Original Research Paper

Analgesic and Anti-inflammatory Activity of *Euphorbia antiquorum* Linn

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Abstract: *Euphorbia antiquorum* (L.) is used as traditional medicine for various ailments in Bangladesh. But the scientific basis for its use especially in pain and inflammation remains largely unknown. Therefore, the present study was designed to evaluate analgesic and anti-inflammatory effect of the aqueous ethanolic extract of the whole plant. The analgesic activity was evaluated by hot plate, acetic acid induced writhing and formalin induced writhing methods in Swiss Albino mice at the doses of 250 and 500 mg kg\(^{-1}\) body weight. The extract was also investigated for the anti-inflammatory effect on Long Evans rats at above mentioned doses using carrageenan induced rat paw edema method. Phytochemical analysis of the extract revealed the presence of tannins, alkaloids, flavonoids, saponins and terpenoids. The extract elicited a significant (p<0.01) analgesic activity in a dose dependent fashion in both the early and late phases of formalin test and also in hot plate and acetic acid induced writhing tests at both the doses employed. In acetic acid induced writhing test, the extract (at 500 mg kg\(^{-1}\)) produced a maximum inhibition of writhing reaction by 51.13% (p<0.001), which is comparable to that of the standard drug Diclofenac sodium (58.80%). The extract also significantly inhibited the licking response at the dose of 500 mg kg\(^{-1}\) in both the early phase (63.64%, p<0.01) and the late phase (66.43%, p<0.01) of formalin test while the standard drug inhibited by 64.20% and 72.03%, respectively. The oral administration of the extract significantly (p<0.001) inhibited inflammatory response induced by carrageenan in a dose dependent manner. The most prominent inhibition of 74.25% (250 mg kg\(^{-1}\)) and 78.44% (500 mg kg\(^{-1}\)) were observed at the 4th hour of study. Presence of various chemical constituents in the extract of *Euphorbia antiquorum* especially tannins, flavonoids, terpenoids and alkaloids might be responsible for the observed analgesic and anti-inflammatory effects. These experimental findings lend pharmacological support to the suggested folkloric uses of the plant in the management and/or control of pain as well as inflammatory conditions.

**Keywords:** *Euphorbia antiquorum*, Medicinal Plant, Phytochemical Screening, Analgesic, Inflammation

Introduction

Inflammation, considered as a primary physiologic defense mechanism, is a pathophysiological response of mammalian tissues to a variety of hostile agents including infectious organisms, toxic chemical substances, physical injury or other noxious stimuli leading to local accumulation of plasma fluid and blood cells (Sobota *et al.*, 2000; Medzhitov, 2010). It involves a complex array of pathological responses such as enzyme activation, mediator release, fluid extravasation, cell migration, tissue breakdown and...
repair (Vane and Bolting, 1995; Salmi and Jalkanen, 2005; Perianayagam et al., 2006). Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases (Sosa et al., 2002) and so it has become the focus of global scientific research. Anti-inflammatory and analgesic therapy is dominated by two major classes of analgesic drugs; opioids and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). However, both classes of drugs produce serious side effects. According to WHO, it is reported that about 80% of the world population is dependent (wholly or partially) on plant-based drugs (Dharmasiri et al., 2003; Park et al., 2004). Therefore, the search for pharmacological agents to overcome these shortcomings has been a major goal in pain research for quite a long time. To this end, medicinal plants have become considerably useful and economically viable. They contain active constituents that are used in the treatment of many human diseases. Currently, researchers are increasingly turning their attention to natural products used in the traditional medicine because they are cheap and have little side effects. According to WHO, it is reported that about 80% of the world population is dependent (wholly or partially) on plant-based drugs (Dharmasiri et al., 2003; Tabuti et al., 2003; WHO, 1996).

