



Pharmacognostic Study of *Ehretia acuminata* R.Br.

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Abstract: *Ehretia acuminata*, commonly known as “Puna” in Pakistan, is from the Boraginaceae family and is used in ecological, medicinal, and agricultural sectors. The current study was conducted to assess the pharmacognostic potency of bark extracts from *E. acuminata*. The crude distilled water, ethanol, and chloroform extracts signified a concentration-dependent increase in intestinal mobility of the experimented animal, and the plant delivered methodical proof for its pharmacological usage as an antispasmodic drug. The bark distilled water, bark ethanol, and bark chloroform extracts revealed antispasmodic potential (11 ± 1 , 9 ± 1 , and 11 ± 1) at 300 mg/kg. The distilled water, ethanol, and chloroform extracts also showed analgesic and muscle relaxant potential in the present study and the results were concentration dependent. The bark distilled water, bark ethanol, and bark chloroform extracts revealed the analgesic potential (10 ± 1 , 16 ± 1 , and 11 ± 1) at 300 mg/kg. The Bark distilled water, Bark ethanol, and Bark chloroform extracts revealed the muscle relaxant potential (6 ± 1 , 5 ± 1 , and 5 ± 1) at 300 mg/kg. While the distilled water, ethanol and chloroform extracts did not show acute toxic effects against the tested animal mice. In this study, bark extracts of *E. acuminata* showed pharmacological potency in experimental animals. The plant delivered scientific proof for its pharmacological usage as an antispasmodic, acutely toxic, muscle relaxant, as well as an analgesic drug.

Keywords: Analgesic, Antispasmodic, Bark extracts, Boraginaceae, *Ehretia acuminata*, Pharmacological potential

1. INTRODUCTION

The genus *Ehretia* comprises about 150 species, predominantly dispersed in torrid regions of tropical Asia, Australia, Africa, and North America [1]. The genus includes pergolas and shrubs. All parts of the plant, including the leaves, stem, offshoots, fruits, roots, and duramen, are used as herbal medicines separately [2]. *Ehretia acuminata*, a native plant of Pakistan vernacularly known as Puna, has a variety of uses; the wood of the plant is widely used for fuel purposes, while the leaves are used as fodder for livestock. Moreover, the tree is also used as an erosion controller in farm forestry and for gunstock purposes, whereas; the unripe fruit is used as pickles in food [3, 4].

In southern China, various parts of the genus, including the leaves, root, fruit, bark, and duramen, are widely used as traditional medicines for inflammation, cough, itches, diarrhea, dysentery, swellings, cachexia, fever, and syphilis [2]. Many species such as *E. acuminata*, *Ehretia laevis*, as well as *Ehretia microphylla*, have been described to be used in numerous traditional and herbal remedies in China as well as India due to satisfactory feedback in various tests such as anti-inflammatory, anti-diabetic, and antibacterial activity [1, 5].

In Zimbabwe, different parts of *Ehretia obtusifolia* are used for the treatment of sore throat, toothaches in infants, menstrual cramps, abdominal spasms and infertility in women [6]. In India,

E. laevis is used to treat headaches and ulcers. It also possesses potent anthelmintic, diuretic, demulcent, expectorant, and astringent properties. The duramen of *E. laevis* has been used as nosh [7]. The plants of the genus *Ehretia* are renowned for possessing the rich traditional medicinal uses, including the treatment of chest pains, abdominal cramps [8], toothaches, cachexia, cough, diarrhea, syphilis, stomach diseases, and eczema [9, 10]. Moreover, they have also been widely used in the treatment of asthma, tonsils, dry cough, pneumonia, malaria, typhoid, epilepsy, wounds, mental problems, and venereal diseases [11], as well as nervous disorders and kidney inflammations [12].

Ehretia genus has also been described to as possessing some of the important secondary metabolites, including flavonoids, lignans, phenolic acids, nitrile glycosides, steroids, triterpenoids, quinonoids, and pyrrolizidine alkaloids [1]. Ehretianone, a novel quinonoid xanthene extracted from the bark of the roots of *Ehretia buxifolia* is reported to hold antisnake venom potential [13]. The main objective of this study is to assess the pharmacognostic potency of bark extracts of *Ehretia acuminata* and to discover their use as an antispasmodic drug, an acute toxic drug, a muscle relaxant drug as well as an analgesic drug for future.

2. MATERIALS AND METHODS

2.1 Accumulation of Plant Material and Identification

The undried bark of *E. acuminata* was taken from the Pakistan Forest Institute, Peshawar. The act of taxonomic identification was done by Ghulam Jelani, the curator of the herbarium at the Botany Department, University of Peshawar, Pakistan. The specimen sample (Voucher No. 59) is preserved in the herbarium of the Botany Department Government Superior Science College Peshawar, Pakistan.

