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Antioxidant, Analgesic and Anti-inflammatory Activities of the Herb *Eclipta prostrata*

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ABSTRACT

The present study was designed to investigate the antioxidant, analgesic and anti-inflammatory potential of the methanolic extract along with its organic soluble fractions of the herb *Eclipta prostrata*. In addition, total phenolic and flavonoid content and total antioxidant capacity were also determined. Antioxidant potential of the extract/fractions was evaluated by DPPH (1,1diphenyl-2-picrylhydrazyl), NO (nitric oxide) and $ONOO^-$ (peroxynitrite) scavenging assay method. Ethyl acetate fractions (EtOAc) showed highest scavenging activity in all the methods with IC_{50} value of 12.98±0.08, 45.98±0.07 and 14.45±0.18 μg mL^{-1} for DPPH, NO and $ONOO^-$ assay method, respectively. In reducing power assay, EtOAc fraction also showed significant ($p<0.001$) activity. Further, the extract/fractions were studied for their analgesic (hot plate, tail immersion and acetic acid induced writhing test) and anti-inflammatory (carrageenan induced paw edema in rats) activities at a dose level of 200 and 400 mg kg^{-1} body weight. Among all the extract/fractions, EtOAc fraction showed a dose dependent and significant ($p<0.005$, $p<0.05$) analgesic activity in all the tested method. EtOAc fractions also reduced the paw edema considerably (86.80% inhibition after 3 h, $p<0.005$, $p<0.05$) in dose dependent manner compared to carrageenan induced rat. Altogether, these results suggest that the MeOH extract and its organic soluble fractions could be used as a potential antioxidant, analgesic and anti-inflammatory agent.

Key words: Free radical, antinociceptive, inflammation, *Eclipta prostrata*

INTRODUCTION

Endogenous free radicals such as superoxide, nitric oxide and hydroxyl free radicals are produced in the human body during the bio-chemical reactions inside the body everyday. In recent years, oxygen derived free radicals have been reported to be closely involved in many biological symptoms such as inflammation, cancer, atherosclerosis and coronary heart disease (Kourounakis *et al*., 1999; Gulcin *et al*., 2002). Various free radicals are also responsible for the induction of short term algesia (Chung, 2004). Moreover, free radicals play an important role in the pathogenesis of inflammation (Winrow *et al*., 1993). Inflammation is the response to injury of cells

and body tissues through different factors such as infections, chemicals, thermal and mechanical injuries (Oyedapo *et al.*, 2008). Various endogenous mediators like histamine, serotonin, bradykinin, prostaglandins etc are most abundant in inflammatory cell and among them prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation. These mediators even in small quantities can elicit pain response. Pain results in dropped muscular activities. Most of the anti-inflammatory drugs now available are potential inhibitors of cyclooxygenase (COX) pathway of arachidonic acid metabolism which produces prostaglandins. Prostaglandins are hyperalgesic, potent vasodialators and also contribute to erythema, edema and pain. Hence, for treating inflammatory diseases analgesic and anti-inflammatory agents are required. (Anilkumar, 2010). Non steroidal anti-inflammatory drugs (NSAIDs) are the most clinically important medicine used for the treatment of inflammation related diseases like arthritis, asthma and cardiovascular disease (Conforti *et al.*, 2009). Having various and severe adverse effects like gastric lesions for NSAIDs, adverse cardiovascular thrombotic effects for selective cyclooxygenase-2 (COX-2) inhibitors (Chowdhury *et al.*, 2009) and tolerance and dependence induced by opiates, use of these drugs as anti-inflammatory and analgesic agents have not been successful in all the cases. Therefore, new anti-inflammatory and analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs (Gupta *et al.*, 2006).

Eclipta prostrata belongs to Astraceae family and very common in tropical and subtropical regions. The herb has been used in the treatment of infective hepatitis in India (Wagner *et al.*, 1986) and snake venom poisoning in Brazil (Melo *et al.*, 1994) and hyperlipidemia in traditional medicine (Kumari *et al.*, 2006). It has been reported that the leaves of this herb are used in case of gastritis and respiratory disorders like cough and asthma (Kobori *et al.*, 2004), hepatic and spleen enlargement and jaundice, antibacterial, antifungal, antimyotoxic and antihemorrhagic (Wiart *et al.*, 2004). In addition, the crude form of the herb is reported to have anti hepatotoxic (Wong *et al.*, 1988) and anti-inflammatory activity and also used in skin diseases (Chandra *et al.*, 1987).

In the previous study, the crude hexane, ethanol and aqueous extract of the leaves of *Eclipta prostrata* was evaluated for its antioxidant and antibacterial activity (Karthikumar *et al.*, 2007). Therefore, the present study was planned to evaluate the possible analgesic, anti-inflammatory and antioxidant potential of methanolic extract along with its organic soluble fractions (dichloromethane and ethylacetate) of the aerial part of *Eclipta prostrata* in different experimental models.

MATERIALS AND METHODS

Plant materials: The aerial parts of the *Eclipta prostrata* were collected from the village Kachuria under Mollahat thana of Khulna district, Bangladesh during the month of October 2009. The sample was identified by Mrs. Mahmuda Begum, Senior Scientific Officer, Bangladesh National Herbarium, Dhaka, where the voucher specimen has been deposited. Its DACB Accession Number is BD/2889.