Euphorbia antiquorum Linn, a plant belonging to the Euphorbiaceae family, is a large shrub or small spiny tree, 4.5-9 m high, with white latex growing throughout the hotter parts of India and Bangladesh and has various medicinal uses. The plant is naturalized and widely cultivated in neighboring regions such as China, Indonesia, Malaysia, Myanmar, Pakistan, Sri Lanka, Thailand and many other tropical zones in the world. The plant is reported to be used traditionally in inflammatory disorders such as rheumatism and gout, to relieve pain in rheumatism and toothache, in nervous diseases, dropsy, palsy and deafness and as a purgative (Kirtikar and Basu, 1980; Jain and De Filipps, 1991). Previous phytochemical studies of the roots, stems and latex of this plant have shown the presence of triterpenoids and diterpenoids (Min et al., 1989; Anjaneyulu and Ravi, 1989; Gewali et al., 1990; Akihisa et al., 2002). The latex has been reported to contain euphol, euphol 3-O-cinnamate, antiquol A, antiquol B, cycloartenol, cycloeucalenol, euphadienol and euphorbol as the triterpene constituents (Gewali et al., 1990). Juice is also reported to contain diterpene esters, euphorbin. Stembark and latex also contain taraxerol, taraxerone, friedelanol and epi-friedelanol (Ghani, 2003a). On account of the ethnomedical claim of the usefulness of the plant in the management of pain and inflammatory conditions, this present study was aimed at investigating the analgesic and anti-inflammatory activities of the aqueous ethanolic extract of E. antiquorum in laboratory animals, which to the best of our knowledge has not been fully scientifically investigated (Harpalani et al., 2011).

Materials and Methods

Collection and Identification of Plants

Euphorbia antiquorum plant was collected from Jahangirnagar University, Savar, Dhaka, Bangladesh during the last week of January 2011. The plant was identified by the experts of Bangladesh National Herbarium (BNH). The specimen was preserved in BNH and Department of Pharmacy, North South University, Bangladesh and it has been assigned the accession number of DACB 35519.

Preparation of Plant Materials

The collected plant was washed with water and separated from undesirable materials or plants or plant parts. They were aerated by fan aeration to be partially dried and were next heated in an oven at below 40°C for two days to be fully dried. The fully dried plant was then ground to make powder form by the help of a suitable grinder. Then the powders were dissolved in 80% ethanol and kept for a period of five days with accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material followed by a second filtration through whatman filter paper. The filtrate (aqueous ethanolic extract) obtained was evaporated by rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) at 5 to 6 rpm and at 68°C temperature. It rendered a gummy concentrate of dark greenish black color that was designated as crude extract or ethanolic extract. The crude ethanolic extract was finally dried by freeze drier and preserved.

Phytochemical Screening

The freshly prepared aqueous ethanolic extract of E. antiquorum was qualitatively tested for the presence of chemical constituents. The phytochemical screening of the extract was carried out according to the method described previously (Ghani, 2003b; Mogana et al., 2011). Qualitative analysis of alkaloids, flavonoids, terpenoids, gum, saponins and tannins were studied using freshly prepared whole plant extract using the following reagents and chemicals: Alkaloids with Wagner’s reagent; flavonoids with conc. HCl; tannins with 5% ferric chloride solution; saponins with ability to produce stable foam; gum and carbohydrate using

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Molisch's reagent and conc. sulphuric acid; terpenoids with chloroform and conc. sulphuric acid. These were identified by characteristic color changes and/or precipitates using standard procedures.

**Animals**

Young Swiss-Albino mice aged about 4-5 weeks with average weight of 25-35 gm and adult Long Evans Rats of either sex having average weight of 100-130 gm were used for the experiment and maintained in the animal house of the Department of Pharmacy, North South University. The animals were originally obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). They were housed in standard cages under standard environmental conditions of room temperature at 24±1°C and 55-65% relative humidity with 12 h dark light cycle and provided with standard pelleted food for rodents and water ad libitum.

**Evaluation of Analgesic Activity**

**Hot Plate Test**

The hot-plate test (Hot/Cold Plate Model-35100-001, UGO Basil, Italy) was employed for measurement of analgesic activity as previously described by Lanhers et al. (1992) and modified by Ojewole (2004). The temperature was regulated at 55±1°C. Mice were divided into four groups consisting of seven animals in each group. The mice of each group were placed in the beaker (on the hot plate) in order to obtain its response to electrical heat induced pain stimulus. Licking of the paws or jumping out of the beaker was taken as an indicator of the animal's response to heat-induced pain stimulus. The time for each mouse to lick its paws or jump out of the beaker was taken as reaction time (in second). Before treatment, the reaction time was taken once. The mean of this determination constituted initial reaction time before treatment of each group of mice. Each of the test mice was thereafter treated with either Distilled Water (DW), Diclofenac sodium (10 mg kg⁻¹ of body weight) or ethanol extract of *E. antiquorum* at the doses of 250 and 500 mg kg⁻¹ body weight orally. Thirty minutes after treatment, the reaction times of each group of mice were again evaluated five times individually in one hour interval.

