

Antinociceptive and anticonvulsant activities of essential oils of *Zanthoxylum armatum*

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Abstract

Patient compliance is the primary goal in the effective management of painful conditions. In clinical practice, synthetic analgesics showed multiple unwanted effects. Various research groups around the world are focusing on the discovery of effective natural analgesic. The present study deals with the screening of essential oils of the leaves of *Zanthoxylum armatum* (ZEO) for acute toxicity, antinociceptive and anticonvulsant activities. ZEO was found substantially safe up to the dose of 2000 mg/kg i.p. in antinociceptive test. When challenged against acetic acid induced writhing, pain amelioration was observed in a dose related manner. Maximum effect (58.41%) was observed at 400 mg/kg. In formalin induced noxious animal, ZEO significantly relieve the pain in both neurogenic (first phase) and inflammatory (late phase) phases. Maximum effect in early and late phases was 49.08% and 54.02% at 400 mg/kg respectively. Like acute toxicity test, ZEO was found free of convulsive effects in pentylenetetrazole (PTZ) induced anticonvulsant. This study provided scientific validation for the traditional uses of plant in painful condition with considerable safety profile.

Keywords: *Zanthoxylum armatum*; essential oil; analgesic; anticonvulsant

Introduction

Zanthoxylum armatum (Dambara) is a small xerophytic tree or shrub belongs to family Rutaceae. It grows wild in foothills starting from about 800 m up to 1500 m in Malakand, Swat, Dir, Hazara, Buner, Muree hills and Rawalpindi (Shinwari et al., 2006). Fruits and seeds of the plant are edible and used as potherb species. The plant is used for the treatment of

Pneumonia and tick infestation (Sindhu et al., 2010). Young shoots are used as toothbrush and useful for curing gum diseases. Fruit is used for toothache, dyspepsia, as a carminative and stomachache. Seeds are used as condiment and flavoring agent (Arshad and Ahmad, 2004; Abbasi et al., 2010). Powdered fruit is mixed with *Mentha* species and table salt, eaten with boiled egg for chest infection and digestive problems (Islam et al., 2009). Recently different parts of the plant screened for various pharmacological activities (Gilani et al., 2010; Barkatullah et al., 2011; Barkatullah et al., 2012).

In continuation of our research work on Pakistani medicinal plants (Khan et al., 2011; Khan et al., 2012a, Muhammad et al., 2012a; Saeed et al., 2010a; Saeed et al., 2010b; Khan et al., 2011c) and considering different therapeutic uses of this plant in traditional system of treatment, the current study was planned to extract essential oils from the leaves of *Z. armatum* followed by analgesic, anticonvulsant and acute toxicity studies in different animal models.

Materials and methods

Essential oil extraction

A modified Clevenger type apparatus were used for the extraction of essential oil from the leaves of *Zanthoxylum armatum* through hydro-steam distillation. Leaves were thoroughly washed, cut into small pieces, placed in distillation flask and subjected to hydrosteam distillation for about 4 h. The steam and vaporized oil were condensed into liquid by a vertical condenser and collected in measuring tube. Being immiscible and lighter than water, the volatile oil separated out as an upper layer. The oil was then separated from water and collected in small bottles, dried with anhydrous sodium sulphate, sealed, labeled and stored in light resistant vials at 4–6°C for further use (Reverchon and Senatore 1992; Lucchesi et al., 2004).

Animals

NMRI mice were used in various experiments. Animals were fed with standard laboratory food and water *ad libitum*. Animals were kept under standard condition of temperature and light. Before the start of experiment animals were acclimatized with laboratory conditions. The rulings of the institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council were maintained during all the experiments performed.

Acute toxicity test

Acute toxicity test for ZEO was carried out to evaluate any possible toxicity. NMRI mice (n = 6) of either sex were tested by administering different doses (500, 1000, 2000 and 3000 mg/kg) of ZEO, while the control group received normal saline (10 ml/kg). All the groups were observed mortality during 24 h (Bruce, 1985; Khan et al., 2010).