Chemicals: Ammonium molybdate, Folin-chiocaltu phenol reagent, were purchased from E. Merck (Germany). 1,1-diphenyl- 2-picryl-hydrazyl (DPPH), ascorbic acid, quercetin, and potassium ferric

cyanide carraggenan, and DL-penicillamine (DL- 2- amino- 3- mercapto- 3- methylbutanoic acid) were purchased from Sigma Chemical Company (St. Louis, MO, USA). The high quality DCFH-DA and DHR 123 (dihydrorhodamine 123) and $ONOO^-$ were purchased from Molecular Probes (Eugene, Oregon, USA) and Cayman (Ann Arbor, MI, USA), respectively. Diclofenac-Na, and Indomethacin were collected from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals and reagents were of analytical grade.

Preparation of plant extract: The aerial parts of the *Eclipta prostrata* were dried in an oven at 37°C and then powdered with a mechanical grinder, passing through sieve No. 40 and stored in an air tight container. The dried powdered material (1.0 kg) was refluxed with MeOH for 3 h. The total filtrate was concentrated to dryness, *in vacuo* at 40°C to render the MeOH extract (300 g). This extract was suspended in H_2O and then successively partitioned with dicholoromethane (CH_2Cl_2) and ethylacetate (EtOAc), to afford the CH_2Cl_2 (120 g) and EtOAC (40 g), fractions along with a residue (120 g) present in aqueous phase.

Animal: Albino mice (25-30 g) and Wistar rats (175-250 g) of both sexes were used for assessing biological activity. The animals were maintained under standard laboratory conditions and had free access to food and water *ad libitum*. The animals were allowed to acclimatize to the environment for 7 days prior to experimental session. The animals were divided into different groups, each consisting of five animals which were fasted overnight prior to the experiments. Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethics Committee, Atish Dipankar University of Science and Technology, Dhaka, Bangladesh.

Acute toxicity study: Acute oral toxicity assay was performed in healthy nulliparous and non pregnant adult female albino Swiss mice (25-30 g) and albino Wistar rats (175-250 g) divided into different groups. The test was performed using increasing oral dose of the MeOH extract and different organic fractions in water (250, 500, 1000 and 1500 mg kg^{-1} body weight), in 20 mL kg^{-1} volume to different test groups. Normal group received water. The mice and rats were allowed to feed *ad libitum,* kept under regular observation for 48 h, for any mortality or behavioral changes.

***In vitro* antioxidant activity**

The amount of phenolic compounds and flavonoids: The total phenolic and flavonoid content of methanolic extract and several organic fractions were determined using Folin-ciocalteu reagent (Yu *et al*., 2002) and aluminium chloride colorimetric method (Chang *et al*., 2002), respectively. The content of total phenolics in the extract and fractions of *T. dioica* was calculated from regression equation of the calibration curve ($y = 0.013x+0.127$, $r^2 = 0.988$) and is expressed as galic acid equivalents (GAE) and the flavonoid contents of the extract and fractions in terms of quercetin equivalent (the standard curve eqation: $y = 0.009x-0.036$).

Determination of total antioxidant capacity: The antioxidant activity of the MeOH extract and several other organic fractions were evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al*. (1999).

Free radical scavenging activity measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH): The free radical scavenging activity of MeOH extract and other fractions, based on the scavenging

activity of the stable 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca *et al.* (2001). The percentage inhibition activity was calculated from:

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

where, A_0 is the absorbance of the control and A_1 is the absorbance of the extract/ standard. IC_{50} value was calculated from the equation of line obtained by plotting a graph of concentration ($\mu g\ mL^{-1}$) versus % inhibition.

Nitric oxide radical scavenging assay: The procedure is based on the method (Sreejayan and Rao, 1997), where sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. IC_{50} value was calculated from the equation of line obtained by plotting a graph of concentration ($\mu g\ mL^{-1}$) versus % inhibition.

Measurement of the $ONOO^-$ scavenging activity: The $ONOO^-$ scavenging activity was measured by monitoring the oxidation of DHR 123, by modifying the method of Kooy *et al.* (1994).

Reducing power activity: The reducing power of *E. prostrata* was determined according to the method previously described by Oyaizu (1986). Increased absorbance of the reaction mixture indicated increased reducing power.

Analgesic activity

Hot plate method: The animals were divided into four groups with five mice in each group. Group I animals received vehicle (1% Tween 80 in water, 10 mL kg^{-1} body weight), animals of Group II received Diclofenac-Na at 10 mg kg^{-1} body weight while animals of Group III and Group IV were treated with 200 and 400 mg kg^{-1} body weight (p.o.) of the crude extract/fractions of *E. prostate*. The animals were placed on Eddy's hot plate kept at a temperature of (55±0.5)°C. A cut off period of 15 second, was observed to avoid damage to the paw (Franzotti *et al.*, 2000). Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to 0, 30, 60 and 90 min after oral administration of the samples (Eddy and Leimback, 1953; Malairajan *et al.*, 2006; Toma *et al.*, 2003).

Tail immersion test: The procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice (Toma *et al.*, 2003).

Acetic acid-induced writhing test: The analgesic activity of the samples was also studied using acetic acid-induced writhing model in mice (Ahmed *et al.*, 2004).

Anti-inflammatory activity

Carrageenan induced paw edema test in rats: Male Wistar rats (175-250 g) were divided into four groups of five animals each. The test groups received 200 and 400 mg kg^{-1}, p.o. of the extract. The reference group received indomethacin (10 mg kg^{-1}, p.o.) while the control group received

3 mL kg^{-1} of distilled water. After 1 h, 0.1 mL, 1% w/v carrageenan suspension in normal saline was injected into the subplantar tissue of the right hind paw (Winter *et al.*, 1962). The paw volume was measured at 1, 2 and 3 h after carrageenan injection using a micrometer screw gauge. The percentage inhibition of the inflammation was calculated from the formula:

$$\% \text{ inhibition} = (1 - D_t/D_0) \times 100$$

where, as D_0 was the average inflammation (hind paw edema) of the control group of rats at a given time, D_t was the average inflammation of the drug treated (i.e., extract/fractions or reference indomethacin) rats at the same time (Gupta *et al.*, 2005).

