Australian Journal of Basic and Applied Sciences, 5(8): 729-733, 2011
ISSN 1991-8178

Analgesic and Anti-inflammatory Potential of Methanolic Extract of *Glinus oppositifolius* L.

[1]Nazia Hoque, [1]Md. Razibul Habib, [2]Mohammad Zafar Imam, [3]Jamiuddin Ahmed and [4]Md. Sohel Rana

[1]Department of Pharmacy, East West University, Dhaka, Bangladesh.
[2]Department of Pharmacy, Stamford University Bangladesh, Bangladesh.
[3]Department of Pharmacy, Noakhali Science and Technology University, Bangladesh.
[4]Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh.

Abstract: *Glinus oppositifolius* (Molluginaceae) is found in different parts of Bangladesh and has been traditionally used in joint pain, inflammation, diarrhea, fever, boils and skin disorders. The present study was undertaken to validate the folkloric use of this plant in painful and inflammatory conditions. The analgesic activity was tested using acetic acid induced writhing and tail immersion test while the anti-inflammatory activity by Carrageenan induced paw edema test. Significant peripheral and central analgesic effect was shown by the extract at both 200 ($p<0.05$) and 400 mg/kg ($p<0.001$) doses. The extract (500 mg/kg) also reduced the paw inflammation of mice ($p<0.001$) induced by carrageenan. These results suggest that the methanolic extract of *G. oppositifolius* possess central and peripheral analgesic and anti-inflammatory activity and the folkloric use of this plant in pain and inflammation is scientifically valid.

Key words: Medicinal plants, Writhing, Tail immersion, pain, inflammation.

INTRODUCTION

Traditional medicinal practice, both ancient and modern, has been considered as the key treatment options for the treatment of various human ailments over the centuries (Arora and Kaur, 2007). Medicines from plants origin comprise the major constituents of most indigenous medicines and a large number of modern medicinal preparations contain one or more ingredients of plant origin (Bannerman, 1982).The use of commonly prescribed analgesic drugs exemplified as opiates and NSAIDs are criticized due to their adverse effects, more over they are not useful in all cases. That's why new compounds with improved pain management potential with minimum side effects are being sought with urgency.

Inflammatory diseases are considered one of the world's major health problems (Bohlin, 1995; Yesillada *et al*., 1997). Inflammation is autoimmune disorder that involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair (Vane and Bolting, 1995; Perianayagam *et al*., 2006). Now a day's inflammation has become the focus of global scientific research because of its implication in virtually all human and animal diseases.

Glinus oppositifolius (Family Molluginaceae) which is locally known as 'Gima shak' in Bangladesh and has been reported in favor of various traditional uses (Burkhill, 1985). Dried stems with leaves of *G. oppositifolius* are used for treating abdominal pain and jaundice, while decoction of fine powder of the aerial parts is used in the treatment of malaria (Diallo *et al*., 1999). *G. oppositifolius* is also reported to possess wound healing remedy (Debes, 1998) and used as folk medicine by traditional healers for treating joint pain, inflammation, diarrhea, intestinal parasites, fever, boils and skin disorders (Diallo, 2000). A bioactive pectic polysaccharide had been isolated from *G. oppositifolius* which has immunomodulating property (Inngerdingen, 2005). As the plant is traditionally used in the treatment of inflammation and various pains, an attempt has been taken to evaluate the anti-inflammatory and analgesic activity of this plant as there is no scientific and methodical investigation so far been documented in literature concerning its analgesic and anti-inflammatory properties.

Corresponding Author: Md. Razibul Habib, Lecturer, Dept. of Pharmacy, East West University, Dhaka, Bangladesh. Contact No.: +880 1718 423 453, E-mail: mrhjewel@gmail.com

MATERIALS AND METHOD

Collection and Identification:

The whole plants were collected from a village near Aricha, Savar Dhaka, Bangladesh and identified by the taxonomist of the National Herbarium of Bangladesh, Mirpur, Dhaka. A voucher specimen of the plant has been deposited (Accession No.: 32528) in the herbarium for further reference.

