INTRODUCTION

Pain had been defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage [1]. However, for animals, it is harder to know whether an emotional experience has occurred. Therefore, this concept is often excluded. Pain in animals, as provided by Zimmerman, was an aversive sensory experience caused by actual or potential injury that elicits protective motor and vegetative reactions, results in learned avoidance and may modify species specific behaviour, including social behaviour [2]. Persistent or chronic pain is one of the major causes for people seeking healthcare. It could significantly interfere with the quality of life and general functioning of the patient. Pain therapies currently available were causing uncomfortable to harmful side effects [3].

Inflammation is the response to injury of cells and body tissues through different factors such as infections, chemicals, and thermal and mechanical injuries [4]. 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 vasodilators and also contribute to erythema, edema, and pain. Hence, for treating inflammatory diseases, analgesic and anti-inflammatory agents are required [5]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most clinically important medicine used for the treatment of inflammation-related diseases like arthritis, asthma, and cardiovascular disease [6]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications due to their efficacy for a wide range of pain and inflammatory conditions [7]. However, the long-term administration of NSAID may induce gastro-intestinal ulcers, bleeding, and renal disorders due to their nonselective inhibition of both constitutive (COX-1) and inducible (COX-2) isoforms of the cyclooxygenases enzymes [8]. Therefore, new anti-inflammatory and analgesic drugs lacking those effects...
are being searched all over the world as alternatives to NSAIDs and opiates [9, 10]. 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 [11, 12]. Hence the search for new, safe and effective analgesic and anti-inflammatory drugs is justified.

*Baccaurea ramiflora* (family: Euphorbiaceae) is native to Southeast Asia region and is found distributed in the sub-Himalayan tract, mainly from Nepal to Sikkim, Darjeeling hills, Arunachal Pradesh, Tripura, Assam, Bhutan, Burma, Penninsular Malaysia, Tibet and Andaman islands [13]. It is an evergreen tree reaching a height of about 5-10 m. Fruit is yellowish and velvety, 2-3 cm in diameter with leathery pericarp, three seeded arillus embedded in pinkish white pulp. The leaf is simple, alternately arranged, with petiole [14]. The common names include Latkan or Bhubi (Bengali), Letuk (Assamese), Leteku (Hindi), Mafai (Thai) and Burmese grape (English). It is used medicinally to treat skin diseases. The whole plant of B. ramiflora is utilized as an antiphlogistic and anodyne against rheumatoid arthritis, cellulitis, and abscesses and to treat injuries [15]. Young leaves of B. ramiflora are used as vegetable, flavoring agent with curries, fresh bark is chewed or juice is used orally for constipation [16].

Microcos paniculata (family: Euphorbiaceae) is a shrub that is abundant in secondary forests and also grown as hedges. Microcos is tall semi-deciduous tree, sometimes shrubby. Leaves 10-15 cm long, elliptic-oblong, acuminate, entire or slightly and irregularly toothed [17]. Flowers small, yellow, in terminal panicles. Fruits globose or slightly obovoid, about 10 mm across. The plant is used in indigestion, eczema, itch, small-plex, typhoid fever, dysentery and syphilitic ulceration of the mouth. Tripuras in Chittagong Hill Tracts use leaves of this plant along with turmeric and shell of snail for the treatment of jaundice [18]. A decoction of the roots is used to treat coughs. A drink prepared from the roasted and boiled leaves is given to children as a vermifuge. It has been used traditionally to prepare herbal medicines and traditional teas, while a limited number of reports concerning the chemical constituents and biological activities of M. paniculata have appeared in the literature [19, 20].

The preliminary phytochemical screening of petroleum ether and chloroform extracts of Microcos paniculata leaves revealed the presence of alkaloids, glycosides, carbohydrates, tannins, phytosterols and flavonoids. Ethanol extract revealed the presence of proteins and saponins. Aqueous extract revealed the presence of saponins. Based on the above findings, *Baccaurea ramiflora* and Microcos paniculata leaves extracts was evaluated for its analgesic and anti-inflammatory effects on experimental induced pain and inflammation.

**MATERIALS AND METHODS**

**Collection of plant materials**

The leaves of *Baccaurea ramiflora* and Microcos paniculata belonging to family Euphorbiaceae were collected from local market of Belonia, Tripura, India during May – July and authenticated (ID No. is BOT/HEB/AC23072011 and BOT/HEB/AC23072512) by Dr. B. K. Datta, Professor of Botany, Plant Taxonomy and Biodiversity Laboratory, Department of Botany, Tripura, India.

