Analgesic, Anti-inflammatory and Antipyretic Activities of Stem Extract of Zizyphus oxyphylla Edgew

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Abstract: The study aims to investigate the stem extract of Zizyphus oxyphylla Edgew for some pharmacological activities. Different tests were performed to check analgesic, anti-inflammatory and anti-pyretic activities. Acetic acid induced model and formalin test were used for analgesic activity followed by carrageenan induced paw edema model, used for checking activity against inflammation, however yeast induced pyrexia model was used for antipyretic activity. Crude extract of Zizyphus oxyphylla has shown significant activity against carrageenan induced paw edema model at 400 and 200 mg/kg dose which was really found influential against inflammation. Similarly, significant activity (P<0.05) was recorded in crude extract as it has shown prominent inhibition against analgesia very close to the standard aspirin in both acetic acid induced writhing test and formalin induced noxious pain test at doses of 100, 200 and 400 mg/kg. Anti-pyretic activity in yeast inducing pyrexia model has revealed that doses of 200 and 400mg/kg have remarkable effects in decreasing the elevated rectal temperature of rats. The crude extract has shown significant results, indicates the need to investigate it for the discovery of more potent compounds for improved medicinal management of pain, inflammation and fever.

Key words: Zizipus oxyphylla · Stem Extract · Analgesic · Anti-inflammatory and Antipyretic activity

INTRODUCTION

The genus Zizyphus worths about 100 species throughout the world [1]. Among those only 6 species are grown in Pakistan. Zizyphus oxyphylla is distributed in Buner, Hazara, Swat and Gari Habibullah [2]. It is traditionally used for treatment of jaundice, pain, inflammation and fever. Leaves on chewing completely anaeasthetize the taste, while fruit is used as emollient and useful expectorant in bronchitis[3]. Literature reveals that methanolic leaf extract of Zizyphus oxyphylla possesses potent antipyretic and antinociceptive activity [2] and significant effects of crude methanolic extract and various fractions of Zizyphus oxyphylla Edgew leaves in case of antibacterial, antifungal, phytotoxic, cytotoxic and insecticidal activities[4]. Several species of Zizyphus have shown various therapeutic activities both in vivo and in vitro such as anti diabetic activity of Zizyphus spina-christi leaves [5] antinoceceptive and antipyretic activities of root bark extract of Zizyphus spina-christi [6], anti spasmodic and antiulcerogenic activity of Zizyphus lotus L. extract [7,8] antimicrobial activity of Zizyphus spina-christi L. fruit [9], Antioxidant and antilisterial activity of Zizyphus jujube [10]. Keeping in view the vast bioactive literature regarding the genus, a study was attempted to check the therapeutic potential of the plant.

MATERIALS AND METHODS

Plant Material: Zizyphus oxyphylla Edgew was collected from Northern Areas of Pakistan (Swat) in February 2011 and identified by the Botany Department, Peshawar University. It was brought to the Agricultural Chemistry Laboratory where a voucher specimen was deposited in Herbarium of the Department.
Preparation and Extraction: The plant material was dried under suitable temperate conditions and later on pulverized for further chemical treatment. The powder plant material was macerated thrice with methanol for 7 days for primary extraction. The filtrate was evaporated using rotary evaporator which has given crude extract, then subjected to pharmacological analysis.

Experimental Animals: Male rats, weighting 170-220 g and mice (Swiss albino), weighting 19-26 g (Experimental Animal house, Pharmacology Department, PCSIR Laboratories, Peshawar) were used in these studies. All animals were housed on particular room temperature in polypropylene cages. The rats were used for antipyretic studies however albino mice were used for anti-inflammatory and analgesic studies. Animals were divided into drug-treated test group, saline treated control group and standard drug groups. Keeping in view the international principles and rules regarding care of lab animals, they were used in all experiments.

Carrageenan-Induced Edema Model: Carrageenan-induced hind paw edema test was performed according to the protocol described by Winter et al., (1962) [11] with some modifications. Test samples of crude extract of Zizyphus oxyphylla as well as the negative and positive controls were administered intra peritoneally 1 hr before producing edema in the paw. Acute inflammation was created by sub planter injection of 0.1ml of 1% suspension of carrageenan in normal saline, in the hind paw of the mice. Plethysmometer was used to measure paw volume at 1, 2, 3, 4 and 5 h after the carrageenan injection. 100mgkg⁻¹, i.p. Indomethacin was used as positive control, while saline was used as a negative control. Inhibition (%) of the inflammation was determined by comparing drug group with both controls (positive and negative). The formula used was:

\[ \% I = 1 - \left( \frac{d_t}{d_c} \right) \times 100 \]

whereas “\( d_t \)” represents difference in paw volume in the drug-treated group (DTG) and “\( d_c \)” stands for difference in paw volume in control group (CG). However, “\( I \)” represents inhibition of inflammation.

Analgesic Activity
Acetic Acid-Induced Abdominal Writhing: Writhing test for analgesic activity was used according to Adzu et al. [6]. Five groups were made having six mice in each of them and they were starved for about 18 h before experiment. First 3 groups have received test crude extract at 400, 200 and 100 mgkg⁻¹ while group 4 has served as saline group (control) and group 5 has served as standard drug (Aspirin) group. Standard drug was given intra peritoneally at a rate of 150mgkg⁻¹. 10 mgkg⁻¹ of aqueous solution of acetic acid (0.7 %) was given to all the mice after half hr following by monitoring of withings in all the groups 8 minutes after the acetic acid injection.