Preparation of Plant Materials:

The plants were dried under shed and then in oven at reduced temperature (<50^0C) to make suitable for grinding purpose. The coarse powders were then stored in air-tight container with necessary markings for identification and kept in cool, dark and dry place. The powdered plant materials were extracted using methanol: water (7:3, v/v) by a Soxhlet apparatus at 50°C. The solvent was completely removed and obtained dried crude extract which was used for investigation.

Collection and Maintenance of the Experimental Animals:

The experiments carried out on Swiss albino mice (3-4 weeks old, weighing 20-25 g) for analgesic test and albino rats (2-3 months old, weighing 130-170 g) for anti-inflammatory study. They were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). All animals were maintained in groups of five at 24 ± 1°C with light/dark cycle of 12:12 hours, relative humidity: 55-65% and had free access to feed (developed by the Bangladesh Council of Scientific and Industrial Research Dhaka.) and water ad libitum. They were starved overnight but allowed fresh water before administration of the plant extracts. All protocols for animal experiment were approved by the institutional animal ethical committee.

Chemicals and Drugs:

Carrageenan was supplied by Sigma Chemical Company, USA, Acetyl salicylic acid and Diclofenac-Na was collected from Square Pharmaceutical Ltd., Bangladesh while Nalbuphine was from Incepta Pharmaceutical Ltd, Bangladesh.

Phytochemical Screening:

In this research work, the freshly prepared crude extract was qualitatively tested for the presence of chemical constituents. Methanolic extract of *G. oppositifolius* was screened for carbohydrates, alkaloids, gums, saponins, flavanoids and steroids. The presence of all these chemical compounds which have significant medicinal values ware identified by characteristic color changes using standard procedures (Ghani, 2003).

Analgesic Study:

The peripheral analgesic activity of MEGO was investigated by the acetic acid induced writhing test (Witkin, 1961) in mice. The central analgesic action of MEGO was evaluated by tail immersion method (Hasan, 2009).

Acetic Acid Induced Writhing Test:

The animals randomly divided into four groups consisted of 5 mice in each group. Group- I was kept as control giving 1% Tween-80 in distilled water (10ml/kg b.w). Group II received Diclofenac-Na as standard drug at a dose of 10mg/kg of body weight (p.o). Group-III and Group-IV received the test compound methanolic extract of *Glinus oppositifolius* at the doses of 200 mg/kg and 400 mg/kg of body weight (p.o) respectively. Thirty minutes after treatment, the mice were given an intraperitoneal (i.p.) injection of 0.7% acetic acid in a volume of 10ml/kg to induce the specific contraction of the body referred to as "writhing". The no. of writhings occurring between 5 and 15 minutes after acetic acid injection was recorded.

Tail Immersion Test:

The animals were treated as discussed above except Nalbuphine was used as standard drug in this experiment. 1-2 cm of mice tail was immersed in warm water kept constant at 55± 10^0C. The first reading was discarded and the reaction time was recorded as a mean of the next two readings. The latent period of the tail flick response was determined at 0, 30, 60 and 90 min after the administration of drugs.

Anti Inflammatory Study:

This method was essentially that of Winter *et al.* (Winter *et al.*, 1962). The animals were randomly divided into three groups consisted of 5 rats in each group.

Group I was kept as control giving 1% Tween-80 in distilled water.
Group II received Acetyl Salicylic acid as standard drug at a dose of 300mg/kg of body weight
Group III received the methanolic extract of *G. oppositifolius* at the doses of 500mg/kg of body weight**.**

The average percent increase in paw diameter (Aiyelero *et al*., 2009) with time was calculated and compared against the control group. Percent inhibition was calculated using the formula:

$$\% \text{ Inhibition of inflammation} = (V_c - V_t)/V_c \times 100,$$

Where, V_c and V_t represents the average paw diameter of control and treated animals respectively.