**Preparation of Extracts**

After collection of the plants, the leaves of both the plants were rinsed thoroughly in tap water and dried in shade for about 20 days under controlled temperature (25 ± 2 °C). Then the crude material was powdered, passed through a 40 mesh sieve and stored in a well closed container for further usage. Coarsely powdered and dried leaves were successively soxhlated using petroleum ether, chloroform, ethanol and water for 72h. The extracts were filtered and the solvents were evaporated to dryness under reduced pressure in a rotary evaporator at 40 °C to 45 °C. A brown residue was recovered from flask with 12% yield of ethanol extract.

**Animals**

Wistar rats (150–250 gm) and Swiss albino mice (20–25 gm) of either sex, brought from Sainath Agencies, Hyderabad, Telangana, India. The rats were acclimatized to the laboratory conditions for a week before the start of the experiments; they were maintained as per the Institutional ethical committee (IAEC) norms. Animals were housed at standard conditions of temperature (22 ± 1°C) and 12/12 h light/dark cycle. They were fed with standard pellet diet and had free access to water. Five animals are used in each group. Permission for conduct of these experiments were obtained from, Institutional Animal Ethics Committee (IAEC) Regd. No. 1662/PO/Re/S/12/CPCSEA.

**Preliminary Phytochemical Screening**

Qualitative preliminary phytochemical screening was carried out for evaluation of tannins, alkaloids, flavonoids, saponins, etc using standard procedures and tests [21].
Acute toxicity study

Acute toxicity study was carried out as per OECD guidelines. An aggregate of fifteen Swiss albino mice were used for studies which were fasted overnight, providing free access to water and were randomly divided into three groups, each containing five mice. The control group (C) was given normal standard diet [22]. The two treated groups were given oral administration of a single dose of ethanolic extract of *Baccaurea ramiflora* 2000 mg/kg (BR2) and also *Microcos paniculata* 2000 mg/kg (MP2). The oral administration was managed by utilizing a curved ball tipped intubation needle affixed to a 2 ml syringe [23]. Immediately after administration the animal’s behavior, toxic signs and mortality were continuously observed for the first thirty minutes and periodically at hourly intervals for during the first twenty four hours, special attention given during the first four hours, and daily thereafter for a total of 14 days [24].

Analgesic activity

Acetic acid induced writhing in mice

Female Swiss albino mice were divided into six groups (n = 6):
- Group 1- Vehicle control
- Group 2- Aspirin (100 mg/kg, p.o.)
- Group 3- EEBR (200 mg/kg, p.o.)
- Group 4- EEMP (400 mg/kg, p.o.)
- Group 5- EEMP (200 mg/kg, p.o.)
- Group 6- EEMP (400 mg/kg, p.o.)

Before 60 min of administration of acetic acid solution at a dose of 10 ml/kg (0.6%, i.p.) the mice were pretreated orally with EEBR, EEMP or Aspirin. The number of abdominal constrictions or writhing (full extension of both hind paws) was cumulatively counted over a period of 15 min. The analgesic activities were expressed as mean number of writhes and percentage inhibition was calculated by the following formula:

\[
\% \text{ Inhibition} = \frac{Wc - Wt}{Wc} \times 100
\]

Where, Wc and Wt are mean number of writhes observed in vehicle control group and treatment group respectively [25].

**EEBR-Ethanol extract of Baccaurea ramiflora, EEMP- Ethanolic extract of Microcos paniculata.**

Hot plate method

The Swiss albino mice were first screened by placing them on hot plate maintained at 55 ± 10°C and the reaction time was recorded in seconds. The pain threshold was considered to be reached when animals showed the signs of paw licking or jumping [26]. Only those mice which reacted within 15 s and which did not show large variations when tested on four separate occasions, each 15 min apart, were used for the test. The time for paw licking or jumping on the hot plate was selected as a reaction time [27].

**The mice were divided into six groups (n=6):**
- Group 1- Vehicle control
- Group 2- Pentazocine (30 mg/kg, p.o.),
- Group 3- EEBR (200 mg/kg, p.o.),
- Group 4- EEBR (400 mg/kg, p.o.),
- Group 5- EEMP (200 mg/kg, p.o.),
- Group 6- EEMP (400 mg/kg, p.o.).

Various responses such as paw licking or jumping were recorded before and after 30, 60, 90, 120 and 150 min following oral administration of EEBR, EEMP or Pentazocine [20]. A cut-off time of 15 s was used to avoid harm to the animals.