**Acetic Acid Induced Writhing Test**

The analgesic activity of the samples was evaluated using acetic acid induced writhing method in mice following the method of Koster et al. (1959) with slight modification (Owoyele et al., 2001; Altun et al., 2009). In this method, acetic acid is administered intraperitoneally to the experimental animals to create pain sensation. The animals were divided into four groups with seven mice in each group. Group I animals received distilled water, Group II received Diclofenac sodium at 10 mg kg⁻¹ while animals of Group III and Group IV were treated with 250 and 500 mg kg⁻¹ of the 80% ethanol extract of *E. antiquorum* after an overnight fast. Test samples and vehicle were administered orally 30 min prior to intraperitoneal administration of 0.7% v/v acetic acid solution. Animals were kept individually under glass jar for observation. Each mouse of all groups was observed individually for counting the number of writhing they made in 10 min commencing just 5 min after the intraperitoneal administration of acetic acid solution. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half-writhings were taken as one full writhing. The number of writhes in each treated group was compared to that of a control group while Diclofenac sodium was used as a reference standard (positive control). The percentage inhibition of writhing was calculated as follows:

\[
\% \text{ Inhibition} = \left(1 - \frac{V_c}{V_t}\right) \times 100
\]

\[V_c = \text{Number of writhing motions in drug-treated mice}
\]

\[V_t = \text{Number of writhing motions in the control group of mice}
\]

**Formalin Test**

The method used was similar to that described previously (Shibata et al., 1989; Viana et al., 1998). The mice were divided into four groups each containing seven mice and were administered with distilled water (1 mL kg⁻¹, i.p.), ethanolic extract of *E. antiquorum* (250 and 500 mg kg⁻¹, i.p) or Diclofenac sodium (10 mg kg⁻¹, s.c). Thirty minutes after this treatment, 50 µL of a freshly prepared 0.6% solution of formalin was injected subcutaneously under the plantar surface of the left hind paw of each mouse. The mice were placed individually in an observation chamber and monitored for one hour. The time (in sec) spent in licking and biting of the injected paw was taken as an indicator of pain response. Antinociceptive effect was determined in two phases. The early phase (phase 1) was recorded during the first 5 min, while the late phase (phase 2) was recorded during the last 20-30 min after formalin injection.

**Anti-Inflammatory Activity by Carrageenan-Induced Rat Paw Edema Method**

The anti-inflammatory activity of the ethanol extract was investigated on carrageenan induced inflammation in rat paw following an established
method (Winter et al., 1962). Rats were randomly divided into four groups, each consisting of seven animals, of which group I was kept as control giving only distilled water. Group II was standard which received Diclofenac sodium (10 mg kg\(^{-1}\)) as the reference standard for comparison while Group III and Group IV were given the test material at the doses of 250 and 500 mg kg\(^{-1}\) body weight respectively. Half an hour after the oral administration of the test materials, 1% carrageenan was injected to the right hind paw of each animal. The volume of paw edema was measured at 0, \(\frac{1}{2}, 1, 2, 3, 4\) and 8 h using Plethysmometer (Model 7141, UGO Basil, Italy) after administration of carrageenan. The left hind paw served as a reference non-inflamed paw for comparison.

The average percent increase in paw volume with time was calculated and compared against the control group. Percent inhibition was calculated using the formula:

\[
\% \text{ Inhibition of paw edema} = \frac{V_c - V_t}{V_c} \times 100
\]

where, \(V_c\) and \(V_t\) represent average paw volume of control and treated animal respectively.

**Statistical Analysis**

The data are expressed as the mean ± SEM and analyzed by one-way analysis of variance (ANOVA) and Dunnett’s \(t\)-test was used as the test of significance. \(P\) value <0.05 was considered as the minimum level of significance. All statistical tests were carried out using SPSS statistical software.