Statistical analysis: All values were expressed as the mean±standard error of three replicate experiments. The analysis was performed by using student's t test. $p<0.001$, $p<0.005$ and $p<0.05$ were considered to be statistically significant.

RESULTS

Acute toxicity study: The extract/fractions of *E. prostrata* were safe up to a dose of 1500 mg kg^{-1} (p.o.) body weight. Behavior of the animals was closely observed for the first 3 h then at an interval of every 4 h during the next 48 h. All extract/fractions did not cause mortality in mice and rats during 48 h observation but little behavioral changes, locomotor ataxia, diarrhea and weight loss were observed. Food and water intake had no significant difference among the group studied.

In vitro antioxidant activity

Total phenolic and flavonoid contents: Table 1 represents the content of both groups in the extract and different organic soluble fractions. The content of total phenolics in the extract and fractions of *E. prostrata* was determined using the Folin-ciocalteu assay, calculated from regression equation of the calibration curve ($y = 0.013x \pm 0.127$, $r^2 = 0.988$) and is expressed as Galic Acid Equivalents (GAE) and the flavonoid contents of the extract and fractions in terms of quercetin equivalent (the standard curve eqation: $y = 0.009x-0.036$). EtOAc fractions showed the highest total phenolic and flavonoid content and was found to be 99.43±1.01 mg g^{-1} plant extract (in GAE) and 200.43±0.72 mg g^{-1} plant extract (in quercetin equivalent), respectively.

Total antioxidant capacity: Percentage yield of methanol extract and different organic fractions of *E. prostrata* and their total antioxidant capacity are given in Table 1. Total antioxidant capacity

Table 1: Yield, total amount of plant phenolic compounds, flavonoids and total antioxidant capacity of methanolic extract and soluble organic fraction of *Eclipta prostrata*

Sample	Yield (%)	Total phenols mg g^{-1} plant extract (in GAE)[a]	Total flavonoids mg g^{-1} plant extract (in QA)[b]	Total antioxidant capacity mg g^{-1} extract (in ASC)[c]
MeOH	32.66	49.64±0.12	105.39±0.32	320.54±0.69
CH_2Cl_2	13.33	9.24±0.19	18.91±0.41	84.92±1.01
EtOAc	4.00	99.43±0.02	200.43±0.23	589.95±0.45

[a]Gallic acid equivalents (GAE, mg g^{-1} of each extract) for the total phenolic content.[b] Quercetin equivalents (mg g^{-1} of each extract) for the total flavonoid content.[c] Ascorbic acid equivalents (mg g^{-1} of each extract) for the total antioxidant capacity. The GAE, QA and ASC values are expressed as Means±SEM of triplicate experiments

of *E. prostrata* is expressed as the number of equivalents of ascorbic acid. Total antioxidant capacity of EtOAc fractions showed the highest and was found to be 589±0.95 mg g^{-1} equivalent of ascorbic acid.

DPPH radical scavenging activity: Table 2 represents the DPPH radical scavenging activity. EtOAc fractions showed the highest DPPH scavenging activity with the IC_{50} value of 12.98±0.08 μg mL^{-1}, followed by MeOH and CH_2Cl_2 fractions with the IC_{50} value of 45.32±0.16 and 89.45±0.23 μg mL^{-1}, respectively. EtOAc fractions showed the similar activity as standard ascorbic acid (IC_{50} 12.76±0.11 μg mL^{-1}).

Nitric oxide (NO•) scavenging activity: Table 2 describes that all the extracts are likely to have the nitric oxide scavenging activity and statistically significant ($p<0.001$). The range of IC_{50} values were 45.98±0.07 to 149.34±0.72 μg mL^{-1}.

Peroxynitrite ($ONOO^-$) scavenging activity: The $ONOO^-$ scavenging activity was measured by monitoring the oxidation of DHR 123. The MeOH extract and its organic soluble fractions exhibited significant $ONOO^-$ scavenging effects in a dose-dependent manner, with IC_{50} values of 14.45±0.18 μg mL^{-1} for EtOAc fraction and 34.97±0.21 for MeOH extract, whereas, penicillamine a well known $ONOO^-$ scavenger, with an IC_{50} value of 6.98±0.32 μg mL^{-1} (Table 2).

Reducing power ability: For the measurement of the reductive ability, we investigated the Fe^{3+} to Fe^{2+} transformation in the presence of extract and organic fractions. Like the antioxidant activity, the reducing power of *E. prostrata* increased with increasing concentration of the sample. Figure 1 shows the reductive capabilities of the *E. prostrata* compared with quercetin, galic acid and ascorbic acid. All *E. prostate* extract/fractions concentrations tested showed higher activities and these differences were statistically significant ($p<0.001$).

Analgesic activity

Hot plate test: In the hot plate test, the results presented in Table 3 showed that all extract/fractions significantly ($p<0.05$) increased pain threshold of mice in a dose dependent manner. The analgesic effect of EtOAc fraction become stronger in 90 min after drug administration, with similar pain inhibition intensity of Diclofenac-Na at 10 mg kg^{-1}, body weight while, the MeOH and CH_2Cl_2, extract/fractions at dose of 400 mg kg^{-1} b.wt. exhibited prominent analgesic activity.