2.2 Extraction Preparation of Plant Material

The collected bark was kept in the shade for 3 weeks at room temperature to be completely dried. After the drying process, grinding was done with the help of a grinder machine. The bark was grounded to be used as extracts in distilled water, chloroform,

and ethanol. The powder materials were weighted by Electrical balances (Gottingen, Germany), and 50 mg powder was taken and dissolved in 500 ml of solvents followed by the measurement of the solvents using graduated cylinders. Each of the given solutions was then prepared in a 1000 ml beaker (Borosilicate glass). All of the solutions were kept in reserve for three days (72 h) in a stirrer (DAIHAN SRICO). After stirring for 3 days, the solutions were then sieved through filter paper (WHATMAN NO. 1), The filtrates were accumulated and poured into respective beakers. Distilled water, chloroform, and ethanol extracts were then evaporated with the help of a Rotatory vacuum machine (HAHNSHIN S/Co, South Korea). The distilled water, chloroform, and ethanol extracts were then poured into the china dishes, which appeared to be in crude form. They were placed in the Water Bath (DAIHAN Scientific, Germany) at 55 °C for 120 minutes to completely purify the extracts. Protected sterile vials were used in order to prevent any impurities. When all of the extracts had completely dried out and got ready, they were then collected from the china dishes and placed in the aforementioned three separate vials with the help of a stirrer, followed by placing them in a refrigerator at 40 °C in order to protect them from bacteria, fungi, or any other contaminants [13].

2.3 Preparation of Serial Dilution

Eppendorf tubes were used in order to prepare a serial dilution. These tubes were tagged from 1 to 3. The first tube was filled with 500 µl stock extract, while, the second and third Eppendorf tubes were filled with distilled water of about 250 µl using a pipette with 500 µl tips. 250 µl of stored extract from the 1st Eppendorf tube was drawn out to second, from second to third by a pipette. The stored extracts persisted in the 1st tube of about 250 µl as default or stock for the diluted extract, followed by the shaking of all the Eppendorf tubes by a Vortex mixer (DAIHAN Scientific, Germany). Finally, the diluted concentrations were formulated at 250 mg/ml, 125 mg/ml, and 62.5 mg/ml [14].

2.4 Analgesic Activity

2.4.1. Acetic acid induced activity

Swiss albino female mice (20-30 g) were brought

from the Veterinary Research Institute Peshawar, Pakistan, for the activities. The mice were kept starved for 4 hours before the initiation of the procedure, followed by the division of the animals into six sets. The first set was administered with normal saline (10 ml/kg I.P) as a negative control, while, the 2nd set was inoculated with the standard drug (Diclofenic sodium) as a positive control (50 ml/kg I.P) third, fourth and fifth sets were provided with 10, 15, and 20 mg/kg I.p. of extract, and the residual 6th set was inoculated with acetic acid. After 30 minutes of saline, diclofenic sodium, and plant extract injections, pain was fostered by introducing 1 % of acetic acid into the peritoneum of mice. The wriggle (abdominal constriction, trunk twirling and expansion of hind limbs) took place in 10 minutes and the result/effect revealed hindrance in percentage [16].

2.5 Muscle Relaxant Activity

2.5.1. Traction test

During the given test, female mice were laid on a string firmly braced from the apex. Normal mice seized the string by feet. However, by allowing them to hover freely, they would hold the cable with at least one hind foot for 4-5 seconds. The incapability of the mice to hold the cord with at least one hind foot indicated a failure in the traction [17]. During the examination, animals were classified into six sets; each administered with either saline (10 ml/kg) or the plant extracts at different doses i.e. (100, 150, and 200 mg/kg).

2.6 Acute Toxicity Activity

Swiss Albino female mice (20-30 g) were used in the said activity for the evaluation of toxic effects [18]. The caged animals accommodated under standard conditions of 12 hours of light/dark cycle, nourished with the food prepared by the Veterinary Research Institute along with water access were habituated to the laboratory environment for 14 days, preceding the experiment. All of the mice were retained famished overnight with a free approach to water followed by the random division into 10 groups, each with six mice which were constantly being scrutinized for the initial 4 hours

and then the subsequent 24 hours for any possible toxic symptoms.

2.7 Antispasmodic Activity

2.7.1. Charcoal movement activity

Swiss Albino female mice (20-30 g) were kept deprived of nourishment for 5 hours before the procedure was initiated. However, they were allowed to drink water. After 60 mins, the mice were treated with standard drugs and plant extracts, followed by the oral administration of 1ml charcoal nosh (3 % deactivated charcoal in 2 % aq-tween 80) to each mouse, succeeded by the treatment of charcoal for 50 minutes. each mouse was then dissected and the interval covered by charcoal food from the pyloric region to caecum was calculated to demonstrate the inhibition induced by the extracts in percentage [18].