**Results**

**Phytochemical Analysis**

Preliminary phytochemical screening of 80% ethanol extract of *E. antiquorum* revealed the presence of various bioactive components among which tannin, flavonoid, alkaloid and terpenoid were the most prominent. The result of phytochemical tests has been summarized in the Table 1.

**Analgesic Activity**

**Hot Plate Test**

Result of hot plate test is shown in Table 2 and Fig. 1. The plant extract at both the doses displayed dose dependent increase in retention time and the maximum effect was observed with 500 mg kg\(^{-1}\) at the 3rd hour of study which was comparable to the standard drug. As shown in Fig. 1b, *E. antiquorum* extract at 500 mg kg\(^{-1}\) revealed progressive increase in latency time compared to control and it was found to increase by more than 100% at 3rd hour of study. The overall result was found to be statistically significant (\(p<0.05\) and \(p<0.001\)).

**Acetic Acid-Induced Writhing Test**

Table 3 shows the effects of the extract on acetic acid-induced writhing in mice. The oral administration of both doses of *E. antiquorum* extract significantly (\(p<0.001\)) attenuated the acetic acid-induced abdominal writhes in mice in a dose dependent fashion. The percent inhibition of writhing response by the extract was 37.12 and 51.13% at 250 and 500 mg kg\(^{-1}\) doses respectively.

**Formalin Test**

The effect of the extract of *E. antiquorum* on formalin induced pain in mice is shown in Table 4 and Fig. 2. The extract dose dependently inhibited the licking response in both the early phase (56.81% at 250 mg kg\(^{-1}\), \(p<0.05\) and 63.64% at 500 mg kg\(^{-1}\), \(p<0.01\)) as well as the late phase (61.54% at 250 mg kg\(^{-1}\), \(p<0.05\) and 66.43% at 500 mg kg\(^{-1}\), \(p<0.01\)) of formalin test and the inhibitory effects were comparable to those of the standard drug.

![Fig. 1. Effect of the hydroethanol extract of *E. antiquorum* on thermally induced pain stimulus in mice, (a) Increase in latency time over a period of 4 h; (b) Percentage increase in retention time compared to control](image-url)
Fig. 2. Anti-nociceptive effect of the ethanol extract of *E. antiquorum* in formalin induced pain stimulus in mice. Data shown are the percentage inhibition of pain sensation compared to control.

Fig. 3. Anti-inflammatory activity of *E. antiquorum* in carrageenan induced edema in rats.

Table 1. Phytochemical analysis of extract of *Euphorbia antiquorum*

| Extract                  | Tannin | Flavonoid | Saponin | Gum | Alkaloid | Terpenoid |
|--------------------------|--------|-----------|---------|-----|----------|-----------|
| 80% Ethanol extract of *E. antiquorum* | +++    | +++       | ++      | +   | +++      | +++       |

(+) indicates presence and (-) indicates absence of phytochemicals.

Table 2. Effect of the aqueous ethanol extract of *Euphorbia antiquorum* on latency to hot plate test

| Treatment             | Dose (mg kg<sup>−1</sup>) | No. of writhing | % Inhibition |
|-----------------------|---------------------------|----------------|--------------|
| Control               | Vehicle                   | 41.75±3.77     | -            |
| Diclofenac sodium     | 10                        | 17.20±0.49***  | 58.80        |
| *E. antiquorum* 250   | 250                       | 26.25±1.43**   | 37.12        |
| *E. antiquorum* 500   | 500                       | 20.40±1.06***  | 51.13        |

Data are represented as the mean ± SEM, (n = 7); ***p<0.001 were considered significant in comparison with control.
Anti-Inflammatory Activity

The oral administration of both doses of the aqueous ethanolic extract of *E. antiquorum* significantly (p<0.05 and p<0.001) inhibited inflammatory response induced by carrageenan in rats in a dose related manner. As shown in Table 5 and Fig. 3, the most prominent inhibition of 74.25% at 250 mg kg⁻¹ and 78.44% at 500 mg kg⁻¹ were observed at the 4th hour of study after which the inhibitory activity was found to decline. The anti-inflammatory effect of the extract at the dose of 500 mg kg⁻¹ was comparable to that of the reference standard.

