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Full Length Research Paper

Analgesic and anti-inflammatory effect of whole *Ageratum conyzoides* and *Emilia sonchifolia* alcoholic extracts in animal models

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This study was designed to investigate the analgesic and anti-inflammatory effects of *Ageratum conyzoides* and *Emilia sonchifolia* alcoholic extracts in animal models. Analgesic effects have been investigated in acetic acid induced writhing model and formalin induced licking model of Swiss albino mice. Anti-inflammatory effect was investigated in carrageenan induced anti-inflammatory paw edema model of Wistar albino rat. Data were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. In a dose-dependent response, *A. conyzoides* and *E. sonchifolia* extracts inhibited 49.85 and 39.47% of acetic acid induced pain at the highest dose 2.0 g/kg body weight (BW). These effects were statistically significant (P < 0.05) as compared to the reference drug, diclofenac sodium (40 mg/kg). *A. conyzoides* reduced 35.48% and *E. sonchifolia* reduced 38.70% of formalin induced pain by 2.0 g/kg which were also statistically significant (P < 0.05) as compared to morphine (0.5 mg/kg). In a time-dependent inhibition of carrageenan induced paw edema model, the extracts of *A. conyzoides* and *E. sonchifolia* promoted 50.23 and 48.11% inhibition of paw edema at the 4th hour of administration, respectively and the effects were statistically significant (P < 0.05). No mortality was observed in acute toxicity test. Observed results demonstrate an effective analgesic and anti-inflammatory potentials of the extracts to be used as complementary and alternative therapy.

Key words: *Ageratum*, *Emilia*, anti-inflammatory, analgesic, carrageenan.

INTRODUCTION

Due to having adverse side effects, like gastric lesions, caused by non-steroidal antiinflammatory drugs (NSAIDs) and tolerance and dependence induced by opiates, the use of these drugs as analgesic agents have not been successful in all the cases. Analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates in this context. The development of novel compounds having analgesic and anti-inflammatory activities with an improved safety profile is still required (Tonk et al., 2012). Therefore, the investigation of the effective plant based analgesic and anti-inflammatory drugs have been paid more attention, because plants derived medicines have made large contribution (Igbinosa et al., 2009) to human health and well being since last few decades, providing a source of inspiration for novel drugs with less expense and no side effects.

Ageratum conyzoides L. (Asteraceae) is an annual herb with a long history of traditional medicinal uses by various cultures worldwide. It has been used in leprosy and purulent ophthalmia in Asia (Katsuri et al., 1973). Its decoction or infusion is given in diarrhea, dysentery,

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intestinal colic, flatulence, rheumatism, fever and pain associated with navel in children (Chopra et al., 2002). It has been found to be bacteriocide, antidysenteric and anti-lithiatic in South America and Africa (Ekundayo et al., 1988).

In Central Africa, it has been commonly used to treat pneumonia, wounds and burns. In some other African countries, the plant has been popularly used in skin diseases, wound healing, mental retardation, infectious diseases, headaches, dyspnea, asthma and uterine troubles (Okunade, 1981). In Cameroon, it is a local remedy for craw-craw, but in Cameroon and Congo, it is used to treat fever, rheumatism and colic (Abbiw, 1990). In Brazil, the aqueous extracts of the leaves and whole plants have been used to treat colic, colds and fevers, diarrhea, rheumatism and spasms (Marques et al., 1988; Brasil et al., 1989). In Brazil, it is also known as a medicinal tea plant used as anti-inflammatory, analgesic, anti-diarrheal and as healers of gynecological diseases (Yamamoto et al., 1991; Sharma and Sharma, 1995).

