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Evaluation of antinociceptive potential of methanolic extract of different parts of *Ehretia serrata* Roxb and *Ehretia obtusifolia in vivo.*

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Abstract

Background: The antinociceptive potential study of different parts of *Ehretia serrata* Roxb and *Ehretia obtusifolia* are least explored till now. These plant parts are usually used as fodder and wood in Asia. The aim of this work was to find the antinociceptive effects of the methanolic extracts of leaves, fruits and stem barks of these plants in mice.

Methods: The antinoceceptive activity of methanolic extract of leaf, fruit, and stem bark of *E. serrata* and *E. obtusifolia* in mice was carried out by means of the hot plate method and by using diclofenac sodium as a standard. Twenty groups of 12 h starved mice were prepared where each group comprised of five mice only. While all these starved mice were allowed free access to clean water. In this study, both male and female Albino mice were used. Analgesiometer was used for this bio-essay.

Results: The results showed that the dose significantly ($P<0.05$) reduced the time spent in pain behaviour in all assessment times (0 min, 30 min and 60 min) hence indicating that the plant possesses antinociceptive potential.

Conclusion: It is confirmed through the findings that *E. serrata* and *E. obtusifloia* exhibit strong antinociceptive action in animal model of hot plate which needs verification in other paradigms too.

Keywords: Antinociceptive activity, *Ehretia serrata*, *Ehretia obtusifolia*, Methanol extracts, Hot-plate method, Glutamate induced nociception.

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Introduction

Pain is unpleasant sensation or discomfort caused by various factors, and it is an indication of diseases in body or clue to something that is not going well in our body [1]. Control of pain is one of the most important uses of drugs [2]. Drugs, which alter, reduce, or remove the pain sensitivity, are referred to as painkillers or analgesics [3]. Plants contain many bioactive compounds that show significant analgesic activity and reduce pain sensitivity with no harmful effects [4]. The Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) which reduce rheumatism and pain sensation also produce noxious effects such as GIT ulceration and bleeding [2,5]. Investigation and research on different plants lead to synthesis of plants-derived drugs [6]. Natural plant phytochemicals and bioactive compounds from different parts of plant are source of drugs used as an analgesic, anti-depressant and anti-inflammatory agents [7].

Ehretia serrata is an intolerant deciduous tree locally named as Koda in Australia and Puran or Punna in Pakistan [8]. *E. serrata* is a native species of India and Pakistan [9]. In Pakistan, *E. serrata* is well distributed in the sub-Himalayan tract from Azad Kashmir westward to Rawalpindi, Islamabad, Murree, Hazara and Swat. It has been successfully planted in areas around Lahore [8]. Timber of *Ehretia serrata* is used in Assam, Barak valley particularly, North East India as a fuel, leaves are used as a fodder in dist. Kotli, Azad Jammu and Kashmir, whereas bark juice is used for fever [10,11]. The

green and unripe fresh fruit of *Ehretia serrata* is used in a form of pickle [12].

Ehretia obtusifolia, on the other hand, is a tiny shrub or bushy tree up to 3 m height that grows in eastern and southern Africa. It is also found in Pakistan and India [9]. It is found in dry places. This species is locally named as Ghadaboty in KPK and Charmror in Punjab [13]. In Zimbabwe, the leaves of *E. obtusifolia* are used in a mixture to treat throat infection and on gums of infants to relive them from teething pains. The crushed root in porridge is used to cure infertility in women and a mixture of the roots is used to retain placenta. Plant root is taken as an analgesic [14].

Therefore, the current work was conducted to explore the antinociceptive potential of the leaf, fruit, and stem bark of *E. serrata* and *E. obtusifolia* in animal model of hot plate test.

Materials and Methods

Preparation of methanol extract

Different parts of *E. serrata* and *E. obtusifolia* such as leaves, fruits, and stem bark were gathered from the Department of Botany, University of Peshawar, Peshawar, Pakistan and were characterized by botanists from herbarium of the Department of Botany, University of Peshawar KPK. The shade-dried leaves, fruit, and stem bark of *E. serrata* and *E. obtusifolia* were powdered and then extracted with 99% methanol by heating under reflux. The filtration of methanolic extract was carried out and the conversion of filtrate to a semisolid mass was brought about by means of rotary evaporation according to the method reported [15,16]. For *in vivo* findings, the extracts were administered orally after mixing them in normal saline. The fresh solutions of the mentioned selected plants were prepared for their use in each experiment.

Experimental animals

Colony bred Swiss mice from Veterinary Research Institute, Peshawar were used in the research experiments. The animals were placed in Polypropylene (PP) cages and under stable environment and food with free access to clean drinking water.

Acute toxicity test

The general safety of test articles were carried in acute toxicity as reported elsewhere [17]. The mice were injected with high doses of 500, 1500 and 3000 mg/kg i. p. and observed for gross behavioural and mortality effects during 24 h.