Formalin Test: Method for formalin induced analgesia was used according to Nisar et al. [12]. Animal grouping was the same as in writhing test. Initially animals were treated with crude extract of Zizyphus oxyphylla at different ratios of about 200, 100 and 50mgkg⁻¹ intra peritoneally followed by injection of 0.05 ml of formalin in the plantar surface of right hind paw within a 30 min delay.

Yeast Induced Pyrexia Model: Antipyretic analysis was performed according to the protocol of Al-Ghamdi [13]. Pyrexia was induced in rats by a sub-cutaneous injection of 10 mlkg⁻¹ of 15% water solution of yeast. Each animal was checked for rectal temperature before the administration of crude extract using clinical digital thermometer. Animals which have shown least increase of 0.5°C in rectal temperature after 24 hrs of yeast injection were separated from the whole. Total five groups were used in which each of them had 6 animals. Three of them were treated with crude extract at a dose rate of 50, 200 and 400 mgkg⁻¹ however rest of the two were subjected to the saline and aspirin (standard). After treatment, temperature of every animal was repeatedly recorded at 0.5, 1, 1.5 and 2 hrs.

Statistical Analysis: The data were expressed as mean ± S.E.M. Data were statistically analyzed by analysis of variance (ANOVA) followed by Student’s t-test and a probability level lower than 0.05 was considered as statistically significant [14].

RESULTS AND DISCUSSION

Anti-Inflammatory Activity: The results obtained from carrageenan induced paw edema test are demonstrated in Fig. 1. It was observed that 400 mg/kg dose of crude extract was really influential against the edema followed by 200 mg/kg. Both of them showed worth mentioning
Fig. 1: Anti-inflammatory activity of *Z. oxiphylla* crude extract in carrageenan induced paw edema model in mice

The two bars should be distinguish in color

Fig. 1 should be inserted before Table 1 inside the text in order since it had been mentioned first in the text

Table 1: Effect of the *Zizyphus oxyphylla* stem extract on acetic acid-induced writhing test in mice

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Writhing count*</th>
<th>Inhibition (%) against writhings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (control)</td>
<td>29±1.28</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>17.25±0.76*</td>
<td>40.51*</td>
</tr>
<tr>
<td>200</td>
<td>7.9±1.84*</td>
<td>72.76*</td>
</tr>
<tr>
<td>400</td>
<td>4.7±0.91*</td>
<td>83.79*</td>
</tr>
<tr>
<td>Aspirin (ASA) 150</td>
<td>3.61±1.367*</td>
<td>87.51*</td>
</tr>
</tbody>
</table>

*Effect was found significant (*P* < 0.05)*

*Values are expressed as mean ± S.E.M. [n = 5]*

Table 2: Effect of *Zizyphus oxyphylla* stem extract on formalin-induced noxious pain activity

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Early phase (0-12 min)</th>
<th>Late phase (15-30 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>2.7±0.4</td>
<td>2.2±0.6</td>
</tr>
<tr>
<td>100</td>
<td>1.7±0.2*</td>
<td>1.3±0.3*</td>
</tr>
<tr>
<td>200</td>
<td>0.9±0.5*</td>
<td>0.8±0.7*</td>
</tr>
<tr>
<td>Aspirin 150</td>
<td>0.2±0.1*</td>
<td>0.2±0.2*</td>
</tr>
<tr>
<td></td>
<td>2.9±0.2</td>
<td>2.9±0.1</td>
</tr>
</tbody>
</table>

*Effect was found significant (*P* < 0.05).*

*Values are expressed as mean ± S.E.M. [n = 5]*

Fig. 2: Effect of *Zizyphus oxyphylla* crude extract on yeast-induced pyrexia in rats
activity against the inflammation in the hind paw of mice. The dose of 50 mg/kg has shown least activity comparatively. Carrageenan induced edema is a multimediated phenomena that liberates variety of mediators. It is said to be biphasic; the first phase (1h) involves the release of serotonin and histamine while the second phase (over 1h) is mediated by prostaglandins, the cyclooxygenase products and the continuity between the two phases is provided by kinins [15-16]. The overall activity of drug was really stronger as compare to saline and standard drug Indomethacin throughout and was found influential in a dose dependent manner. The curative time span of first two hour of 400 mg/kg drug was found remarkable in treating the inflammatory disorders.

Yeast Induced Pyrexia: The results of the yeast induced pyrexia test are presented in Fig. 2. Treatment of rats with crude extract of *Zizyphus oxyphylla* at the doses of 50, 200 and 400 mg/kg has significantly decreased the rectal temperature of the rats in a dose dependent manner. The Antipyretic effect was observed from the first hour which was further maintained for 2 hrs after administration of the extract. Doses of 200 and 400 mg/kg showed maximum deduction in elevated rectal temperature of rats as compare to 50mg/kg dose indicates the potential effect of extract of *Zizyphus oxyphylla* in decreasing the prostalgland. Aspirin has decreased the fever in rats throughout the whole period of experiment as shown in Fig. 2.

CONCLUSION

*Zizyphus oxyphylla* has been reported to have various pharmacological activities and has been extensively used for various ailments such as inflammatory disorders, GIT infections, urinary tract infections and hepatic disorders in folkloric use. In view, *Zizyphus oxyphylla* was investigated both phytochemically and pharmacologically in search of finding new pharmacological activities. Various *in-vivo* tests (analgesic, anti-inflammatory, anti-pyretic) has revealed that stem extract can be effective to treat not only inflammatory diseases but also as pain killers and coping against pyrexia. So, further phytochemical studies are required to isolate and identify those chemical compounds, which are responsible for the observed activity.

ACKNOWLEDGMENTS

The author is very thankful to Department of Agricultural Chemistry, Agriculture University, Peshawar, Pakistan for providing research facilities.

REFERENCES