Other folk remedies including anti-itch, sleeping sickness, mouthwash for toothache, antitusive, vermifuge, tonic and killing lice (flowers) are widely known (Kapur, 1993). The leaves have been used on cuts, sores, as an anti-inflammatory agent (Abena et al., 1993; Moura et al., 2005) and insecticide (Ramachandran and Nair, 1981), an antidote to snake poison (Jain and Sahu, 1993), an anti-tetanus, in headache, boils (Siddiqui and Husain, 1992), skin diseases (Sankaran and Alagesaboopathi, 1995), ringworm infection (Anuradha et al., 1986), typhoid, malarial fever, uterine problems (Singh, 1988), throat infection, painful gums, abscess for early suppuration, wound healing and leucorrhoea (Sharma et al., 1985). The root is used in lithiasis and infant diarrhea (Hemadri and Rao, 1989). Its potent nematocidal and pests control effects are also documented (Gravena et al., 1993). It is the only plant used in HIV/AIDS disease (Igoli et al., 2005). A lot of biologically important chemical components both from essential oil and crude extracts of different parts of this plant have been isolated and identified. Prominently, 51 essential oil components belonging to the mono and sesquiterpenes (Ekundayo et al., 1988), 21 flavonoids, especially the polymethoxylated flavones (Gonzalez et al., 1991), some triterpenes like friedelin, stigmasterol and spinasterol and alkaloids, basically, lycopsamine and echinatine have been extracted from this plant. Some chromene, chromone and coumarine have also been isolated from this plant (Okunade, 1981).

Emilia sonchifolia (Lin.) is a bushy annual herb distributed mainly in Asian countries (Warrier et al., 1995). It has been traditionally used as important medicinal plant in most tropical and subtropical countries, including Bangladesh. Different parts of the plant have been used in the treatment of asthma, inflammation, intermittent fever, breast cancer, ophthalmia, cuts and wounds (Chopra et al., 1986). Leaf juice is used in eye inflammation, night blindness and sore throat. Antimicrobial activity of aqueous extract and cytotoxic, anti-Ehrlich ascitic carcinoma (EAC) and anti-Dalton's lymphoma ascites (DLA) activities of alcoholic extracts have been reported (Shylesh and Padikkala, 2000). Decoction of this plant is used as a febrifuge in infantile tympanities and bowel compliant. Hydroalcoholic extract of the arial parts of *E. sonchifolia* has been reported for its antinociceptive effect (Couto et al., 2011). A preliminary study on the anti-inflammatory effect of *E. sonchifolia* has also been reported (Muko and Ohiri, 2000). Some known compounds like simiral, beta-sitosterol, stigmasterol, palmitic acid and honey acid were obtained from the whole plant of *E. sonchifolia* (Gao et al., 1993). Few pyrrolizidine alkaloids, senkirkine and doronine were isolated from the aerial parts of *E. sonchifolia (*Cheng and Röder, 1986).

Evidences showed that different parts of these plants have been used for several acute and chronic diseases in many corners of the world. However, insufficient numbers of researches have endeavored to clarify the aforementioned folk and traditional uses of these plants. This study investigated the analgesic and anti-inflammatory effects of alcoholic extracts of the whole *A. conyzoides* and *E. sonchifolia* in animal models.

MATERIALS AND METHODS

Collection of plant material

The whole *A. conyzoides* and *E. sonchifolia* plants were collected from Chittagong, Bangladesh. The plants were taxonomically identified by Dr. Shaikh Boktear Uddin, Associate Professor and Taxonomist, Department of Botany, University of Chittagong, Bangladesh. Voucher specimen (Accession no; *A. conyzoides*-36073 and *E. sonchifolia*-36075) of each has been preserved for further documentation.

Preparation of extracts

Fresh *A. conyzoides* and *E. sonchifolia* whole plants were washed with distilled water immediately after collection. The plants were chopped into small pieces, air dried at room temperature (26 ± 1)°C for about 10 days and ground into powder (grinder purchased locally) to store in an airtight container. The resulting powder (750 g) of each was extracted in ethanol for 7 days at room temperature. Extracts were individually evaporated using rotary evaporator (RE200 Bibby Sterling, UK) to dryness to give 48 g (yield 6.4% w/w) of *A. conyzoides* and 44 g (yield 5.87% w/w) of *E. sonchifolia* blackish-green pastes which were kept at 4°C until further use.

Experimental animals

Six-week-old Swiss albino mice of both sexes weighing 25 to 30 g and seven-week-old Wistar albino rats of the both sexes weighing 150 to 200 g were obtained from animal house of Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratories, Chittagong. The animals were housed individually in stainless steel wire meshed plastic cages in a controlled temperature (25 ± 2)°C and humidity (55 to 60%) controlled room with a 12 h light-dark cycle. The animals were supplied with standard rat pellet diet and drinking water *ad libitum* during the entire period of the study. Maintenance of animals and experimentations were carried out

according to the regulations of the Institutional Animal Ethics Committee (034- 2011/Animal).