Table 2: Antioxidant activities of the *E. prostrata* extract on DPPH, $ONOO^-$ and NO

Sample	[a]DPPH IC_{50} (μg mL^{-1})	[b]$ONOO^-$ IC_{50} (μg mL^{-1})	[c]NO IC_{50} (μg mL^{-1})
MeOH	45.32±0.22*	34.97±0.61*	75.89±0.15*
CH_2Cl_2	89.45±0.12	> 80	149.34±0.72#
EtOAc	12.98±0.08*	14.45±0.18**	45.98±0.07**
Ascorbic acid	12.76±0.11		5.47±0.22
Quercetin			15.24±0.12
L-penicillamine		6.98±0.32	

[a]DPPH is the free radical scavenging activity (IC_{50}: μg mL^{-1}), [b]$ONOO^-$ is the inhibitory activity of authentic peroxynitrite (IC_{50}: μg mL^{-1}), [c]NO is the inhibition of NO production (IC_{50}: μg mL^{-1}), *$p<0.001$ by student's test for values between the sample and the control. **$p<0.005$ by student's test for values between the sample and the control. # Not significant

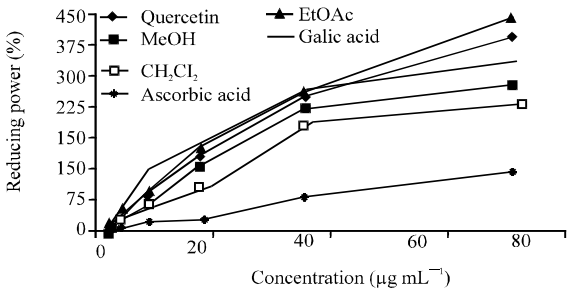

Fig. 1: Reducing power of MeOH extract and fractions of *E. prostrata* and standards (ascorbic acid, quercetin and galic acid) by spectrophotometric detection of Fe^{3+} to Fe^{2+} transformation. Results are mean±SEM of three parallel measurements

Table 3: Effect of *E. prostrata* extract/fractions on hotplate latency in mice

		Mean latency (sec)				Inhibition (%)		
Groups	Dose (mg kg^{-1})	0 min	30 min	60 min	90 min	30 min	60 min	90 min
Control	Vehicle	2.71±0.190	2.79±0.271	2.65±0.222	2.82±0.169			
Diclofenac-Na	10	2.81±0.286	8.12±0.299*	11.81±0.829*	15.05±0.361*	65.64	77.56	81.26
MEP1	200	2.84±0.237	5.27±0.820*	7.12±0.681*	11.1±0.535*	47.06	62.78	74.59
MEP2	400	2.98±0.213	5.23±0.832*	7.78±0.698*	11.45±0.809*	46.65	65.94	75.37
CEP1	200	2.13±0.317	4.12±0.797*	6.23±1.291*	9.75±0.313*	32.28	57.46	71.08
CEP2	400	2.38±0.452	4.41±0.644*	6.76±0.392*	10.01±1.350*	36.73	60.80	71.83
EEP1	200	2.43±0.236	6.30±0.353*	9.87±0.354*	14.08±0.245*	55.71	73.15	79.97
EEP2	400	2.78±0.105	7.01±0.472*	10.56±0.644*	14.84±0.482*	60.20	74.91	81.00

Values are Mean±SEM (n = 5); *p<0.05 by student's test for values between the sample and the vehicle treated group. MEP: Methanolic extract, CEP: Dichloromethane fraction, EEP: Ethylacetate fraction of E. prostrata. 1: 200 mg kg^{-1} b.wt, 2: 400 mg kg^{-1} b.wt

Tail immersion test: Table 4 depicts the analgesic activity shown by extract/fractions by tail flick method. The extract/fractions showed dose dependent analgesic activity against radiant heat induced algesia. High doses (400 mg kg^{-1}) showed significant difference in the analgesic activity when compared with control group. Maximum analgesic effect was observed at 90 min interval. The result was statistically significant ($p<0.005$).

Acetic acid induced writhing in mice: The extract/fractions effectively reduced the number of abdominal muscle contractions induced by 0.7% acetic acid solution. EtOAc fractions had a dose dependent protection ranging from 45.69 to 68.78% at 200 and 400 mg kg^{-1} b.wt., respectively against vehicle treated control group and the result were statistically significant ($p<0.05$) in chemically induced algesia (Fig. 2).

Anti-inflammatory activity

Carrageenan induced paw edema test: Table 5 shows the results of the anti edematous effect of orally administered MeOH extract and its organic fractions of the herb of *E. prostrate* on carrageenan induced paw edema in rats. All extract/fractions showed dose dependent anti-inflammatory activity and statistically significant ($p<0.05$; <0.005). EtOAc fractions showed 86.80% inhibition of edema at a dose of 400 mg kg^{-1} b. wt. in 3h after the administration of carrageenan.

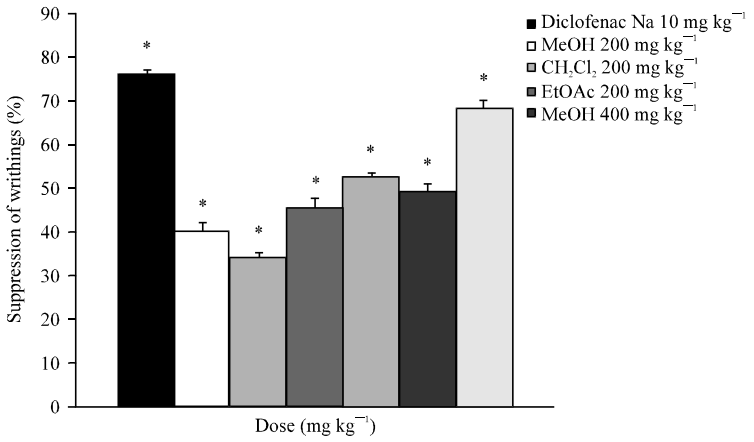

Fig. 2: Effect of *E. prostrata* extract/fractions on acetic acid induced writhings in mice. Observations were taken 30 min after oral administration of test agents or 15 min after diclofenac Na. Values are Mean±SEM (n=5); *p<0.05 by student's test for values between the sample and the vehicle treated group