**Discussion**

Three different analgesic testing methods were employed in the current investigation with the objective to identify possible peripheral and central effects of the aqueous ethanolic extract of *E. antiquorum*. To gain an insight into the central anti-nociceptive effect of the extract, the hot plate test (Marchioro et al., 2005), possibly acting on a descending inhibitory pain pathway (Richardson et al., 1998), was used. The hot plate test produces two kinds of behavioral responses, namely paw licking and jumping. Both of these are considered to be supraspinally integrated responses (Chapman et al., 1985). It has generally been regarded that the μ receptor is associated with pain relief and has been shown to be potent in regulating thermal pain (Dhawan et al., 1996). The extract of the plant at both doses (250 and 500 mg kg⁻¹) as well as standard diclofenac sodium (10 mg kg⁻¹) demonstrated a longer latency time than the control group in a dose related manner. It is an established fact that any agent that causes a prolongation of the hot plate latency using this test must be acting centrally (Ibironke and Ajiboye, 2007).

Therefore, by considering several reports and our current results, the antinociceptive activity of the extract, at least in part, is likely to be mediated centrally.

Acetic acid induced abdominal writhing, the visceral pain model, is a sensitive method to evaluate peripherally acting analgesics. The crude extract of the plant showed significant analgesic effect at both dose levels compared to the reference drug diclofenac sodium against acetic acid induced pain in mice. Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting in release of free arachidonic acid from tissue phospholipid via cyclooxygenase and prostaglandin biosynthesis (Duarte et al., 1988). This model of response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathway (Ribeiro et al., 2000; Voilley, 2004). The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Duarte et al., 1988). Results of the present study show that the plant extract produced significant analgesic effect, which may be due to the inhibition of the synthesis of the arachidonic acid metabolite.

Formalin test is also a well-established valid model for the study of central sensitization events at the spinal level after peripheral inflammatory state (Diaz and Dickenson, 1997). The advantage of using the formalin model of noiception is that it can discriminate between central and peripheral pain components (Tjølsen et al., 1992). The test consists of two different phases: The first one (neurogenic phase) is generated in the periphery through the activation of nociceptive neurons by the direct action of formalin and the second phase (inflammatory phase) occurs through the activation of the ventral horn neurons at the spinal cord. The late phase is due to inflammation causing a release of

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**Table 4. Analgesic effect of the hydroethanol extract of *Euphorbia antiquorum* using the formalin test**

| Treatment | 0-5 min (early phase) | % Inhibition | 20-30 min (late phase) | % Inhibition |
|-----------|-----------------------|--------------|------------------------|--------------|
| Control   | 44.00±9.70            |              | 35.75±9.51             |              |
| Standard  | 15.75±2.89 **         | 64.20        | 10.00±3.51 **          | 72.03        |
| *E. antiquorum* (250 mg kg⁻¹) | 19.00±3.07 * | 56.81 | 13.75±1.11* | 61.54 |
| *E. antiquorum* (500 mg kg⁻¹) | 16.00±4.18 ** | 63.64 | 12.00±1.87** | 66.43 |

Values are mean ± SEM, (n = 7); *p<0.05, **p<0.01 were considered significantly different in comparison with control.

**Table 5. Effect of the 80% ethanol extract of *Euphorbia antiquorum* on carrageenan induced rat paw edema**

| Treatment | ½ h | 1 h | 2 h | 3 h | 4 h | 8 h |
|-----------|-----|-----|-----|-----|-----|-----|
| Control   | 0.63±0.048 | 0.75±0.046 | 1.41±0.06 | 1.79±0.08 | 1.67±0.08 | 1.59±0.07 |
| Diclofenac sodium 10 mg kg⁻¹ | 0.37±0.037** | 0.36±0.04 | 0.57±0.064* | 0.37±0.094** | 0.32±0.07*** | 0.68±0.04** |
| (41.26) | (52.00) | (59.57) | (79.33) | (80.84) | (57.23) |
| *E. antiquorum* 250 mg kg⁻¹ | 0.45±0.05 | 0.46±0.06 | 0.68±0.019* | 0.49±0.13** | 0.43±0.06*** | 0.86±0.04* |
| (28.57) | (38.67) | (51.77) | (72.63) | (74.25) | (45.91) |
| *E. antiquorum* 500 mg kg⁻¹ | 0.42±0.028** | 0.41±0.036 | 0.59±0.016* | 0.41±0.035*** | 0.36±0.033*** | 0.77±0.027*** |
| (33.33) | (45.33) | (58.16) | (77.09) | (78.44) | (51.57) |