Antinociceptive activity

Acetic acid induced writhing test

The analgesic activity was carried out using NMRI mince (18–22 g) of either sex. Animal were divided in to five groups (n=6). Group I and II were injected with normal saline (10 ml/kg, i.p.) and Ibuprofen (150 mg/kg, i.p.), while remaining groups were treated with ZEO (100, 300 and 400 mg/kg, i.p.) after the above treatment animals were injected i.p. with acetic acid (1%). The abdominal constriction (writhing) was counted for 10 min after 5 min of acetic acid injection (Khan et al., 2009; Khan et al., 2010).

Formalin induced flinching behaviour test

The method used in our study for the assessment of formalin-induced flinching behaviour in normal rats was described previously (Khan et al., 2011). In this method, 0.05 ml of formalin (2.5% formaldehyde) was injected into the plantar surface of the right hind paw, 30 min after treating the animals with the extracts (50, 100 and 200 mg/kg i.p.). Nociceptive behavior was quantified as rat walking or can stand on injected paw; paw partially elevated; total elevation of injected paw, injected paw licking or biting. Formalin injection induced a stereotyped response characterized by two well distinct phases; phase I started almost immediately and was short lasting (0-5 min) followed, by prolonged tonic phase II lasting (15-30 min). Ibuprofen (150 mg/kg i.p.) was used as a standard drug.

Pentylenetetrazole (PTZ) induced anticonvulsant test

The animals were divided into five groups (n=6), group I was treated with normal saline (10 ml/kg), group II was treated with diazepam (5 mg/kg, i.p.), while remaining groups were treated with ZEO (200, 300 and 400 mg/kg i.p). After 30 min of treatment all animals were injected pentylenetetrazole (PTZ) 80 mg/kg (s.c.). Each animal was observed for onset and mortality (Khan et al., 2012c).

Statistical analysis

Results are expressed as mean ± S.E.M. One-way ANOVA was used for comparison test of significant differences among groups followed by Dunnet’s multiple comparison post test. A level of significance ($P < 0.05$ or 0.01) was considered for each test.

Results

Effect of acute toxicity test

All animals were safe and no gross behavior was observed up to dose of 2000 mg/kg, while the animal locomotion was greatly diminished at higher doses. No mortality was obser-

Table 1. Effect of acute toxicity test of the essential oils of *Zanthoxylum armatum* (ZEO).

Treatment	Dose	No of animal died per 6	% Mortality
Normal saline	10 ml/kg	6/0	–
	500 mg/kg	6/0	–
ZEO	1000 mg/kg	6/0	–
	2000 mg/kg	6/0	–
	3000 mg/kg	6/3	50

Table 2. Effect of the essential oils of *Zanthoxylum armatum* (ZEO) in acetic acid-induced writhing in mice.

Group	Dose	No of writhes
Saline	10 ml/kg	70.70±3.45
ZEO	100 mg/kg	54.60±3.40
	200 mg/kg	45.10±4.10*
	400 mg/kg	29.40±3.10**
Ibuprofen	150 mg/kg	28.10±2.50**

Values are reported as mean ± S.E.M. for group of six animals. The data were analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. * $P < 0.05$, ** $P < 0.01$.

Table 3. Effect of the essential oils of *Zanthoxylum armatum* (ZEO) in formalin induced flinching behaviour.

Group	Dose (mg/ kg)	Early phase (0-5 min)	Late phase (15-30 min)
Saline	10 mL/ kg	71.10±4.80	73.30±5.47
	100	57.30±3.30	55.50±4.47
ZEO	200	49.90±2.24*	47.60±5.50*
	400	36.20±4.25*	33.70±3.81**
Ibuprofen	150	44.30± 3.40*	17.10 ±3.75**

Values are reported as mean ± S.E.M. for group of six animals. The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. * $P < 0.05$, ** $P < 0.01$.

ved after 24 h at the test dose of 2000 mg/kg, while 50% was observed at the dose of 3000 mg/kg as shown in table 1.