Reagents and drugs

Acetic acid, morphine hydrochloride (99.50%) and absolute ethanol were purchased from Sigma-Aldrich, Munich, Germany. Morphine was dissolved in saline solution just before use. Diclofenac sodium (powder form) was kindly donated by GlaxoSmithKline Ltd., Bangladesh. A commercially available concentrated formalin solution (Sigma-Aldrich, Munich, Germany) was diluted with saline to the appropriate concentration (2.5%). Carrageenan (lambda form, FMC Marine Colloids Division, Sigma-Aldrich, Poole, UK) was used for paw edema test.

Assay for analgesic effect

Acetic acid induced writhing test

The analgesic activity of *A. conyzoides* and *E. sonchifolia* extracts were measured by the acetic acid induced writhing test in Swiss albino mice as described by Koster et al. (1959). Briefly, the inhibitions of writhing produced by the extracts were determined by comparing the inhibitions produced by the control group. Diclofenac sodium at a dose of 40 mg/kg (BW) was used as standard analgesic agent (positive control). Intraperitoneal injection of (1%) acetic acid at a dose of 2.3 ml/kg was used to create pain sensation. The number of writhing and stretching was counted over 20 min (20 min after the application of acetic acid). The extracts and distilled water (control) were administered orally 30 min before acetic acid injection as treatment and control respectively. The percent of analgesic action was determined by the following formula.

$$\text{Analgesic effect (\%)} = \frac{\text{Mean writing count (control group-treated group)}}{\text{Mean writhing count of control group}} \times 100$$

Formalin test

The procedure was similar to that described by Gaertner et al. (1999). The plant extracts (1.0, 1.5 and 2.0 g/kg), reference drug morphine (0.5 mg/kg) and distilled water were administered orally 30 min before formalin injection. A 20 µl of 2.5% formalin (0.92% formaldehyde) made in phosphate buffer (pH 7.4) was injected under the right hind paw surface of the experimental mice. Each mouse was placed individually in a cage and observed from 0 to 5 min, followed by the injection of formalin to analyze the first phase of formalin induced pain (neurogenic pain). The length of time the animal spent for licking the injected paw was counted with a chronometer and was considered as location of pain. The percentage of inhibition was calculated by the following formula:

$$\text{Inhibition (\%)} = \frac{L_{control} - L_{treated}}{L_{control}}$$

where, L represents licking.

Assay for anti-inflammatory effect

Anti-inflammatory activities of two extracts were determined by using carrageenan induced paw edema test in the hind paw of rat as reported previously (Winter et al., 1962). Briefly, the initial volume of right hind paw of each rat was measured using plethysmometer (7150, UGO Basile, Italy) and then 0.1 ml of 1% (w/v) carrageenan was subcutaneously injected into the subplantar region of right hind paw in order to induce acute inflammation. The extracts (0.5, 1.0 and 1.5 g/kg), standard anti-inflammatory drug diclofenac sodium (40 mg/kg) and distilled water were administered orally 30mins before the subplantar injection of carrageenan to treated, positive control and normal control groups, respectively. The volume of right hind paw was measured at 1st, 2nd, 3rd and 4th hour after the carrageenan injection. The inhibition of paw edema was calculated according to the following formula:

$$\text{Inhibition (\%)} = \frac{(Ct - Co)\ \text{control} - (Ct - Co)\ \text{treated}}{(Ct - Co)\ \text{control}} \times 100$$

where C_t is the right hind paw thickness volume (in mm^3) at time t and C_o is the right hind paw thickness volume (in mm^3) before carrageenan injection.

Acute toxicity test

Wistar albino rats maintained under standard laboratory condition were used for acute toxicity study. A total of five animals received a single oral dose (0.5, 1.0, 2.0, 3.0 and 4 g/kg BW) of the extract has been used. Animals were fasted over-night prior to administration. After administration of the extracts, food was withheld for further 3 to 4 h. Animals were observed individually once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observation including changes in skin and fur, eyes and mucous membrane, respiratory and circulatory rate, autonomic and central nervous system (CNS) changes were made (Zaoui et al., 2002).

