation*. **10**, 384-386.

Aydin, S., Demir, T., Ozturk, Y. and Baser, K.H. 1999. Analgesic activity of *Nepeta italica* L. *Phytother. Res.* **13**, 20-23.

Banglapedia, 2012. Healthcare System. National Encyclopedia of Bangladesh (online edition); http:// www.banglapedia.org/HT/H_0132.htm; Access Date: 26 May 2016.

Chen, J., Wang, X., Qu, Y.G., Chen, Z.P., Cai, H., Liu, X., Xu, F., Lu, T.L. and Cai, B.C. 2012. Analgesic and anti-inflammatory activity and pharmacokinetics of alkaloids from seeds of *Strychnos nux-vomica* after transdermal administration: effect of changes in alkaloid composition. *J. Ethnopharmacol*. **139**, 181-188.

Devraj, A. and Karpagam, T. 2011. Evaluation of anti-inflammatory activity and analgesic effect of *Aloe vera* leaf extract in rats. *Int. Res. J. Pharm*. **2**, 103-110.

Duarte, I.D., Nakamura, M. and Ferreira, S.H. 1988. Participation of the sympathetic system in acetic acid-induced writhing in mice. *Braz. J. Med. Biol. Res*. **21**, 341-343.

Faruk, M.A., Khan, M.F., Mian, M.Y., Rahman, M.S. and Rashid, M.A. 2015. Analgesic and anti-diarrheal activities of *Aganosma dichotoma* (Roth) K. Schum. in Swiss-Albino mice model. *Bangladesh Pharm. J.* **18**, 15-19.

Gene, R.M., Segura, L., Adzet, T., Marin, E. and Inglesias, J. 1998. Heterotheca inuloides: anti-inflammatory and analgesic effects. *J. Ethnopharmacol*. **60**, 157-162.

Harris, N.C. and Ryall, R.W. 1988. Mustard oil excites but does not inhibit nociceptive dorsal horn neurones in the rat: a presumed effect on A-delta fibres. *Br. J. Pharmacol*. **94**, 180-184.

Jiarui, C. and Friis, I.C. 2003. Wilmot-Dear M. Boehmeria. *Flora of China*. **5,** 164-174.

Kaushik, D., Kumar, A., Kaushik, P. and Rana, A.C. 2012. Analgesic and anti-inflammatory activity of *Pinus roxburghii* Sarg. *Adv. Pharmacol. Sci*. 1-6.

Khan, M.F., Rabbi, S.N.I., Hossain, M.A. and Rashid, M.A. 2014. Membrane Stabilizing and Thrombolytic Activities of *Sida rhombifolia* L. *Bangladesh Pharm. J*. **17**, 43-45.

Khan, .M.F, Khan, Z.I., Uddin, M.R., Rahman, M.S. and Rashid, M.A. 2015 In vivo hypoglycemic and alloxan induced antidiabetic activity of *Xeromphis uliginosa* Retz. *Afr. J. Pharm. Pharmacol*. **9**, 363-366.

Medicinal plants of Bangladesh. http://www.mpbd.info/ plants/boehmeria-malabarica.php. Date of access: 26.08.2015.

Omale, J. and Okafor, P.N. 2008. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata*. *Afr. J. Biotechnol.* **7**, 3129-3133.

Rahman, M.A., Uddin, S.B. and Wilcock, C.C. 2007. Medicinal plants used by Chakma tribe in hill tracts districts of Bangladesh. *Ind. J. Traditional Knowledge*. **6**, 508-517.

Sarker, M., Das, S.C., Saha, S.K., Mahmud, Z.A. and Bachar, S.C. 2012. Analgesic and anti-inflammatory activities of flower extracts of *Punica granatum* Linn. (Punicaceae). *J. Appl. Pharma. Sci.* **2**, 133-136.

Shinde, U.A., Phadke, A.S., Nair, A.M., Mungantiwar, A.A., Dikshit, V.J. and Saraf, M.N. 1999. Membrane stabilizing activity - a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. *Fitoterapia* **70**, 251-257.

Van Wagenen, B.C., Larsen, R., Cardellina, J.H. II, Ran dazzo, D., Lidert, Z.C. and Swithenbank, C. 1993. Ulosantoin, a potent insecticide from the sponge *Ulosa ruetzleri*. *J. Org. Chem*. **58**, 335-337.