Anti-inflammatory activity

**Carrageenan-induced paw edema in rats**

The wistar rats were divided into six groups (n = 6):
- Group 1- Carrageenan control,
- Group 2- Diclofenac (10 mg/kg, p.o.),
- Group 3- EEBR (200 mg/kg, p.o.),
- Group 4- EEBR (400 mg/kg, p.o.),
- Group 5- EEMP (200 mg/kg, p.o.),
- Group 6- EEMP (400 mg/kg, p.o.).

Acute inflammation was produced by sub plantar injection of 0.1 ml of 1% lambda Carrageenan (Sigma Chemical Co., USA) suspension in sterile normal saline in the left hind paw of each rat. Rats were pretreated orally with EEBR, EEMP and diclofenac (10 mg/kg p.o.) 1 h before carrageenan injection [28].

The rat paw volume up to the ankle joint was measured using Plethysmometer from 0-6 h at an interval of 1 h. The mean changes in injected paw volume with respect to initial paw volume were calculated. Percentage inhibition of paw volume between treated and control group was calculated using following formula

\[
\% \text{ Inhibition} = (1 - \frac{Vt}{Vc}) \times 100
\]

Where, Vc and Vt represent mean increase in paw volume in control and treated groups, respectively [29].

**Cotton pellet-induced granuloma in rats**

The effect of EEBR and EEMP in sub-acute inflammation was assessed using cotton pellet granuloma in rats [30, 31].

The rats were divided into six groups (n = 6):
- Group 1- Vehicle control
- Group 2- Diclofenac (10 mg/kg, p.o.),
- Group 3- EEBR (200 mg/kg, p.o.),
- Group 4- EEBR (400 mg/kg, p.o.),
- Group 5- EEMP (200 mg/kg, p.o.),
- Group 6- EEMP (400 mg/kg, p.o.).

Autoclaved cotton pellets weighing 35 ± 1 mg each were implanted subcutaneously through small
incision made along the axilla or flank region of the rats anesthetized with anesthetic ether. EEBR, EEMP, and diclofenac (10 mg/kg p.o.) were administered once daily for seven consecutive days from the day of cotton pellet insertion. On the eighth day all rats were sacrificed and the cotton pellets covered by the granulomatous tissue were excised from animal body and dried in hot air oven at 60°C for 24 h and weighed [32].

STATISTICAL ANALYSIS

Values were expressed as mean ± SEM and statistically analysis was carried out using Graph Pad 5.0 software (Graph Pad, San Diego, USA) by applying One Way ANOVA with Dunnett’s test or Two Way ANOVA with Bonferroni test, p< 0.05 was considered to be significant.

RESULTS

Phytochemical analysis

Preliminary phytochemical qualitative analysis of EEBR, EEMP showed the presence of alkaloids, saponins, flavonoids, tannins in the extract.

Acute toxicity study

The acute toxic study results of the ethanolic extract of leaves of Baccara ramiflora and Microcos paniculata showed no noticeable signs of acute toxicity and mortality when given orally at dose of 2000 mg/kg body weight. Hence, the extracts was found to be safe at the dose of 2000 mg/kg body weight and hence 1/10th and 1/5th of the same i.e. 200 mg/kg and 400 mg/kg were selected for screening dose for further studies.

Analgesic activity

Acetic acid induced writhing in mice

Administration of acetic acid produced 66 ± 1.8 writhes in mice. Administration of EEBR, EEMP at 200 mg/kg and 400 mg/kg significantly (p< 0.001) decreased the number of writhings by 19.69%, 31.81%, 18.18% and 30.30% respectively when compared to vehicle control group. Aspirin (100 mg/kg) significantly (p< 0.001) reduced the number of writhings by 54.54% when compared to vehicle control group (Table 1).

Table 1: Effect of EEBR, EEMP in acetic acid induced writhing in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Writhing</th>
<th>Percent inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>---</td>
<td>66±1.8</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>100mg/kg</td>
<td>30±1.8***</td>
<td>54.54</td>
</tr>
<tr>
<td>EEBR</td>
<td>200mg/kg</td>
<td>53±1.5***</td>
<td>19.69</td>
</tr>
<tr>
<td>EEMP</td>
<td>400mg/kg</td>
<td>45±1.5***</td>
<td>31.81</td>
</tr>
<tr>
<td>EEP</td>
<td>200mg/kg</td>
<td>54±1.6***</td>
<td>18.18</td>
</tr>
<tr>
<td>EEMP</td>
<td>400mg/kg</td>
<td>46±1.5***</td>
<td>30.30</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM for six animals and analysed by One-way ANOVA followed by post hoc Dunnett’s test **p< 0.01, ***p< 0.001 when compared to vehicle control.