Antinociceptive effect

Effect of ZEO in acidic acid induced writhing test

Abdominal constriction induced by acetic acid was significantly antagonized at 200 and 400 mg/kg, when ZEO was administrated intraperitoneally (table 2). The effect was in a dose dependent manner. Maximum pain reduction (58.41%) was noted at 400 mg/kg as shown in figure 1. The antinociceptive effect of ZEO was comparable to standard drug, ibuprofen (150 mg/kg i.p.).

Effect of ZEO in formalin induced flinching behaviour

Results of ZEO in formalin induced flinching behaviour in both phases are illustrated in table 3. It demonstrated significant antinociceptive effect in both phases. Pain attenuation was in a dose dependent manner and maximum pain reduction was 49.08% and 54.02% in early and late phases respectively. In the early phase, pain reversal of ZEO was even more prominent than ibuprofen (150 mg/kg i.p.) at 400 mg/kg i.p. However, in late phase ibuprofen was dominant.

Effect of ZEO in pentylenetetrazole (PTZ) induced anticonvulsant activity

When ZEO was tested in pentylenetetrazole (PTZ) induced anticonvulsant activity, it did not produced any significant at test does (100, 200 and 400 mg/kg i.p.).

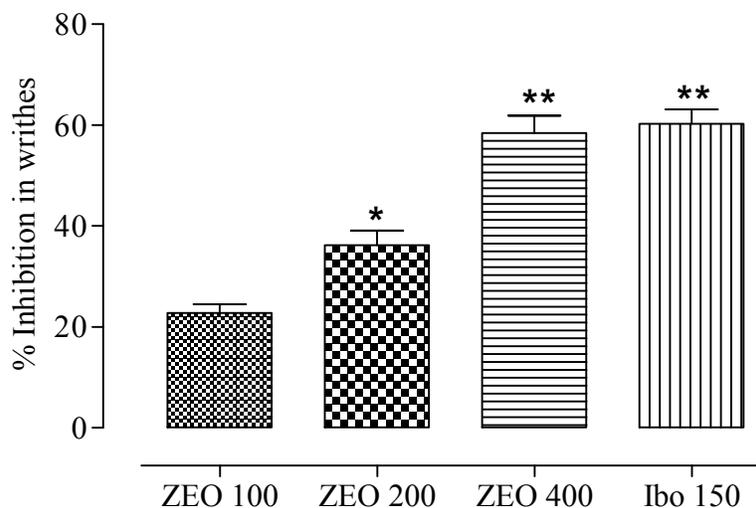


Figure 1. Protection (%) of the essential oils of *Zanthoxylum armatum* (ZEO) in acetic acid-induced writhing in mice. Values are reported as mean \pm S.E.M. for group of six animals. The data were analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. * $P < 0.05$, ** $P < 0.01$.

Discussion

The current study deals with the acute toxicity test, antinociceptive and anticonvulsant activities of essential oils extracted from the leaves of *Zanthoxylum armatum* (ZEO) in different animal models. ZEO was found safe up to the dose of 200 mg/kg i.p. and thus provided strong foundation for further experimental studies. Of various pharmacological tests available for the assessment of antinociceptive activity of test articles, two chemically induced noxious paradigms i.e. acetic acid induced writhing and formalin induced flinching behavior were selected. Noxious mechanism for acetic acid has been recommended liberation of