2.8 Statistical Analysis

The result of this activity was achieved with the use of one-way ANOVA by Dunnet's numerous juxtapositions. The acquired result was then contrasted with the vehicle observational group. * $P < 0.01$ was considered to be statistically significant. The percent inhibition was calculated by the following formula;

$$\% \text{ inhibition} = [(A-B)/A] \times 100$$

Where A = Average number of writhing of the control group.

While B = Average number of the writhing of the test group

The result of the traction test was accomplished with the help of one-way ANOVA by Dunnet's various comparisons. A level of importance of $P < 0.05$ was considered numerically substantial.

The result of the Antispasmodic Activity was described as mean \pm S.E.M (The standard error of the mean) succeeded by execution of Statistical analysis by Student's t test. A level of significance of $P < 0.05$ was considered statistically substantial.

3. RESULTS & DISCUSSION

3.1 Analgesic Activity of *Ehretia acuminata* on Acetic Acid Persuaded Writhing in Mice

The distilled water bark extracts of *E. acuminata* showed significant analgesic activity ($*P < 0.01$). The activity was dose dependent. It showed 19 ± 1 no. of writhes at the dosage of 100 mg/kg. Similarly, both doses showed 15 ± 1 and 10 ± 1 no. of writhes at 200 and 300 mg/kg. The ethanol bark extracts of *E. acuminata* revealed noteworthy analgesic activity. The activity was dose dependent. It showed 21 ± 1 no. of writhes at 100 mg/kg. Similarly, both doses showed 17 ± 1 and 16 ± 1 no. of writhes at 200 and 300 mg/kg. The chloroform bark decoction of *E. acuminata* set forth notable analgesic activity. The activity was dose-dependent. It showed 22 ± 1 no. of writhes at 100 mg/kg. Similarly, both doses showed 15 ± 1 and 11 ± 1 no. of writhes at 200 and 300 mg/kg Figure 1.

Numerous researchers described analgesic activities of multiple medicinal herbs that delivered significant effects on the tested animals which fully substantiate our findings e.g., Khan *et al.* evaluated the analgesic effects of methanol extracts of various parts of *Ehretia serrata* and *E. obtusifolia* and showed significant results which supports our result [19]. Al-Snafi performed analgesic activities of *Cordia myxa* and reported substantial result similar to our discovery [20]. Other researchers also executed analgesic activities of *E. microphylla* and revealed consequential findings that correspond to our results [21].

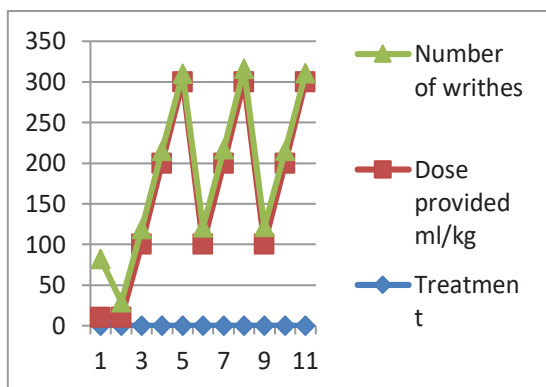


Fig. 1. Analgesic activity of *E. acuminata* bark.

3.2 Muscle Relaxant Activity

The distilled-water bark extract of *E. acuminata* showed significant muscle relaxant activity ($P < 0.05$). The activity was dose dependent. The muscle relaxant potential was 11 ± 1 at 100 mg/kg and 9 ± 1 at 200 mg/kg, while at 300mg/kg; the muscle relaxant potential was 6 ± 1 and showed significant results. The ethanol bark decoction of *E. acuminata* illustrated a noteworthy muscle relaxant experiment. The activity was dose-dependent. The muscle relaxant potential was 11 ± 1 at 100 mg/kg and 7 ± 1 at 200 mg/kg while at 300mg/kg, the result was 5 ± 1 and showed significant results. The chloroform bark extract of *E. acuminata* showed significant muscle relaxant activity. The activity was dose-dependent. The muscle relaxant potential was 12 ± 1 at 100 mg/kg and 8 ± 1 at 200 mg/kg while at 300mg/kg, the muscle relaxant potential was 5 ± 1 and showed significant results Figure 2. AlBayaty explored the muscle relaxant potential of *Cordia myxa* (Boraginaceae) extract in the isolated tracheal smooth muscle of sheep. The results support our findings [22].