Data are represented as the mean ± SEM, (n = 7); Values in parentheses indicate percent inhibition of paw edema; *p<0.05, **p<0.01, ***p<0.001 were considered significantly different in comparison with control.
serotonin, histamine, bradykinin and prostaglandins, which at least to some degree can cause the sensitization of the central nociceptive neurons (Verma et al., 2005). Morphine, a typical narcotic analgesic drug, can inhibit nociception in both phases (Shibata et al., 1989) while NSAIDs, by acting supraspinally, can also reduce the pain in both phases (Martindale et al., 2001). In the present study, the crude extract produced antinociception against both neurogenic and inflammatory phases of formalin test. Taken together, it was observed that the plant extract possessed both central and peripheral analgesic effects against all the different models used.

The present study provides further evidence that the aqueous ethanolic extract of *E. antiquorum* has anti-inflammatory activity in acute inflammation model in rats. Carrageenan-induced inflammation is most commonly used as an experimental model for evaluating the anti-inflammatory potency of compounds or natural products that has been reported to exhibit a high degree of reproducibility (Kale et al., 2007). Moreover, we used a plethysmometer to measure the paw edema volume which is a consistent method to study the relative changes in rat paw edema (Abudoleh et al., 2011). The probable mechanism of action of carrageenan-induced inflammation is bi-phasic; the first phase is attributed to the release of histamine, serotonin and kinins in the first hour while the second phase is attributed to the release of prostaglandins and lysosome enzymes in the following 2 to 4 h (Brooks and Day, 1991). It is reported that the second phase is sensitive to most clinically effective anti-inflammatory drugs (Vinegar et al., 1969). The results of present study indicate that the extract significantly inhibited carrageenan-induced acute inflammation in the 4th hour of study and the finding was comparable to that of the standard diclofenac sodium which at least to some degree can cause the sensitization of the central nociceptive neurons (Fawole et al., 2010). There are also reports on the role of tannins in anti-nociceptive activity (Ramprasath et al., 2006). Flavonoids, also known as nature’s tender drugs, possess abundant biological and pharmacological activities. For instance, analgesic and anti-inflammatory effects have been observed in flavonoids (Kim et al., 2004; Duke’s, 2006; Küpeli and Yesilada, 2007). It is also reported that flavonoids such as rutin, quercetin and luteolin produced significant antinociceptive and anti-inflammatory activities (Pathak et al., 1991; Pelzer et al., 1998; Duke’s, 2006). Besides, alkaloids are well known for their ability to inhibit pain perception (Uche and Aprioku, 2008). Alkaloids have been shown to possess anti-inflammatory activity by inhibiting the action of arachidonic acid metabolism via the cyclooxygenase and 5-lipoxygenase pathways (Barik et al., 1992; Hajare et al., 2001). Studies have also demonstrated that terpenoids produced significant analgesic and anti-inflammatory activities (Safayhi and Sailer, 1997; Calixto et al., 2000; Moody et al., 2006). They are known to exert their anti-inflammatory effect by inhibiting phospholipase A2, a key enzyme of arachidonic acid metabolism, thereby stopping prostaglandin synthesis (Barar, 2000).

**Conclusion**

Based on previous studies and our current investigation, we conclude that the analgesic and anti-inflammatory effect of the plant extract may be due to the presence of flavonoids, tannins, alkaloid or terpenoids. These experimental findings lend pharmacological support to the suggested folkloric uses of the plant in the management and/or control of pain as well as inflammatory conditions. However, further studies are in progress in our laboratory to isolate the active constituents responsible for the observed effect and to elucidate the possible mechanisms of action responsible for the analgesic and anti-inflammatory activities of the aqueous ethanolic extract.

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**Author’s Contributions**

Banibrata Das: Designed the research plan, supervised the study, analyzed the data, contributed to the writing and critically reviewing the manuscript.

Samina Alam: Carried out all experiments, participated in data-analysis and contributed to the drafting of the manuscript.