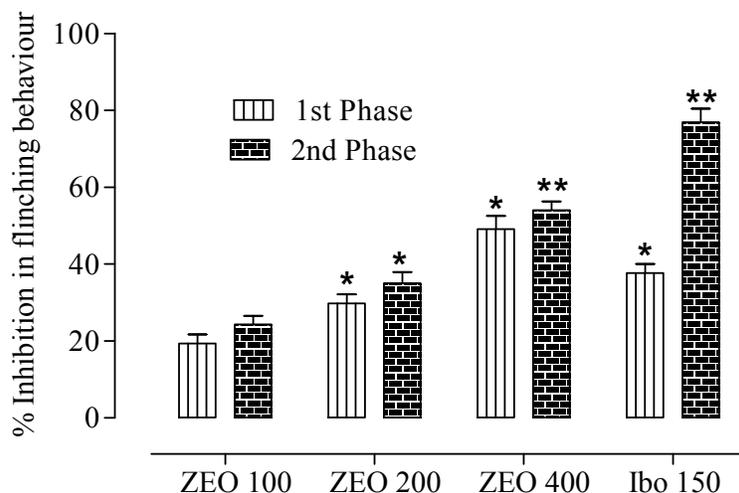


Figure 2. Protection (%) of the essential oils of *Zanthoxylum armatum* (ZEO) in formalin induced flinching behaviour. Values are reported as mean \pm S.E.M. for group of six animals. The data were analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. * $P < 0.05$, ** $P < 0.01$.

different endogenous mediators such as bradykinin, serotonin, histamine, substance P (Mazid et al., 2010; Ahmad et al., 2011; Muhammad et al., 2012b). Hyperalgesia induced by the injection of acetic acid is symbolized by contraction of the abdominal muscle accompanied by an extension of the forelimbs and body elongation. These peripheral nociceptive fibers are sensitive to both narcotics analgesic and non-steroid anti-inflammatory drugs (Wibool et al., 2008). ZEO significantly attenuated the abdominal constriction provoked by the acetic acid in a dose dependent manner. For that reason, one possible mechanism of antinociceptive activity of ZEO could be due to the blockade of the effect or the release of endogenous substances (arachidonic acid metabolites) that excite pain nerve endings.

From a mechanistic point of view, the acetic acid induced writhing test is deficient in specificity; different mechanism may be implicated in the reduction of muscular constriction such as sympathetic system through the release of biogenic amines, cyclooxygenases and their metabolites inhibition and through opioids receptors mechanisms (Andrade et al., 2007). This deficit can be overcome using the formalin test. It is frequently used as a primary behavioral screening test for the assessment of the antinociceptive activity of compounds used in moderate, long lasting clinical pain. (Bukahri et al., 2004). Formalin injection test produced a distinct biphasic response. First neurogenic phase (0-5 min) occurs within seconds of formalin injection as a direct result of chemical stimulation of peripherally localized TRPA-1 containing nociceptors (McNamara et al., 2007). The second, later tonic phase (25-30 min) occurs as a result of increased primary afferent drive with subsequent sensitization of nociceptive spinal neuron. From a mechanistic point of view, different analgesics may act differently. Centrally acting drugs such as opioid inhibit both phases equally but peripherally acting drugs, such as cyclooxygenase inhibitors (Khan et al., 2011) inhibits only the late phase.

When ZEO was challenged against formalin induced nociceptive stimulus, it produced prominent antagonism in both phases. The effect was dose related; maximum pain reduction was being obtained at highest tested dose. Therefore, the antinociceptive activity of ZEO in formalin test could be strongly attributed to the antagonism of peripherally acting as well as centrally acting pain mediators. On the other hand, ibuprofen as a standard drug showed specifically more dominant effect in the last phase like other non steroidal antiinflammatory drugs.

ZEO was also tested in pentylenetetrazole (PTZ) induced anticonvulsant test; it did not produced any significant activity at test doses (100, 200 and 400 mg/kg i.p.) and therefore, ruled out the presence of any convulsant component. In summary, it is revealed by the results that ZEO has strong antinociceptive potential; suggesting partial mediation of both peripheral and central blockade of noxious stimulus. Our experimental findings are consistent with the traditional claim of the plant in the treatment of painful conditions with substantial safety profile.

Conflict of Interest

There is no conflict of interest associated with the authors of the paper.

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