Table 4: Effect of *E. prostrata* extract/fractions on tail withdrawal reflux in mice

		Mean latency (sec)				Inhibition (%)		
Groups	Dose (mg kg^{-1})	0 min	30 min	60 min	90 min	30 min	60 min	90 min
Control	Vehicle	2.71±0.106	2.5±0.185	2.9±0.371	2.82±0.191			
Diclofenac-Na	10	2.81±0.147	7.09±0.493*	9.19±0.407*	11.23±0.089*	64.74	68.44	74.89
MEP1	200	2.84±0.339	4.69±0.203*	6.01±0.477*	7.5±0.202*	46.70	51.75	62.40
MEP2	400	2.98±0.397	5.01±0.217*	6.39±0.213*	7.76±0.459*	50.10	54.62	63.66
CEP1	200	2.13±0.172	4.01±0.333*	4.56±0.534*	6.39±0.180*	37.66	36.40	55.87
CEP2	400	2.38±0.435	4.78±0.550*	4.98±0.207*	6.29±0.502*	47.70	41.77	55.17
EEP1	200	2.43±0.448	5.23±0.346*	6.19±0.130*	7.98±0.282*	52.20	53.15	64.66
EEP2	400	2.78±0.297	4.89±0.213*	6.67±0.448*	8.43±0.198*	48.88	56.52	66.55

Values are Mean±SEM (n=5); *p<0.005 by student's test for values between the sample and the vehicle treated group. MEP: Methanolic extract, CEP: Dichloromethane fraction, EEP: Ethylacetate fraction of E. prostrata. 1: 200 mg kg^{-1} b.wt, 2:400 mg kg^{-1} b.wt.

Table 5: Effect of *E. prostrata* extract/fractions on carrageenan induced paw edema in rats

		Oedema diameter (mm)			Inhibition (%)		
Groups	Dose (mg kgG^{1})	1 h	2 h	3 h	1 h	2 h	3 h
Control	Vehicle	12.89±0.009	13.54±0.080	15.23±0.009			
Indomethacin	10	5.34±0.012*	3.92±0.019*	1.33±0.005**	58.57	71.05	91.27
MEP1	200	9.39±0.002*	6.83±0.009*	3.92±0.003*	27.15	49.56	74.26
MEP2	400	8.21±0.004*	5.32±0.018*	3.14±0.019**	36.31	60.71	79.38
CEP1	200	10.64±0.009	7.81±0.003*	5.48±0.009*	17.46	42.32	64.02
CEP2	400	9.82±0.002*	7.21±0.005*	5.23±0.012**	23.82	46.75	65.66
EEP1	200	6.21±0.003*	4.19±0.003**	2.80±0.005*	51.82	69.05	81.62
EEP2	400	4.83±0.019*	4.12±0.011*	2.01±0.002**	62.53	69.57	86.80

Values are Mean±SEM (n = 5); *p<0.05 by student=s test for values between the sample and the vehicle treated group. MEP: Methanolic extract, CEP: Dichloromethane fraction, EEP: Ethylacetate fraction of E. prostrata. 1: 200 mg kg^{-1}. b.wt, 2: 400 mg kg^{-1} b.wt

DISCUSSION

To determine the efficacy of natural antioxidants either as pure compounds or as plant extract, a great number of *in vitro* methods have been developed in which antioxidant compounds act by several mechanisms. The phosphomolybdenum method was based on the reduction of Mo(VI) to Mo(V) by the compounds having antioxidant property and is successfully used to quantify Vitamin E in seeds (Prieto *et al.*, 1999). DPPH• is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Nakayama, 1994) and is usually used as a substrate to evaluate the antioxidant activity of a compound (Chang *et al.*, 2002). Based on the data obtained from this study, DPPH radical scavenging activity of EtOAc fractions (IC_{50} 12.98±0.08 μg mL^{-1}) of *E. prostrata* was similar to the standard (IC_{50} 12.76±0.11μg mL^{-1}). These findings agree with previous reports on scavenging of free radicals (Rao *et al.*, 2009). Moreover, it was revealed that organic soluble fraction of *E. prostrata* did show the proton donating ability and could serve as free radical inhibitor or scavenger. A direct correlation between antioxidant capacity and reducing power of certain plant extracts has been reported. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Duh *et al.*, 1999). Polyphenolic compounds, like flavonoids, tannins and phenolic acids, commonly found in plants have been reported to have multiple biological effects, including antioxidant activity (Kahkonen *et al.*, 1999). Phenolic compounds are understood to induce the cellular antioxidant system; increase approximately 50% cellular glutathione concentration. Flavonoids are important in the modulation of γ-glutamylcysteine synthase in both cellular antioxidant defenses and detoxification of xenobiotics (Muchuweti *et al.*, 2007). A flavonoid diosmetin, two isoflavonoids 3'-hydroxybiochanin A and 3'-O-methylorobol have been isolated from the *E. prostrata* (Lee *et al.*, 2009) and the total phenol and flavonoid content was found to be the highest in EtOAc fractions and this may be the cause for the highest antioxidant activity in different model.