Rajib Bhattacharjee: Contributed to the statistical analysis and writing of the manuscript.

Biplab Kumar Das: Participated in data-analysis and reviewing the manuscript.

**Ethics**

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues are involved.
References

Abudoleh, S., A. Disi, E. Qunaibi and T. Aburjai, 2011. Anti-arthritic activity of the methanolic leaf extract of *Urtica pilulifera* L. on albino rats. Am. J. Pharmacol. Toxicol., 6: 27-32. DOI: 10.3844/ajptsp.2011.27.32

Akihisa, T., E.M.K. Wijeratne, H. Tokuda, F. Enjo and M. Toriumi *et al.*, 2002. Eupha-7,9(11),24-trien-3β-ol (“Antiquol C”) and other triterpenes from *Euphorbia antiquorum* latex and their inhibitory effects on Epstein-Barr virus activation. J. Natural Products, 65: 158-162. DOI: 10.1021/np010377y

Altun, M.L., G.S. Çitoğlu, B.S. Yılmaz and H. Özbek, 2007. Anti-inflammatory activity of *Viburnum opulus* leaves. J. Ethnopharmacol., 112: 300-304. DOI: 10.1016/j.jep.2007.03.024

Anjaneyulu, V. and K. Ravi, 1989. Terpenoids from *Euphorbia antiquorum* produced by subcutaneous formalin inflammation. New Engl. J. Med., 324: 1716-1725. PMID: 8981566

Barik, B.R., T. Bhowmik, A.K. Dey, A. Patra and A. Chatterjee *et al.*, 1992. Premnazolone and isoxazolalkaloid of *Premna integrifolia* and *Gmelina arborea* with anti-inflammatory activity. Fitoterapia, 53: 295-299.

Brooks, P.M. and R.O. Day, 1991. Nonsteroidal anti-inflammatory drugs-differences and similarities. New Engl. J. Med., 324: 1716-1725. DOI: 10.1056/NEJM199106133242407

Calixto, J.B., A. Beirith, J. Ferreira, A.R.S. Santos and V.C. Filho, 2002. Naturally occurring antinoceptive substances from plants. Phytotherapy Res., 14: 401-418. DOI: 10.1002/1099-1573(200009)14:6<401::AID-PTR762>3.0.CO;2-H

Chapman, C.R., K.L. Casey, R. Dubner, R.H. Gracely *et al.*, 1985. Pain measurement: An overview. Pain, 22: 1-31. DOI: 10.1016/0304-3959(85)90145-9

Dharmasiri, M.G., J.R.A.C. Jayakody, G. Galhena, S.S.P. Liyanage and W.D. Ratnasooriya, 2003. Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*. J. Ethnopharmacol., 87: 199-206. DOI: 10.1016/S0378-8741(03)00159-4

Dhawan, B.N., F. Cesselin, R. Raghubir, T. Reisine and P.B. Bradley *et al.*, 1996. International union of pharmacology. XII. classification of opioid receptors. Pharmacol. Rev., 48: 567-592. PMID: 8981566

Diaz, A. and A.H. Dickenson, 1997. Blockade of spinal N- and P-type, but not L-type, calcium channels inhibits the excitability of rat dorsal horn neurones produced by subcutaneous formalin inflammation. Pain, 69: 93-100. PMID: 9060018

Duarte, I.D., M. Nakamura and S.H. Ferreira, 1988. Participation of the sympathetic system in acetic acid-induced writhing in mice. Braz. J. Med. Biol. Res., 21: 341-343. PMID: 3203167

Duke's, 2006. Phytochemical and ethnobotanical databases.

Fawole, O.A., S.O. Amoo, A.R. Ndhlala, M.E. Light and J.F. Finnie *et al.*, 2010. Anti-inflammatory, anticholinesterase, antioxidant and phytochemical properties of medicinal plants used for pain-related ailments in South Africa. J. Ethnopharmacol., 127: 235-241. DOI: 10.1016/j.eph.2009.11.015

Gewali, M.B., M. Hattori, Y. Tezuka, T. Kikuchi and T. Namba, 1990. Constituents of the latex of *Euphorbia antiquorum*. Phytochemistry, 29: 1625-1628. DOI: 10.1016/0031-9422(90)80134-3

Ghani, A., 2003a. Medicinal Plants of Bangladesh. 2nd Edn., Asiatic Society of Bangladesh, Dhaka, ISBN-10: 9845123481, pp: 225.