Hot plate method

The extracts were evaluated for their antinociceptive potential in mice using the hot plate method reported by Shethy et al. and by modified method as well [18]. Twenty groups of 12 h starved mice were prepared where each group comprised of five mice each. While all these starved mice were allowed free access to clean water. In this study, both male and female Albino mice were used. Analgesometer were used for this bio-essay. Each mouse was placed on a hot plate kept at 55 ± 10°C. The initial latency time, which denotes the time taken for mice to show reaction to the pain stimulus determined with a stop watch was recorded. Response to pain stimulus included raising and licking of hind foot or jumping. The cut off time was fixed for 20 s, and served as a control pain reaction time. Response of latency of the animal was noted down in hot plate for three hours with 30-min interval after treatment [19].

Statistical investigation

The analgesic potential values were expressed as "mean increase in latency after drug administration ± SEM" in seconds. Statistical analysis was performed with the aid of Student's t-test for significance with the aid of GraphPad Prism-6 (GraphPad Software, San Diego, California USA, http://www.graphpad.com). Differences were considered significant at $p \leq 0.05$ and highly significant at $p \leq 0.01$. The procedure of Alcaraz was followed in all statistical protocols [20].

Results and Discussion

The main objective of current study was to investigate the analgesic potential of the methanolic extracts of fruit, leaf, and stem bark of *E. serrata* and *E. obtusifolia* plants. Presented in Tables 1 and 2 are results of our investigation of the analgesic effect of these extracts in mice. Our findings showed, that the methanol extract of the leaf, fruit, and stem bark of both the plants exhibit pronounced analgesic effect in mice by reducing pain, and that not only the duration but also the intensity, of analgesia induced by different parts were found dose-dependent. The significant analgesic effect of methanolic extracts of different plant parts resulted in pain relief. The results showed dose dependency analgesia caused by both intensity and duration of different plant parts methanolic extracts. The complex response to an acute and a non-inflammatory nociceptive input is measured through the hot plate test which is normally considered as one of the best models for the evaluation of central nociceptive potential [21]. The prolongation of hot plate latency caused by any agent using the reported test, must be acting centrally as it is considered as an established fact [22]. Thus, the central activity of the methanol plant extracts was investigated. It is important to mention here that not only the peripheral but also the central mechanism of pain is found inhibited by narcotic analgesics [23-25].

Extracts of *Ehretia serrata* and *Ehretia obtusifolia* revealed effective analgesic potency at 100, 200 and 300 mg/kg dose levels as shown in Tables 1 and 2. Results in Table 1 exhibited that the leaf extract of *Ehretia serrata* show highly significant analgesic activity at 100 mg/kg dose both after 30 min (18.533 ± 3.2353) of the test and (20.186 ± 2.3695) after 1 h. On the other hand, the analgesic activity shown by leaf extract of *Ehretia serrata* at 200 mg/kg was 11.446 ± 0.5217) at 30 min and highly significant (22.500 ± 1.6072) after 1 h, whereas at a dose of 300 mg/kg the leaf extract exhibited more significant result both after 30 min (14.333 ± 1.4529) and (14.766 ±

0.8838) after 1 h. Moreover, results showed that the fruit extract of *Ehretia serrata* at a dose of 100 mg/kg showed significant analgesic activity (14.100 ± 1.3051) at 30 min and highly significant (22.430 ±0.7605) at 1 has given in Table 1.

Table 1. *Analgesic effect of leaf, fruit and stem bark methanol extracts of E. serrata.*

Treatment	Dose (mg/kg)	Time before dose administration (0 min)	Time after dose administration (30 min)	Time after dose administration (60 min)
Normal saline		70.6667 ± 4.50925		
Dichlofenac sodium	10 mg/kg	9.2667 ± 0.733	7.00 ± 0.5773	10.00 ± 0.57735
Leaf	100 mg/kg	8.4667 ± 0.9955	18.533 ± 3.2353**	20.186 ± 2.3695**
	200 mg/kg	7.8333 ±0.1202	12.633 ± 0.3383	22.500 ± 1.6072**
	300 mg/kg	10.546 ± 2.1641	14.333 ± 1.4529**	14.766 ± 0.8838**
Fruit	100 mg/kg	8.066 ± 1.01700	14.100 ± 1.3051*	22.430 ± 0.7605**
	200 mg/kg	10.066 ± 0.6691	15.50 ± 0.00000**	20.253 ± 0.4511**
	300 mg/kg	12.366 ± 2.1070	13.90 ± 0.20810*	20.823 ± 1.4669**
Stem bark	100 mg/kg	7.800 ± 0.70000	17.166 ± 3.7024**	21.396±3.2352**
	200 mg/kg	7.733 ± 0.66910	14.666 ± 0.6227**	17.89 ± 0.47480*
	300 mg/kg	10.633 ± 2.1987	14.226 ± 1.2013*	18.386 ± 1.0123**

Values are reported as mean ± SEM for groups of five animals each. Data were analysed by one way ANOVA followed by Dunnett's test. *Significant at P<0.05; **Highly significant at P<0.01.