Statistical analysis

Values for analgesic activity were expressed as "mean increase in latency after drug administration ± standard deviation (SD)" in terms of seconds, whereas values for anti-inflammatory activity were expressed as "mean increase in paw volume ± SD". Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. The values were considered significantly different at $P < 0.05$.

RESULTS

The extracts showed a dose-dependent analgesic actions on acetic acid induced writhing response of abdominal stretching (Table 1). This analgesic effect in terms of writhing response was significant ($P < 0.05$) at all the doses of the extract in comparison to the control group; however, the activity was less as compared to diclofenac sodium. *A. conyzoides* extract was found more effective than *E. sonchifolia*.

The results of the present study revealed that the extracts significantly ($P < 0.05$) reduced the licking time inhibiting the formalin induced pain (Table 2). The percentage inhibition for morphine was much higher than that of both extracts; however, effect of *E. sonchifolia* extract was higher in the reduction of licking time than that of *A. conyzoides*.

Table 1. Effect of *A. conyzoides* and *E. sonchifolia* extracts on acetic acid-induced writhing response in mice.

Dose	Writhing response (count/20 min)		Analgesic (%)	
	ACEx	ESFx	ACEx	ESFx
Control	69.0 ± 1.9[a]	76.0 ± 0.70[a]	-	-
1.0 g/kg	55.0 ± 1.3[b]**	61.0 ± 1.0[b]*	20.28	19.73
1.5 g/kg	45.2 ± 0.9[c]***	55.0 ± 1.3[c]*	34.49	27.63
2.0 g/kg	34.6 ± 2.0[d]***	46.0 ± 1.1[d]*	49.85	39.47
DS40 (mg/kg)	16.5±1.3[e]***		76.09	

Data are shown as mean ± SD of five animals in each group. ACEx: *A. conyzoides* extract; ESFx: *E. sonchifolia*; DS: Diclofenac sodium. Values with superscript letters b-e in each column are significantly different from each other.The value of superscript letter a for control. Data were analyzed by one-way ANOVA followed as compared to Tukey's post hoc test for multiple comparisons (significance level, ***P < 0.00, **P < 0.01, *P < 0.05).

Table 2. Effect of *A. conyzoides* and *E. sonchifolia* extracts on formalin-induced licking response in mice.

Dose	Licking time (min)		Inhibition (%)	
	ACEx	ESFx	ACEx	ESFx
Control	74.4 ± 1.15	74.4 ± 1.15	-	-
1.0 g/kg	61.0 ± 1.0[a]*	59.4 ± 1.7[a]*	18.01	20.16
1.5 g/kg	55.0 ± 1.6[b]*	51.8 ± 1.2[b]*	26.07	30.37
2.0 g/kg	48.0 ± 1.4[c]*	45.6 ± 3.4[c]*	35.48	38.70
MP (0.5 mg/kg)	39.0±0.7[d]*		50.00	

Data are shown as mean ± SD of five animals in each group. ACEx: *A. conyzoides* extract; ESFx: *E. sonchifolia*; DS: Diclofenac sodium; MP: Morphine. Values with superscript letters are significantly different as follows: a, b and c as compared to control and b as compared to positive control. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test for multiple comparisms (significance level, *P < 0.05).

Table 3. Effect of *A. conyzoides* extract on the increase of carrageenan-induced paw edema in albino rat.

Dose	Hind paw edema (mm^3)			
	1 h	2 h	3 h	4 h
Control	0.37±0.0	0.64 ± 0.0	0.76 ± 0.0	0.88 ± 0.1
ACEx 0.5 g/kg	-	0.56 ± 0.0[ns]	0.65 ± 0.0	0.69 ± 0.0[ns]
ACEx 1.0 g/kg	0.31 ± 0.0[ns]	0.50 ± 0.0[ns]	0.55 ± 0.0[a]*	0.60 ± 0.0[a]*
ACEx 1.5 g/kg	0.28 ± 0.0[ns]	0.38 ± 0.0[b]**	0.44 ± 0.0[b]*	0.44 ± 0.0[b]*
DS (40 mg/kg)	0.23 ± 0.0[c]*	0.35 ± 0.0[c]*	0.32 ± 0.0[c]**	0.35 ± 0.0[c]*

Data are shown as mean ± SD of five animals in each group. ACEx: *A. conyzoides* extract; DS: Diclofenac sodium, ns=not significant as compared to control and positive control. Values with superscript letters are significantly different as follows: a and b as compared to control and c as compared to positive control. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons (significance level *P < 0.05). "(-)" Indicates no paw edema.

