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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, flavanoids, 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; for instance, NSAIDs cause gastric lesions, renal damages and respiratory depression while opiates induce tolerance and dependence (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 spinous 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 nervine 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. Stem-bark 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 40ºC for two days to be fully dried. The fully dried plant was then grinded 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 ethanol 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

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-writhing 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:

$$\% \text{ Inhibition} = (1-V_t/V_c) \times 100$$

V_t = Number of writhing motions in drug-treated mice

V_c = 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 mice. 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. Anti-nociceptive 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, ½, 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.

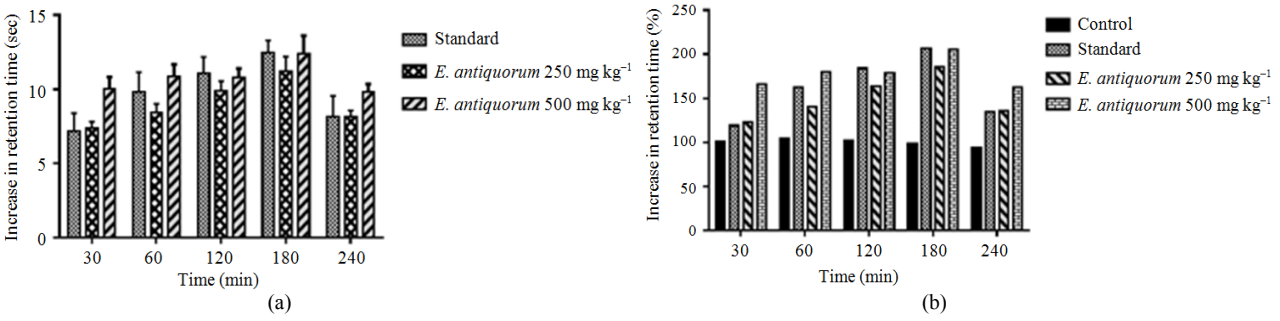

Fig. 1. Effect of the hydroethanol extract of *E. antiquorum* on thermally induced pain stimulus in mice, (a) Increase in latency time over a period of 4 h; (b) Percentage increase in retention time compared to control

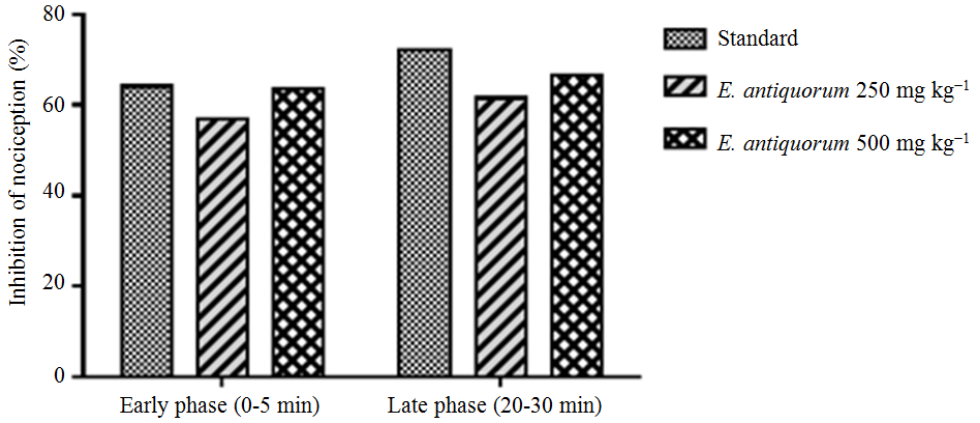

Fig. 2. Anti-nociceptive effect of the ethanol extract of *E. antiquorum* in formalin induced pain stimulus in mice. Data shown are the percentage inhibition of pain sensation compared to control