However, at 200 and 300 mg/kg doses, the fruit extract of *E. serrata* showed significant results (15.50 ± 0.00000) and (13.90 ± 0.20810) at 30 min and highly significant (20.253 ± 0.4511 and 20.823 ± 1.4669) after 1 h, respectively (Table 1). Results also showed that the analgesic effect of stem bark extract of *E. serrata* at 100 and 200 mg/kg doses are significant (17.166 ± 3.7024 and 14.666 ± 0.6227) at 30 min after which the activity began to increase with passage of time and showed more significant results (21.396 ± 3.2352 and 17.89 ± 0.47480) after 1 h (Table 1).

Ehretia obtusifolia leaf extract showed insignificant analgesic activity at 100 mg/kg and 200 mg/kg after 30 min with the values 10.433 ± 0.3844 and 11.446 ± 0.5217), respectively, whereas and highly significant effects were observed after 1 h (19.900 ± 1.9157 and 17.506 ± 0.1189) as displayed in Table 2.

Table 2. *Analgesic effect of leaf, fruit, and stem bark methanol extracts of E. obtusifolia.*

Treatment	Dose (mg/kg)	Time before dose administration (0 min)	Time after dose administration (30 min)	Time after dose administration (60 min)
Normal saline		70.6667 ± 4.50925		
Dichlofenac sodium	10 mg/kg	9.2667 ± 0.733	7.00 ± 0.5773	10.00 ± 0.57735
Leaf	100 mg/kg	7.833 ± 0.4409	10.433 ± 0.3844	19.900 ± 1.9157**
	200 mg/kg	9.000 ± 0.7211	11.446 ± 0.5217	17.506 ± 0.1189*
	300 mg/kg	7.833 ± 0.6119	17.496 ± 3.0343**	25.273 ± 1.1042**
Fruit	100 mg/kg	12.266 ± 1.092	16.056 ± 0.9032**	20.220 ± 2.8853**
	200 mg/kg	9.566 ± 1.0520	13.2900 ± 0. 531*	14.056 ± 2.2420*
	300 mg/kg	10.33 ± 1.1623	14.890 ± 0.8083*	12.666 ± 0.4409
Stem bark	100 mg/kg	8.400 ± 0.3000	18.223 ± 1.7410**	23.733 ± 2.8591**
	200 mg/kg	7.466 ± 0.8570	13.766 ± 3.3448*	17.466 ± 3.0168**
	300 mg/kg	11.166 ± 1.833	12.456 ± 0.7919*	20.536 ± 2.2838**

Values are reported as mean ± SEM for groups of five animals each. Data were analyzed by one way ANOVA followed by Dunnett's test. *Significant at P<0.05; **Highly significant at P<0.01.

The pain killing effect of leaf increases as time and dose increase. On the other hand, leaf extract of *Ehretia obtusifolia* at a dose of 300 mg/kg showed highly significant after 30 min and 1 h with the values 17.496 ± 3.0343 and 25.273 ±1.1042 respectively. The Fruit extract displayed highly significant results at 100 a dose of mg/kg both after 30 min and 1 h with the values 16.056 ± 0.9032 and 20.220 ± 2.8853 respectively, whereas at 200 and 300 mg/kg doses, significant results with the values 13.2900 ± 0. 531 and 14.890 ± 0.8083, respectively, after 30 min were obtained, and after 1 h the values were 14.890 ± 0.8083 and 12.666 ± 0.4409. Stem bark of *Ehretia obtusifolia* showed highly significant values at level of 100 mg/kg both after 30 min and 1 h with the values 18.223 ± 1.7410, 23.733 ± 2.8591, respectively, as shown in Table 2. At 200 and 300 mg/kg doses the values were13.766 ± 3.3448 and 12.456 ± 0.7919 after 30 min of administration, and were 17.466 ± 3.0168 and 20.536 ± 2.2838, respectively after 1 h. The glutamate was found to play a significant role in nociceptive processing in central as well as in peripheral nervous systems, therefore, the effect of *Ehretia obtusifolia* against nociception induced by glutamate is of great interest [26].

Conclusion

The present results indicate that the methanolic extract of leaf, fruit, and stem bark of *E. serrata* and *E. obtusifolia* have significant analgesic effects. The extract will, therefore, be of possible potential benefit in the control of pain. However, more detailed studies are required to establish the safety, efficacy, and active constituents of these plants, along with the mechanism of action.

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Conflict of Interest

No conflict of interest is declared among authors.

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