Hot plate method

Pretreatment of mice with EEBR, EEMP at 200mg/kg and 400 mg/kg significantly (p< 0.001) increased the pain latency, there was dose dependent increase in latency time in response to thermal stimulation, whereas pentazocine (30 mg/kg, i.p) also significantly (p< 0.001) increased the response latency at 30, 60, 90, and 120 minutes of administration (Table 2).

Table 2: Effect of EEBR, EEMP on Hot plate method

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Paw Withdrawal latency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 Min</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>---</td>
<td>3.35±0.21</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>30mg/kg</td>
<td>3.58±0.38</td>
</tr>
<tr>
<td>EEBR</td>
<td>200mg/kg</td>
<td>3.92±0.65</td>
</tr>
<tr>
<td>EEBR</td>
<td>400mg/kg</td>
<td>3.86±0.73</td>
</tr>
<tr>
<td>EEMP</td>
<td>200mg/kg</td>
<td>3.90±0.63</td>
</tr>
<tr>
<td>EEMP</td>
<td>400mg/kg</td>
<td>3.84±0.70</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM for six animals and analysed by Two way ANOVA followed by Bonferroni post-hoc test, *p< 0.05, ***p< 0.001 when compared to healthy control.

Anti-inflammatory activity

Carrageenan induced paw edema in rats

Treatment with EEBR, EEMP at a dose of 200 mg/kg, and 400 mg/kg exhibited a significant decrease in paw volume. EEBR, EEMP at a dose of 200 mg/kg, and 400 mg/kg also showed significant (p< 0.001) decrease in paw volume at 3rd and 5th h. Diclofenac (10 mg/kg) exhibited a significant (p< 0.001) reduction in paw volume.
in paw volume at 3rd and 5th h as compared to vehicle control. The percentage inhibition of change in paw volume of EEBR, EEMP at a dose of 200 mg/kg, and 400 mg/kg was found to be 35.45%, 55.90 %,35.90% and 55.00 % respectively at 3rd h. However the maximum percentage inhibition was found to be at 5th h 57.89%, 81.95 %, 56.76% and 81.57 % for EEBR, EEMP at a dose of 200 mg/kg, and 400 mg/kg respectively. The percentage inhibition of diclofenac (10 mg/kg) was found to be 75.45 % and 92.48 % at 3rd & 5th h respectively when compared with carrageenan control animals (Table 3).

Table No 3: Effect of EEBR, EEMP in Carrageenan induced paw edema in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Change in paw volume(ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1h</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>---</td>
<td>0.82±0.07</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10mg/kg</td>
<td>0.42±0.06*(48.78)</td>
</tr>
<tr>
<td>EEBR</td>
<td>200mg/kg</td>
<td>0.62±0.08(24.39)</td>
</tr>
<tr>
<td>EEM</td>
<td>400mg/kg</td>
<td>0.49±0.06(40.24)</td>
</tr>
<tr>
<td>EEBR</td>
<td>200mg/kg</td>
<td>0.64±0.07(21.95)</td>
</tr>
<tr>
<td>EEMP</td>
<td>400mg/kg</td>
<td>0.46±0.06(43.90)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM for six animals and analysed by Two way ANOVA followed by Bonferroni post – hoc test, *p< 0.05, ***p< 0.001 when compared to carrageenan control. The figures in parenthesis indicate the percent inhibition.

Cotton pellet-induced granuloma in rats

In cotton pellet granuloma, EEBR, EEMP at a dose of 200 mg/kg, and 400 mg/kg significantly (p< 0.001) inhibited the granuloma formation when compared to vehicle control group. The degree of inhibition was dose dependent. The EEBR, EEMP at a dose of 200 mg/kg, and 400 mg/kg inhibited the granuloma formation by 21.21%, 35.60%, 22.72% and 36.36% respectively. Diclofenac (10 mg/kg) significantly (p< 0.001) inhibited the granuloma formation by 50.75% (Table 4).