Ghani, A., 2003b. Medicinal Plants of Bangladesh. 2nd Edn., Asiatic Society of Bangladesh, Dhaka, ISBN-10: 9845123481, pp: 500.

Hajare, S.W., S. Chandra, J. Sharma, S.K. Tandan and J. Lal *et al.*, 2001. Anti-inflammatory activity of *Dalbergia sissoo* leaves. Fitoterapia, 72: 131-139. DOI: 10.1016/S0367-326X(00)00272-0

Harpalani, A.N., A.D. Taranalli, K.V. Otari, R.V. Karadi and R.V. Shete, 2011. Anti-inflammatory and antiarthritic potential of aqueous and alcoholic extracts of *Euphorbia antiquorum*. Linn. Pharmacologyonline, 2: 287-298.

Ibironke, G.F. and K.I. Ajiboye, 2007. Studies on the anti-inflammatory and analgesic properties of *Chenopodium ambrosioides* leaf extract in rats. Int. J. Pharmacol., 3: 111-115. DOI: 10.3923/ijp.2007.111.115

Jain, S.K. and R.A. DeFilipps, 1991. Medicinal Plants of India. 1st Edn., Reference Publ., Algonac, pp: 408.

Kale, M., A.V. Misar, V. Dave, M. Joshi and A.M. Mujumdar, 2007. Anti-inflammatory activity of *Dalbergia lanceolata* bark ethanol extract in mice and rats. J. Ethnopharmacol., 112: 300-304. DOI: 10.1016/j.jep.2007.03.024

Kim, H.P., K.H. Son, H.W. Chang and S.S. Kang, 2004. Anti-inflammatory plant flavonoids and cellular action mechanisms. J. Pharmacol. Sci., 96: 229-245. DOI: 10.1254/jphs.CRJ04003X

Kirtikar, K.R. and B.D. Basu, 1980. Indian Medicinal Plant. BishenSingh Pub., Dehradun.

Koster, R., M. Anderson and E.J. De Beer, 1959. Acetic acid for analgesic screening. Fed. Proc., 18: 412-412.

Küpeli, E. and E. Yesilada, 2007. Flavonoids with anti-inflammatory and antinoceptive activity from *Cistus laurifolius* L. leaves through bioassay-guided procedures. J. Ethnopharmacol., 112: 524-530. DOI: 10.1016/j.jep.2007.04.011
Vane, J.R. and R.M. Botting, 1995. New insights into the mode of action of anti-inflammatory drugs. Inflamm. Res., 44: 1-10. DOI: 10.1007/BF01630479

Verma, P.R., A.A. Joharapurkar, V.A. Chatpalliwar and A.J. Asnani, 2005. Antinociceptive activity of alcoholic extract of *Hemidesmus indicus* R.Br. in mice. J. Ethnopharmacol., 102: 298-301. DOI: 10.1016/j.jep.2005.05.039

Viana, G.S.B., T.G. do Vale, V.S.N. Rao and F.J.A. Matos, 1998. Analgesic and antiinflammatory effects of two chemotypes of *Lippia alba*: A comparative study. Pharm. Biol., 36: 347-351. DOI: 10.1076/phbi.36.5.347.4646

Vinegar, R., W. Schreiber and R. Hugo, 1969. Biphasic development of carrageenin edema in rats. J. Pharmacol. Exp. Ther., 166: 96-103. PMID: 5776026

Voilley, N., 2004. Acid-sensing ion channels (ASICs): New targets for the analgesic effects of Non-Steroid Anti-Inflammatory Drugs (NSAIDs). Curr. Drug Target Inflamm. Allergy, 3: 71-79. PMID: 15032643

WHO, 1996. WHO Guideline for the Assessment of herbal medicines. WHO Expert Committee on specification for pharmaceutical preparation. Technical Report series no. 863, Geneva.

Winter, C.A., E.A. Risley and G.W. Nuss, 1962. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Proc. Soc. Exp. Biol. Med., 111: 544-547. DOI: 10.3181/00379727-111-27849