Tables 3 and 4 showed a time-dependent increase of rat hind paw edema with the administration of carrageenan along with extracts and diclofenac sodium. However, paw edema was inversely increased with the increase of extract dose. Reduction of paw edema was significant (P < 0.05) at the doses of 1.0 and 1.5 g/kg as compared to the control. Effect of *A. conyzoides* extract was comparable with diclofenac sodium (Table 4).

Table 4. Effect of *E. sonchifolia* extract on the increase of carrageenan induced paw edema in albino rat.

Dose	Hind paw edema (mm^3)			
	1 h	2 h	3 h	4 h
Control	0.85 ± 0.02	0.88 ± 0.02	0.78 ± 0.02	0.69 ± 0.00
ESFx 0.5 g/kg	-	0.79 ± 0.02^{a}*	0.70 ± 0.03^{ns}	0.61 ± 0.02^{ns}
ESFx 1.0 g/kg	0.75 ± 0.00^{b}**	0.76 ± 0.01^{b}**	0.62 ± 0.01^{b}***	0.51 ± 0.02^{b}***
ESFx 1.5 g/kg	0.71 ± 0.02^{c}**	0.68 ± 0.01^{c}***	0.51 ± 0.01^{c}***	0.35 ± 0.01^{c}***
DS (40 mg/kg)	0.23 ± 0.0^{d}***	0.35 ± 0.0^{d}***	0.32 ± 0.0^{d}***	0.35 ± 0.0^{d}***

Data are shown as mean ± SD of five animals in each group. ESFx: *E. sonchifolia* extract; DS: Diclofenac sodium, ns=not significant compared to control and positive control. Values with superscript letters are significantly different as follows: a, b and c as compared to control and d as compared to positive control. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons (Significance level, ***P < 0.001, **P < 0.01, *P < 0.05). "(-)" Indicates no paw edema.

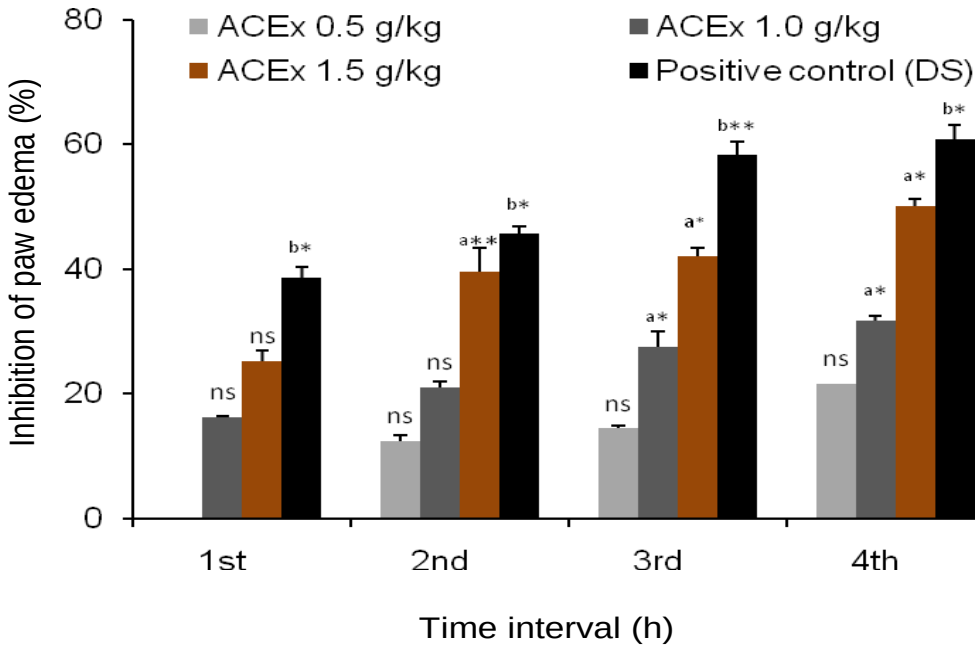

Figure 1. Percentage inhibition (%) of paw edema in time–dependent doses of *A. conyzoides* extract. Data are shown as mean ± SD of five animals in each group. ACEx: *A. conyzoides* extract; DS: Diclofenac sodium, ns=not significant compared to control and positive control. Values with superscript letters are significantly different as follows: a as compared to control and b (positive control). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons (Significance level, *P < 0.05, **P < 0.01).