The brain and spinal cord play a major role in central pain mechanisms. The dorsal horn of the spinal cord is endowed with several neurotransmitters and receptors including: substance P, somatostatin, neuropeptide Y, inhibitory amino acid, nitric oxide, endogenous opioids and the monoamines which are the major targets for pain and inflammation (McCurdy and Scully, 2005).The hotplate method and tail immersion test were considered to be selective to examine compounds acting through opoid receptor; all the extract/fractions increased pain threshold which mean basal latency which indicates that it may act *via* centrally mediated analgesic mechanism. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain (Elisabetsky *et al.*, 1995; Pal *et al.*, 1999). The extract inhibits pain with both mechanisms, suggesting that the plant extract may act as a narcotic analgesic. On the otherhand, acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from the tissue phospholipid (Ahmed *et al.*, 2006). The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics The response is thought to be mediated by peritoneal mast cells (Ribeiro *et al.*, 2000), acid sensing ion channels (Voilley, 2004) and the prostaglandin pathways (Hossain *et al.*, 2006). Flavonoids may increase the amount of endogenous serotonin or may interact with 5-HT_2A and 5-HT_3 receptors which may be involved in the mechanism of central analgesic activity (Annegowda *et al.*, 2010). Previous researchers reported the presence of several therapeutically valued flavonoids from the *E. prostrata* (Lee *et al.*, 2009). Moreover, EtOAc extract showed highest analgesic activity in all the

experimental model which may be due to its high flavonid content as well as free radical scavenging activity, as these free radicals are involved during pain stimulation and antioxidants showed reduction in such pain (Kim *et al*., 2004).

Carrageenan induced oedema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1-2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages (Antonio and Brito, 1998; Gupta *et al*., 2006). Since the extract/fractions significantly inhibited paw edema induced by carrageenan in the second phase and this finding suggests a possible inhibition of cyclooxygenase synthesis by the extract and this effect is similar to that produced by non-steroidal anti-inflammatory drugs such as indomethacin, whose mechanism of action is inhibition of the cyclooxygenase enzyme. Flavonoids and saponins are well known for their ability to inhibit pain perception as well as anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation (Sawadogo *et al*., 2006). This hypothesis is strongly supported by the previous study, which has shown that *Eclipta prostrata* possess anti-inflammatory activity due to the presence of saponins and high flavonoids contents (Arunachalam *et al*., 2009). In addition, the release of several ROS (Koblyakov, 2001) and excessive nitric oxide (NO) due to the activation of neutrophils during tissue damage and inflammation, which is responsible a variety of disease states like cerebral ischemia, atherosclerosis, cancers, migraine parkinsonism etc. (Bhandare *et al*., 2010). Recent findings (Srivastava *et al*., 2000; Viana *et al*., 2003) suggest that tannic acid and polyphenols are potent inhibitors of NO synthase activity and NO production. As EtOAc fraction showed significant free radical as well as NO and $ONOO^-$ scavenging activity, so this can be responsible for the reduction of inflammation in the carrageenan induced paw edema in rats.

The ability of flavonoids to inhibit eicosanoid biosynthesis has been documented. Eicosanoids, such as prostaglandins are involved in various immunological responses and are the end products of the cyclooxygenase and lipoxygenase pathways (Jothimanivannan *et al*., 2010). Further flavonoids are able to inhibit neutorophils degranulation and thereby decrease the release of arachidonic acid (Hoult *et al*., 1994). Thus the presence of flavonoids in the extract/fractions of *E. prostrate* might be responsible for the anti-inflammatory and analgesic activity in Swiss albino mice and rats as well as in vitro antioxidant activity.

CONCLUSION

In conclusion, the results of the present study, indicate that the MeOH extract and its various organic soluble fractions exhibits interesting antioxidant properties, as well as significant analgesic and anti-inflammatory effect which may be due to the presence of phenolic compounds and flavonoids in the extract/fractions. Now our next aim is to explore the isolation and characterization of lead compound liable for aforementioned activity from this plant.

REFERENCES

Ahmed, F., M.S.T. Selim, A.K. Das and M.S.K. Choudhuri, 2004. Anti-inflammatory and antinociceptive activities of *Lippia nodiflora* Linn. Pharmazie, 59: 329-330.

Ahmed, F., M.H. Hossain, A.A. Rahman and I.Z. Shahid, 2006. Antinociceptive and sedative effects of the bark of *Cerbera odollam* Gaertn. J. Oriental Pharmacy Exp. Med., 6: 344-348.

Anilkumar, M., 2010. Ethnomedicinal Plants as Anti-Inflammatory and Analgesic Agents. In: Ethnomedicine: A Source of Complementary Therapeutics, Chattopadhyay, D. (Ed.). Research Signpost, India, ISBN-13: 978-81-308-0390-6, pp: 267-293.

Annegowda, H.V., M.N. Mordi, S. Ramanathan and S.M. Mansor, 2010. Analgesic and antioxidant properties of ethanolic extract of *Terminalia catappa* L. Leaves. Int. J. Pharmacol., 6: 910-915.

Antonio, A.M. and A.R.M.S. Brito, 1998. Oral anti-inflammatory and anti-ulcerogenic activities of a hydroalcoholic extract and partitioned fractions of *Turnera ulmifolia* (Turneraceae). J. Ethnopharmacol., 61: 215-228.

Arunachalam, G., N. Subramanian, G.P. Pazhani and V. Ravichandran, 2009. Anti-inflammatory activity of methanolic extract of *Eclipta prostrate* L. (Astearaceae). Af. J. Pharm. Pharmacol., 3: 97-100.

Bhandare, A.M., A.D. Kshirsagar, N.S. Vyawahare, A.A. Hadambar and V.S. Thorve, 2010. Potential analgesic, anti-inflammatory and antioxidant activities of hydroalcoholic extract of *Areca catechu* L. nut. Food Chem. Toxicol., 48: 3412-3417.