3.3 Acute Toxicity

The distilled water bark extract of *E. acuminata* showed significant acute toxicity. The experiment was dose dependent. The result showed no mortality at 100 mg/kg, 200 mg/kg and 300 mg/kg. The bark ethanol decoction of *E. acuminata* revealed remarkable acute toxicity. The activity was dose dependent. The result showed no mortality at the dosage of 100 mg/kg, 200 mg/kg and 300 mg/kg. The chloroform bark extract of *E. acuminata*

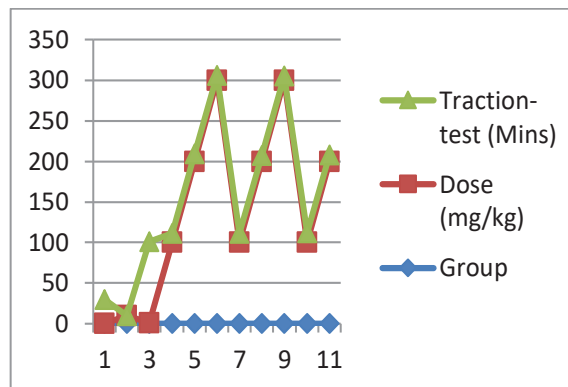


Fig. 2. Muscle relaxant activity of *E. acuminata* bark

showed significant acute toxicity. The activity was dose dependent. The result showed no mortality at 100 mg/kg, 200 mg/kg and 300 mg/kg Figure 3. Another scientist examined the acute toxic effects of *Heliotropium indicum* Linn. (Boraginaceae). Inoculation of HIEA did not cause any death in the experimented animals throughout 24 hours' duration of the acute toxicity which corresponds to our findings [23].

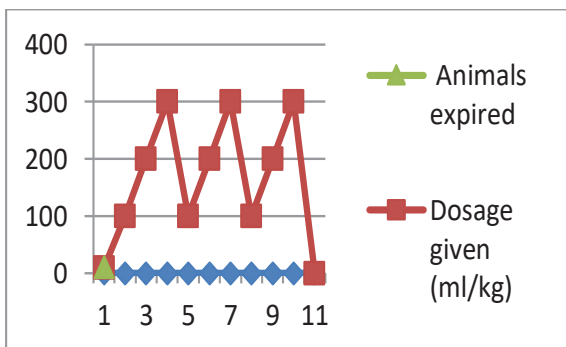


Fig. 3. Acute toxicity of *E. acuminata* bark

3.4 Antispasmodic Activity

The distilled -water bark extract of *E. acuminata* showed significant antispasmodic activity ($P < 0.05$). The activity was dose dependent. The distance covered by charcoal was 19 ± 1 and 15 ± 1 , at 100, 200 mg/kg while at 300mg/kg the 11 ± 1 distance covered by charcoal which showed more significant. The bark ethanol decoction of *E. acuminata* revealed notable antispasmodic activity. The activity was dose dependent. The distance covered by charcoal showed 19 ± 1 and 13 ± 1 at 100 mg/kg and 200mg/kg while at 300 mg/kg showed 19 ± 1 distance covered by charcoal, which showed more significant. The chloroform bark extract of *E. acuminata* showed significant antispasmodic activity. The activity was dose dependent. The distance covered by charcoal showed 20 ± 1 and 17 ± 1 at 100 mg/kg and 200 mg/kg while at 300mg/kg showed 11 ± 1 distance covered by charcoal which showed more significant Figure 4. A group of scientists assessed the Methanol extracts of *Onosma griffithii* and its different parts for possible antispasmodic effects on rabbits' intestine. Rabbits of both sexes (1.0 -2.0 kg) were operated in the experiments. Studies were carried out on rabbits' jejunum (The middle part of the small intestine) preparations [24]. The results showed significant antispasmodic effects which support our findings as well.

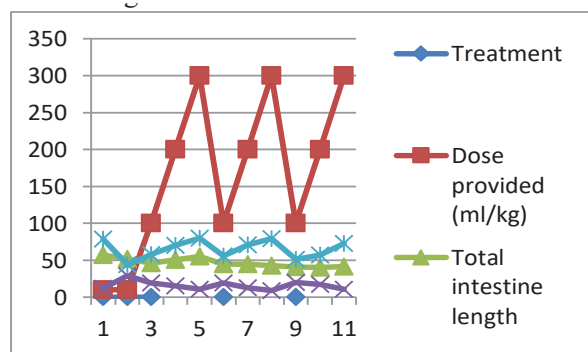


Fig. 4. Antispasmodic activity of *E. acuminata* bark

4. CONCLUSION

The present study was conducted to assess the pharmacognostic potential of bark extracts of *E. acuminata* including four activities, i.e., analgesic activity, muscle relaxant activity, antispasmodic activity, as well as acute toxicity activity, in all of which showed significant dose dependent effects; the higher the dose, the greater the effect. While acute toxicity of *E. acuminata* extracts did not show any effect, the plant provided significant evidence for its pharmacological use as a medicinal plant and can be used as an analgesic, muscle relaxant, and an antispasmodic drug in the future.

5. ETHICAL STATEMENT

The animal used in our research has been approved by the Ethical committee of the veterinary research institute of Peshawar.

6. DISCLOSURE STATEMENT

We wish to confirm that there are no known conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

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