The extracts also showed a time-dependent anti-inflammatory action in carrageenan induced paw edema model (Figures 1 and 2). Highest anti-inflammatory effects were achieved by 1.5 g/kg of both extracts at the 4th hour of administration. *A. conyzoides* promoted 50.23% and *E. sonchifolia* promoted 48.11% of inhibition which were statistically significant (P < 0.05) as compared to the control.

DISCUSSION

This research was carried out to evaluate the analgesic and anti-inflammatory effect of whole ethanolic extracts of *A. conyzoides* and *E. sonchifolia.* Both extracts showed a dose-dependent response with significant inhibition of acetic acid and formalin induced pain. The extracts also showed a time-dependent inhibition of carrageenan

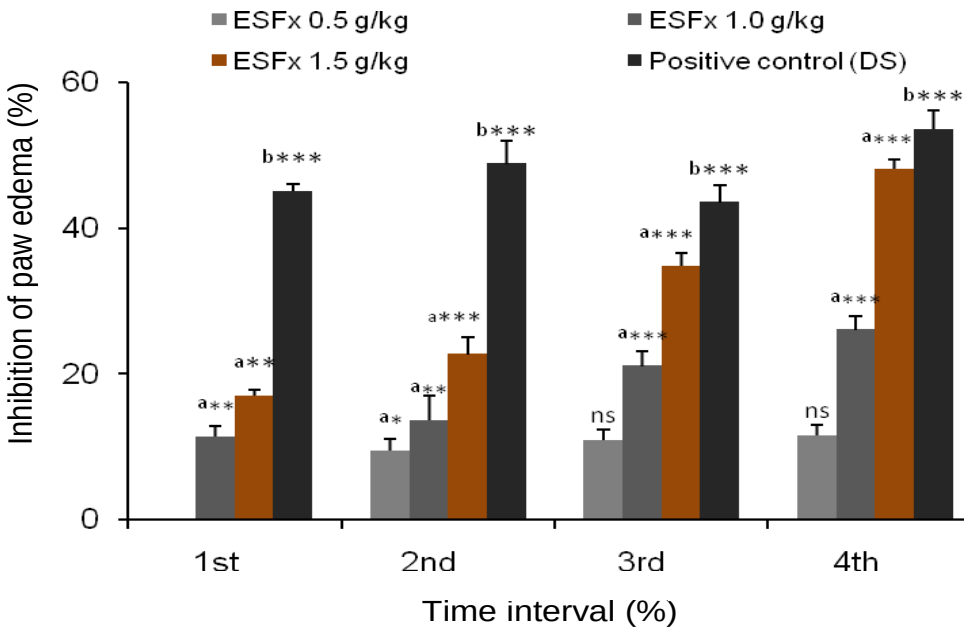

Figure 2. Percentage inhibition (%) of paw edema in time–dependent doses of *E. sonchifolia* extract. Data are shown as mean ± SD of five animals in each group. ESFx: *E. sonchifolia* extract; DS: Diclofenac sodium, ns=not significant compared to control (positive control). Values with superscript letters are significantly different as follows: a as compared to b positive control. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons (Significance level, *P < 0.05, **P < 0.01, ***P < 0.001).

induced paw edema. *A. conyzoides* was found more effective than *E. sonchifolia.* No toxicity was found for the extracts.

Acetic acid induced writhing in mice attributed visceral pain, finds much attention of screening analgesic drugs (Hasan et al., 2010). The writhing method was found effective to evaluate peripherally active analgesics. The crude extracts of both plants (*A. conyzoides* and *E. sonchifolia*) showed significant analgesic action as compared to the reference drug diclofenac sodium, but *A. conyzoides* was found to exhibit higher analgesic activity than *E. sonchifolia* against acetic acid induced pain in mice at all the dose levels, that is, 1.0, 1.5 and 2.0 g/kg BW.