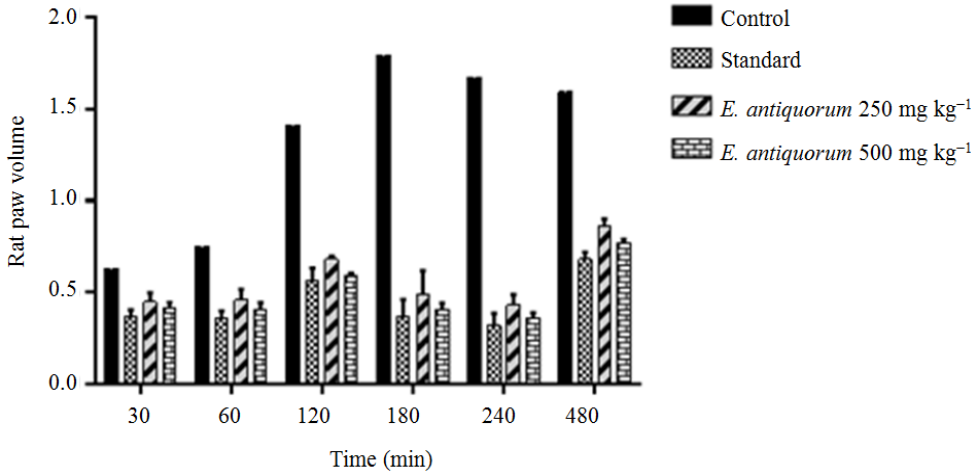

Fig. 3. Anti-inflammatory activity of *E. antiquorum* in carrageenan induced edema in rats

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	0 Hour	½ Hour	1 Hour	2 Hours	3 Hours	4 Hours
Control	6.78±0.611	6.08±0.948	6.32±0.773	6.14±0.644	5.96±0.614	5.68±0.608
Standard	5.30±0.890	7.18±1.223	9.82±1.333	11.10±1.076***	12.44±0.858***	8.14±1.433
E. antiquorum 250 mg kg^{-1}	5.62±0.399	7.40±0.436	8.46±0.571	9.88±0.675*	11.22±0.988***	8.18±0.403
E. antiquorum 500 mg kg^{-1}	7.94±1.227	10.02±0.806	10.88±0.797*	10.80±0.611**	12.42±1.200***	9.82±0.551

Data are represented as the mean ± SEM, (n = 7); *p<0.05, **p<0.01, ***p<0.001 were considered significant in comparison with control

Table 3. Effect of the *Euphorbia antiquorum* extract on acetic acid-induced writhing in mice

Treatment	Dose (mg kg^{-1})	No. of writhing	% Inhibition
Control	Vehicle	41.75±3.77	-
Diclofenac sodium	10	17.20±0.49***	58.80
E. antiquorum	250	26.25±1.43**	37.12
E. antiquorum	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

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** (41.26)	0.36±0.04 (52.00)	0.57±0.064* (59.57)	0.37±0.094** (79.33)	0.32±0.07*** (80.84)	0.68±0.04** (57.23)
E. antiquorum 250 mg kg^{-1}	0.45±0.05 (28.57)	0.46±0.06 (38.67)	0.68±0.019* (51.77)	0.49±0.13** (72.63)	0.43±0.06*** (74.25)	0.86±0.04* (45.91)
E. antiquorum 500 mg kg^{-1}	0.42±0.028** (33.33)	0.41±0.036 (45.33)	0.59±0.016* (58.16)	0.41 ±0.035*** (77.09)	0.36±0.03*** (78.44)	0.77±0.02** (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

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 nociception 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

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 also substantially suppressed the biphasic response of carrageenan-induced inflammation. So, the anti-inflammatory effect of *E. antiquorum* extract may be due to its suppressive action on prostaglandin, protease or lysosome synthesis or activity.

Phytochemical analysis of the ethanolic extract of *E. antiquorum* revealed the presence of tannins, alkaloids, flavonoids, saponins and terpenoids. Strong occurrence of tannins in extract has been shown to possess potent anti-inflammatory properties (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.

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