Acute Toxicity Studies:

In acute toxicity study no toxic symptoms were observed for all formulations up to dose 2g/kg body weight. All animals behaved normally. No neurological or behavioral effects noted. Mice were observed for 14 days. The signs observed were motor activity, rare reactions, phonation, pain sensibility, sound sensibility, tact sensibility, social behavior, abnormal tail, aggressive behavior, ataxia, convulsions, muscle tone, paralysis, eye irritation, cornea reflex, pupil reflex to light, defecation and salivation. No mortality was found up to 14 days study.

Statistical Analysis:

Statistical analysis for animal experiments was carried out using one-way ANOVA followed by Dunnett's multiple comparison tests using SPSS 17. The results obtained were compared with the control group. p <0.05, <0.001 were considered to be statistically significant.

RESULTS AND DISCUSSION

Phytochemical Screening:

The plant extract gave positive reaction for Carbohydrates, Alkaloids, Tannins, Flavonoids and saponins. Steroids, Gums and Reducing sugar were absent in MEGO (Table 1).

Table 1: Results of Phytochemical screening of MEGO.

Extract	Carbohydrates	Tannin	Alkaloids	Gums	Reducing sugars	Saponins	Flavonoids	Steroids
MEGO	++	+	++++	-	-	+	+	+

MEGO denotes for methanolic extract of *G. oppositifolius.* (+): Present, (-): Absent.

Analgesic Study:

Acetic Acid Induced Writhing Test:

The results of acetic acid induced writhing test of the MEGO are shown in Table 2. The table shows that the MEGO reduced the number of writhes by 47.47% (16.6 ± 2.356) and 54.43% (14.4 ± 2.952) for 200 mg/kg and 400 mg/kg of MEGO respectively whereas the reference drug reduced the number of writhes by 80.38% (6.20 ± 0.785).

Table 2: Results of Acetic acid induced writhing in mice of MEGO.

Groups	Treatment	Dose, route	No. of writhing	% Inhibition
Group-I	1% Tween 80 in water	0.4 ml/mouse, p.o.	31.6 ± 3.222	-
Group-II	Diclofenac Na	10 mg/kg, p.o.	6.20 ± 0.785**	80.38
Group-III	*G. oppossitifolis*	200 mg/kg, p.o.	16.6 ± 2.356*	47.47
Group-IV		400 mg/kg, p.o.	14.4 ± 2.952**	54.43

Values are presented as mean ± SEM, (n = 5); * p < 0.05, ** p < 0.001, Dunnet test as compared to control.

Tail Immersion Test:

The results of tail immersion test (55± 10^0C) at 0, 30, 60 and 90 minutes after oral administration of two doses (200 and 400 mg/kg) of MEGO are shown in Figure 1. Treatment with the vehicle did not have any significant effect on the latency of tail immersion. MEGO at all doses tested, showed significant and dose-related increases in tail immersion duration.

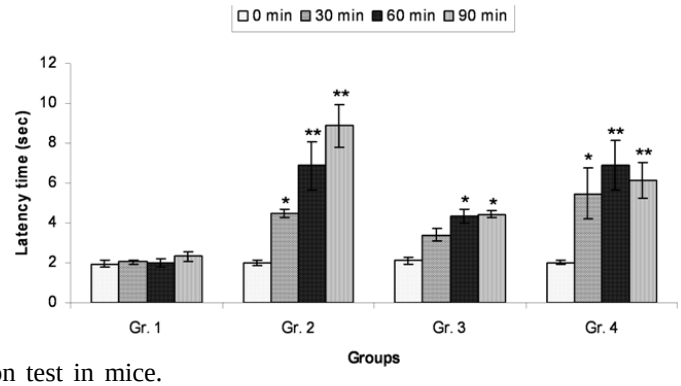

Fig. 1: Tail immersion test in mice.
Values are mean ±SEM, (n = 5); *: p<0.05, **: p<0.001.

Dunnett's test as compared to control. Group I animals received vehicle (1% Tween 80 in water), Group II received Nalbuphine 10 mg/ kg body weight, Group III and Group IV were treated with 200 and 400 mg/kg body weight (p.o.) of MEGO.