Table 4: Effect of EEBR, EEMP on cotton pellet-induced granuloma in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Dry weight of granuloma (mg)</th>
<th>Percent inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>---</td>
<td>132±4.5</td>
<td>---</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10mg/kg</td>
<td>65±2.6***</td>
<td>50.75</td>
</tr>
<tr>
<td>EEBR</td>
<td>200mg/kg</td>
<td>104±4.5***</td>
<td>21.21</td>
</tr>
<tr>
<td>EEBR</td>
<td>400mg/kg</td>
<td>85±4.1***</td>
<td>35.60</td>
</tr>
<tr>
<td>EEMP</td>
<td>200mg/kg</td>
<td>102±4.3***</td>
<td>22.72</td>
</tr>
<tr>
<td>EEMP</td>
<td>400mg/kg</td>
<td>84±4.1***</td>
<td>36.36</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM for six animals and analysed by One way ANOVA followed by Dunnett’s test, ***p< 0.001 when compared to vehicle control.

DISCUSSION

The use of traditional medicine is widespread and plants still present a large source of structurally novel compounds that might serve as leads for development of novel drugs. The present investigation was carried out to scientifically evaluate the traditional claim of *Baccaurea ramiflora* and *Microcos paniculata* as an algesic and anti-inflammatory. On acute oral toxicity the extract was found to be safe up to 2000 mg/kg. Phytochemical screening showed the presence of alkaloids, saponins, flavonoids, tannins in the extract. Analgesic activity of EEBR, EEMP was evaluated using acetic acid induced writhing test and hot plate model to characterize peripheral and central analgesic activity. EEBR, EEMP exhibited significant and marked analgesic actions in both the models. Acetic acid is very sensitive method for screening analgesic effect and causes increase in PGF2 and PGF2a in peritoneal fluid. EEBR, EEMP produced significant inhibition of wriths at the dose of 200 mg/kg (p< 0.001) and 400 mg/kg (p< 0.001) compared to vehicle control.

The hot plate test has been found to be suitable for evaluation of centrally acting analgesics. The hot plate test measures the response to a brief, noxious stimulus thus bears a closer resemblance to clinical pain. The increase in reaction time in the hot plate test suggests the central analgesic effect of *Baccaurea ramiflora* and *Microcos paniculata*. The ability of EEBR, EEMP in analgesic activity may be due to the involvement of endogenous prostaglandins. This means that EEBR, EEMP exerted both peripheral and central analgesic activity for the transmission of painful message in mice.

Carrageenan induced paw oedema which is a classical model of acute inflammation has been widely used in the study of steroid and non-steroid anti-inflammatory drugs. Carrageenan-induced
inflammation has a significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation. Carrageenan is a family of linear sulphated polysaccharides extracted from the red seaweed marine alga Chondrus crispus. Lambda carrageenan is used in animal models of inflammation to test anti-inflammatory activity because dilute carrageenan solutions (1-2%) injection causes swelling and pain. The edema produced by subplantar injection of carrageenan in rat hind paw is biphasic over 4 or more hours. The early phase is attributed due to release of serotonin and histamine while later phase is sustained by prostaglandins and leukotrienes and continuity between two phases is provided by kinins. The second phase is sensitive to most clinically effective anti-inflammatory drugs. The EEBR, EEMP was found to significantly inhibit carrageenan induced rat paw edema in the late phase regulated by prostaglandins and leukotrienes.

Cotton Pellet induced granuloma in rats is a chronic model of inflammation which has been widely used to assess activity of anti-inflammatory drugs on proliferative phase of inflammation. Proliferation of macrophages, neutrophils, fibroblasts and multiplication of small blood vessels which are the basic sources of highly vascularized reddish mass is termed as granulation tissue is seen during repair process of inflammation. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma and the dry weight correlates well with the amount of granulomatous tissue formed. In the present study significant activity of EEBR, EEMP was seen against cotton pellet induced granuloma in rats indicating ability of EEBR, EEMP in reducing number of fibroblasts and synthesis of collagen and mucopol saccharide, natural proliferative events of granulation tissue formation. The presence of flavonoid compounds in the extracts may be responsible for the anti-inflammatory activities in both the models. Therefore analgesic and anti-inflammatory activity of EEBR, EEMP can be attributed to its phytochemical compounds present in the extract.

CONCLUSION
EEBR, EEMP showed analgesic activity in acetic acid induced writhing model (peripheral) and hot plate test (centrally). It also showed anti-inflammatory activity in carrageenan (acute) and cotton pellet induced granuloma model (sub-acute). This activity can be contributed to the phytochemicals present in the extract like alkaloids, saponins, flavanoids and tannins.

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