Braca, A., N.D. Tommasi, L.D. Bari, C. Pizza, M. Politi and I. Morelli, 2001. Antioxidant Principles from *Bauhinia terapotensis*. J. Nat. Prod., 64: 892-895.

Chandra, T., J. Sadique and S. Somasundaram, 1987. Effect of *Eclipta alba* on inflammation and liver injury. Fitoterapia, 58: 23-32.

Chang, C.C., M.H. Yang, H.M. Wen and J.C. Chern, 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal., 10: 178-182.

Chowdhury, M.A., K.R.A. Abdellatif, Y. Dong, D. Das, M.R. Suresh and E.E. Knaus, 2009. Synthesis of celecoxib analogues possessing a N-difluoromethyl-1,2-dihydropyrid-2-one 5-lipoxygenase pharmacophore: biological evaluation as dual inhibitors of cyclooxygenases and 5-lipoxygenase with anti-inflammatory activity. J. Med. Chem., 52: 1525-1529.

Chung, J.M., 2004. The role of Reactive Oxygen Species (ROS) in persistent pain. Mol. Interv., 4: 248-250.

Conforti, F., S. Sosa, M. Marrelli, F. Menichini and G.A. Statti *et al.*, 2009. The protective ability of Mediterranean dietary plants against the oxidative damage: The role of radical oxygen species in inflammation and the polyphenol, flavonoid and sterol contents. Food Chem., 112: 587-594.

Duh, P.D., Y.Y. Tu and G.C. Yen, 1999. Antioxidant activity of water extract of Harng Jyur (*Chrysanthemum morifolium* Ramat). Lebensmittel Wissenschaft und Technol., 32: 269-277.

Eddy, N.B. and D. Leimback, 1953. Synthetic analgesic. II. Dithienyl butenylanddithienyl butylamines. J. Pharmacol. Exp. Ther., 107: 385-393.

Elisabetsky, E., T.A. Amador, R.R. Albuquerque, D.S. Nunes and A.C.T. Cavalho, 1995. Analgesic activity of *Psychotria colorata* (Willd. ex R. and S.) Muell. Arg. alkaloids. J. Ethnopharmacol., 48: 77-83.

Franzotti, E.M., C.V.F. Santos, H.M.S.L. Rodrigues, R.H.V. Mourao, M.R. Andrade and A.R. Antoniolli, 2000. Anti-inflammatory, analgesic activity and acute toxicity of *Sida cordifolia* L. (Malva-branca). J. Ethnopharmacol., 72: 273-277.

Gulcin, I., M.E. Buyukokuroglu, M. Oktay and O.I. Kufrevioglu, 2002. On the *in vitro* antioxidative properties of melatonin. J. Pineal Res., 33: 167-171.

Gupta, M., U.K. Mazumder, P. Gomathi and V.T. Selvan, 2006. Antiinflammatory evaluation of leaves of *Plumeria acuminate*, 2006. BMC Complementary Alternative Med., 6: 36-36.

Gupta, M., U.K. Mazumder, R.S. Kumar, P. Gomathi, Y. Rajeshwar, B.B. Kakoti and V.T. Selven, 2005. Anti-inflammatory, analgesic and antipyretic effects of methanol extract from *Bauhinia racemosa* stem bark in animal models. J. Ethnopharmacol., 98: 267-273.

Hossain, M.M., M.S. Ali, A. Saha and M. Alimuzzaman, 2006. Antinociceptive activity of whole plant extracts of *Paederia foetida*. Dhaka Univ. J. Pharm. Sci., 5: 67-69.

Hoult, J.R., M.A. Moroney and M. Paya, 1994. Actions of flavonoids and coumarins on lipoxigenase and cyclooxygenase. Methods Enzymol., 234: 443-454.

Jothimanivannan, C., R.S. Kumar and N. Subramanian, 2010. Anti-inflammatory and analgesic activities of ethanol extract of aerial parts of *Justicia gendarussa* Burm. Int. J. Pharmacol., 6: 278-283.

Kahkonen, M.P., A.I. Hopia, H.J. Vuorela, J.P. Rauha, K. Pihlaja, T.S. Kulaja and M. Heinonen, 1999. Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem., 47: 3954-3962.

Karthikumar, S., K. Vigneswari and K. Jegatheesan, 2007. Screening of antibacterial and antioxidant activities of leaves of *Eclipta prostrata* (L). Sci. Res. Essay, 2: 101-104.

Kim, H.K., S.K. Park, J.L. Zhou, G. Taglialatela, K. Chung, R.E. Coggeshall and J.M. Chung, 2004. Reactive oxygen species (ROS) play an important role in a rat model of neuropathic pain. Pain, 111: 116-124.

Koblyakov, V.A., 2001. Free radicals and inflammation (progress in inflammation research series, 1999, 260 p.). Biochemistry, 66: 937-938.

Kobori, M., Z. Yang, D. Gong, V. Heissmeyer and H. Zhu *et al*., 2004. Wedelolactone suppresses LPS-induced caspase-11 expression by directly inhibiting the IKK complex. Cell Death Differ., 11: 123-130.

Kooy, N.W., J.A. Royall, H. Ischiropoulos and J.S. Beckman, 1994. Peroxynitrite-mediated oxidation of dihydrorhodamine 123. Free Radical Biol. Med., 16: 149-156.

Kourounakis, A.P., D. Galanakis, K. Tsiakitzis, E.A. Rekka and P.N. Kourounakis, 1999. Synthesis and pharmacological evaluation of novel derivatives of anti-inflammatory drugs with increased antioxidant and anti-inflammatory activities. Drug Dev. Res., 47: 9-16.