Anti-inflammatory Test:

The results of anti-inflammatory studies of the MEGO and aspirin is shown in the Table 3. The MEGO shows strong and significant ($p < 0.01$, $p < 0.001$) anti-inflammatory property compared to the standard drug Aspirin. The MEGO shows 14.89% of inhibition of inflammation whereas the aspirin shows 46.80% of inhibition.

Table 3: Effect of MEGO in Carrageenan induced paw edema in rat

Groups	Treatment	Dose (mg/kg)	Right hind paw edema (mm)				
			0 h	1 h	2 h	3 h	4 h
Group I	Control	-	6.01±0.08	7.41±0.16	8.20±0.10	9.09±0.09	9.07±0.09
Group II	Aspirin	300	6.04±0.06	6.89±0.07*	6.52±0.13**	6.28±0.06**	6.16±0.05**
Group III	MEGO	500	6.03±0.07	7.11±0.07	7.62±0.09*	7.37±0.14**	7.18±0.06**

Values are presented as mean ± SEM, (n = 5); * $p < 0.01$, ** $p < 0.001$, Dunnett's test as compared to control.

Phytochemical screening of the methanolic extract of the plant under investigation showed that it contains flavones amongst other secondary metabolites. Flavonoids are a class of phenolic compounds widely distributed in plants. These compounds have medical functions such as diuretic, laxative, antispasmodic, anti-hypertensive and antiflammatory actons (Mellors and Tappel, 1966). Thus the anti inflammatory activity elicited by this extract may be due partly to its flavonoidal contents, since flavonoids have been shown to have anti-inflammatory activity. Aspirin, a nonselective inhibitor of both cyclooxgenase isoforms (COX 1 and COX 2), exhibits anti-inflammatory action by inhibiting prostaglandins, thromboxane and prostacyclin synthesis involved in inflammation (Katzung, 2004).The carrageenan-induced rat paw inflammation is useful for investigation of the systemic anti-inflammatory activity of drugs. This model is commonly used as an experimental animal model because of its sensitivity in detecting orally active anti-inflammatory agents particularly in the acute phase of inflammation (Dirosa *et al*., 1971; Dirosa, 1972). This could be concluded that the anti-inflammatory effect of the MEGO might be related to the inhibition of mediators that corresponds to its action on each phase of inflammation similar to that of aspirin.

Pretreatment of mice with the MEGO inhibited writhing induced by acetic acid. When this acid is administrated i.p. it induces the release of prostaglandins and sympathetic system mediators (Duarte, 1988). Writhing induced by chemical substances is due to sensitization of nociceptors by prostaglandins. This test is useful for evaluating mild analgesic non-steroidal anti-inflammatory compounds (Ferreira *et al*., 1974). The anti-inflammatory and analgesic mechanisms of the MEGO may also be related to prostaglandin synthesis inhibition, as described for the anti-inflammatory mechanism of aspirin (Ferreira *et al*., 1973).

In Conclusion, considering the results of the present study, it can be said that the results support the traditional use of the plant *G. oppositifolius* in painful and inflammatory conditions. However, further studies should be carried out to evaluate exact mechanisms involve in the analgesic and anti-inflammatory action of the plant and need to isolate the active compound(s) responsible for these pharmacological activities.

REFERENCES

Aiyelero, O.M., Z.G. Ibrahim and A.H. Yaro, 2009. Analgesic and anti-inflammatory properties of the methanol leaf extract of *Ficus ingens* (moraceae) in rodents. Nig. Journ. Pharm. Sci., 8(2): 79-86.

Arora, D.S. and G.J. Kaur, 2007. Antibacterial activity of some Indian medicinal plants. J Nat. Med., 61: 313-317.

Bannerman, R.H., 1982. Traditional Medicine in Modern Healthcare. World Healthcare, 3(1): 10-15.

Bohlin, L., 1995. Structure-activity studies of natural products with anti-inflammatory effects. In: Hostettmann, K. (Ed.), Phytochemistry of Plants used in Traditional Medicine. Clarendon Press, Oxford, pp: 137-161.

Burkhill, H.M., 1985. The Useful plants of West Trophical Africa, second ed, Royal Botanical Gardebs, Kew.