Acetic acid induced writhing method is very sensitive and able to detect anti-nociceptive effects of compound(s) at dose level that may appear to be inactive in other methods, like tail flick test. Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipid (Ahmed et al., 2006) via cyclooxygenase (COX) and prostaglandin biosynthesis (Duarte et al., 1988). In other words, the acetic acid induced writhing has been associated with increased level of prostanoids, especially PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products (Dhara et al., 2000). The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability (Zakaria et al., 2008). This prostaglandin synthesis is inhibited, through a peripheral mechanism of pain inhibition, by the agents which reduce the number of writhing substantially render the analgesic effect (Ferdous et al., 2008).

The significant pain reduction of both plant extracts might be due to the presence of analgesic principles, achieved by the chemical constituents of the extracts, acting with the prostaglandin pathways. It was found that the extract of *A. conyzoides* was possessed with some phytochemical metabolites, especially flavonoids (Moreira et al., 2007) which were reported to have a role in analgesic activity primarily by targeting prostaglandins (Rajnarayana et al., 2001). Both *A. conyzoides* and *E. sonchifolia* were also documented to present triterpenes and alkaloids (Gao et al., 1993), while alkaloids and tannins are well known for their ability to inhibit pain perception (Uche et al., 2008; Vanu et al., 2006).

Formalin test is a useful vehicle, particularly for the screening of novel compounds, since it encompasses inflammatory, neurogenic and central mechanisms of nociception (Lee et al., 2000). The test is sensitive for various classes of analgesic drugs of two distinct phases, reflecting different types of pain. The early phase (initial pain) conducted by our study, reflects a direct effect of formalin on nociceptors (neurogenic pain), while centrally acting narcotics inhibit both the phases and peripherally

acting drugs inhibit only the late phase of formalin induced pain (Hunskaar and Hole, 1987; Elisabetsky et al., 1995). However, the effects of the extracts used in this study were reflected through the comparable reduction, with analgesic drug morphine, of the time spent in licking and biting of the injected paw after the administration of *A. conyzoides* and *E. sonchifolia* extracts.

Carrageenan induced rat paw edema model is a suitable test for evaluating anti-inflammatory properties for natural drugs, because of its sensitivity in detecting orally active anti-inflammatory agents, particularly in the acute phase of inflammation (DiRosa et al., 1971). Development of edema in the paw of rat after injection of carrageenan is a biphasic event (Vinegar et al., 1969). The initial phase observed during the first hour is attributed to the release of histamine and serotonin. The second phase of edema is due to the release of prostaglandins, protease and lysosome (Asongalem et al., 2004; Silva et al., 2005). This leads to a dilation of the arterioles and venules to an increased vascular permeability which consequently makes edema (Ozaki, 1990). Although, the mediators including histamine, 5-HT, the kinins and their complements, have become the recent focus of attention as they are the metabolites of arachidonic acid (AA). Alone or in appropriate combination, AA products of COX pathway are capable of producing the characteristic signs of inflammation, vasodilatation, hyperemia, pain, edema and cellular filtration. The COX products, particularly prostaglandin E2 (PGE2), contribute to increased blood flow through a vasodilatation action, but the lipooxygenase (LOX) pathway is necessary for vascular leakage and edema consequently on cellular infiltration. In our study, carrageenan induced inflammation was significantly ($P < 0.05$ and $P < 0.01$) reduced in all phases of the experiments by treatment with extracts, but *A. conyzoides* produced more pronounced effects than *E. sonchifolia* which is very compatible with their local uses. Whatever the mechanism, it is assumed that at least some of the earlier discussed mediators, either partially or completely, are subjects of inhibition by the extracts of *A. conyzoides* and *E. sonchifolia*. No toxicity or abnormality in acute toxicity test supports the safe use of the extracts.

Conclusion

The analgesic and anti-inflammatory effect against the chemical models of these extracts established the scientific basis of the use of *A. conyzoides* and *E. sonchifolia* as popular folklore medicine. Although, the mechanisms are not studied in this research, but the active secondary metabolites present in crude extracts perhaps operate in a synergistic manner to show such effects. Further study is suggested to identify the chemical constituents linked mechanism of action.

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