Kumari, C.S., S. Govindasamy and E. Sukumar, 2006. Lipid lowering activity of Eclipta prostrate in experimental hyperlipidemia. J. Ethnopharmacol., 105: 332-335.

Lee, M.K., N.R. Ha, H. Yang, S.H. Sung and Y.C. Kim, 2009. Stimulatory constituents of *Eclipta prostrata* on mouse osteoblast differentiation. Phytother. Res., 23: 129-131.

Malairajan, P., G. Geetha, S. Narasimhan and K.J. Jessi, 2006. Analgesic activity of some Indian medicinal plants. J. Ethnopharmacol., 106: 425-428.

McCurdy, C.R. and S.S. Scully, 2005. Analgesic substances derived from natural products (natureceuticals). Life Sci., 78: 476-484.

Melo, P.A., M.C. Nascimento, W.B. Mors and G. Surez-Kurtz, 1994. Inhibition of the mytotoxic and hemorrhagic activities of crotalid venoms by *Eclipta prostrata* (*Astraceae*) extracts and constituents. Toxicon, 32: 595-603.

Muchuweti, M., E. Kativu, C.H. Mupure, C. Chidewe, A.R. Ndhlala and M.A.N. Benhura, 2007. Phenolic composition and antioxidant properties of some spices. Am. J. Food Technol., 2: 414-420.

Nakayama, T., 1994. Suppression of hydroperoxide-induced cytotoxicity by polyphenols. Cancer Res., 54: 1991s-1993s.

Oyaizu, M., 1986. Studies on product of browning reaction prepared from glucose amine. Jpn. J. Nutr., 44: 307-315.

Oyedapo, O.A., C.O. Adewunmi, E.O. Iwalewa and V.O. Makanju, 2008. Analgesic, antioxidant and anti-inflammatory related activities of 2^1-hydroxy-2,4 -dimethoxychalcone and 4-hydroxychalcone in mice. J. Boil. Sci., 8: 131-136.

Pal, S., T. Sen and A.K. Chaudhuri, 1999. Neuropsychopharmacological profile of the methanolic fraction of *Bryophyllum pinnatum* leaf extract. J. Pharm. Pharmacol., 51: 313-318.

Prieto, P., M. Pineda and M. Aguilar, 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal. Biochem., 269: 337-341.

Rao, D.B., C.R. Kiran, Y. Madhavi, P.K. Rao and T.R. Rao, 2009. Evaluation of antioxidant potential of *Clitoria ternate* L. and *Eclipta prostrate* L. Indian J. Biochem. Biophy., 46: 247-252.

Ribeiro, R.A., M.L. Vale, S.M. Thomazzi, A.B. Paschoalato, S. Poole, S.H. Ferreira and F.Q. Cunha, 2000. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. Eur. J. Pharmacol., 387: 111-118.

Sawadogo, W.R., R. Boly, M. Lompo, N. Some, C.E. Lamien, I.P. Guissou and O.G. Nacoulma, 2006. Antiinflammatory, analgesic and antipyretic activities of *Dicliptera verticillata*. Int. J. Pharmacol., 2: 435-438.

Sreejayan and M.N. Rao, 1997. Nitric oxide scavenging by curcuminoids. J. Pharm. Pharmacol., 49: 105-107.

Srivastava, R.C., M.M. Husain, S.K. Hasan and M. Athar, 2000. Green tea polyphenols and tannic acid act as potent inhibitors of phorbol ester-induced nitric oxide generation in rat hepatocytes independent of their antioxidant properties. Cancer Lett., 153: 1-5.

Toma, W., J.S. Graciosa, C.A. Hiruma-Lima, F.D.P. Andrade, W. Vilegas and A.R.M. Souza-Brita, 2003. Evaluation of the analgesic and antiedematogenic activities of *Quassia amara* bark extract. J. Ethnopharmacol., 85: 19-23.

Viana, G.S.B., M.A.M. Bandeira and F.J.A. Matos, 2003. Analgesic and antiinflammatory effects of chalcones isolated from *Myracrodruon urundeuva* allemao. Phytomedicine, 10: 189-195.

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

Wagner, H., B. Geyer, K. Yoshinobu and S.R. Govind, 1986. Coumestans as the main active principles of the liver drugs *Eclipta alba* and *Wedelia calendulacea*. Planta Med., 5: 370-374.

Wiart, C., S. Mogana, S. Khalifah, M. Mahan and S. Ismail 2004. Antimicrobial screening of plants used for traditional medicine in the Stat of Perak, Peninsular Malaysia. J. Fitotera, 75: 68-73.

Winrow, V.R., P.G. Winyard, C.J. Morris and D.R. Blake, 1993. Free radicals in inflammation: Second messengers and mediators of tissue destruction. Br. Med. Bull., 49: 506-522.

Winter, C.A., E.A. Risley and G.W. Nuss, 1962. Carrageenan induced oedema in hind paw of the rats as an assay of anti-inflammatory drug. Proc. Soc. Exp. Biol. Med., 111: 544-547.

Wong, S.M., S. Antus, A. Gottsegen, B. Fessler, G.S. Rao, J. Sonnenbichler and H. Wagner, 1988. Wedelolactone and coumestan derivatives as new antihepatotoxic and antiphlogistic principles. Arzneimittelforschung, 38: 661-665.

Yu, L., S. Haley, J. Perret, M. Harris, J. Wilson and M. Qian, 2002. Free radical scavenging properties of wheat extracts. J. Agric. Food Chem., 50: 1619-1624.