Debes, S.C., 1998. En medisineplante fra mali, *Glinus oppositifolius*. Masrer's thesis. School of Oslo, Norway.

Diallo, D., B. Hveem, M.A. Mahmoud, G. Berge, B.S. Paulsen and A. Maiga, 1999. An ethnobotanical survey of herbal drugs of Gourma district, Mali. Pharmacetucal Biol., 37(1): 80-91.

Diallo, D., 2000. Ethnopharmacological survey of medicinal plants in Mali and phytochemical study of four of them: *Glinus oppositifolius* (aizoaceae), *Diospyros abyssinica* (Ebenaceae), *Entada africana* (Mimosacee), *Trichilia emetica* (meliaceae). These de Doctorate. Faculte de Science, University de Lausanne, Switzerland.

Dirosa, M., J.P. Giroud and D.A. Willoughby, 1971. Studies of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. J. Pathol., 104: 15-29.

Dirosa, M, 1972. Biological properties of carrageenan. J. Pharm. Pharmacol., 24: 89-102.

Duarte, J.D.G., M. Nakamura and S.H. Ferreira, 1988. Participation of the sympathetic system in the acetic acidn induced writhing in mice. Braz. J. Med. Biol. Res., 21: 341-3.

Ferreira, S.H., J.R. Vane, 1974. New aspects of the mode of action of non- steroidal anti-inflammatory drugs.-Ann. Rev. Pharmacol., 14: 57-73.

Ferreira, S.H., S. Moncada and J.R. Vane, 1973. Further experimentation to establish that the analgesic action of the aspirin like drugs depends on the inhibition of the prostaglandin biosynthesis. Brit. J. Pharmacol., 47: 48-58.

Ghani, A., 2003. Medicinal Plants of Bangladesh. The Asiatic Society of Bangladesh. 2nd Revised Edn., Dhaka, Bangladesh, ISBN: 9845123481, pp: 603.

Hasan, S.M.R., M. Jamila, M.M. Majumder, R. Akter, M.M. Hossain, M.E.H. Mazumder, M.A. Alam, R. Jahangir, M.S. Rana and S. Rahman, 2009. Analgesic and antioxidant activity of the Hydromethanolic extract of *Mikania scandens* (L.) Willid. Leaves. Am. J. Pharm. Tox., 4: 1-7.

Inngerdingen, K.T., S.C. Debes, M. Inngjerdingen, S. Hokputsa, S.E. Harding and B. Rolstad, 2005. Bioactive pectic polysaccharide from *Glinus oppositifolius* (L) Aug. DC., a Malian medicinal plant, isolation and partial characterization. J. Ethnopharmacol., 101: 204-14.

Katzung, B.G., 2004. Basic & Clinical Pharmacology. 9th ed. Mc Graw-Hill, New York, pp: 576-603.

Mellors, A. and A.L. Tappel, 1966. The inhibition of mitochondrial peroxidation by ubiquinone and ubiquinol. J. Biol. Chem., 241: 4353-6.

Perianayagam, J.B., S.K. Sharma and K.K. Pillai, 2006. Anti-inflammatory activity of *Trichodesma indicum* root extract in experimental animals. J. Ethnopharmacol., 104: 410-414.

Vane, J.R. and R.M. Bolting, 1995. New insights into the mode of action of anti- inflammatory drugs. Inflammation Res., 44: 1-10.

Yesilada, E., O. Ustun, E. Sezik, Y. Takishi, Y. Ono and G. Honda, 1997. Inhibitory effects of Turkish folk remedies on inflammatory cytokines: interleukin-1, interleukin- 1ß and tumor necrosis factor. J. Ethnopharmacol., 58: 59-73.

Witkin, L.B., C.F. Hebner, F. Gaddi, E. O'Keefe, P. Spitaletta and A.J. Plumer, 1961. Pharmacology of 2-amino-indane hydrochloride. A potent non-narcotic analgesic. J. Pharmacol. Exp. Ther., 133: 400-8.

Winter, C.A., E.A. Risley and G.W. Nuss, 1962. Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med., 111: 544-7.