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Anti-Inflammatory and Antioxidant Activities of Zanthoxylum Armatum Stem Bark

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Anti-Inflammatory and Antioxidant Activities of Zanthoxylum Armatum Stem Bark

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Abstract - The present study is an endeavour to evaluate anti inflammatory and antioxidant activities of ehtanolic extract of steam bark of Zanthoxylum armatum. In vivo anti inflammatory activity was evaluated in wistar species of rats by using carrageenin induced paw edema, where as in vitro antioxidant activity was performed by DPPH free radical method. The plant extract exhibited significant anti-inflammatory and antioxidant activities.

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I. Introduction

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli, an uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses (Kumar et al., 2004). Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can easily be induced (Sosa et al., 2002). The side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical uses (Mattison et al., 1998). Therefore, the development of newer and more potent anti-inflammatory drugs with lesser side effects is necessary. Reactive oxygen species (ROS) are responsible for variety of pathological conditions (Aruoma, 1998). Innate defense system of human body may not be sufficient for curing the damage caused by continued oxidative stress. Thus there is need to supply the antioxidants exogenously to balance their level in the human body. May synthetic antioxidant, such as butylated hydroxyl toluene (BHT), butylated hydroxyanisole (BHA) antioxidants (Yesilyurt et al., 2008). Therefore recently there has been an upsurge of interest in natural products as antioxidants, as they can inhibit the free radical reaction and protect the human body from various diseases.

Zanthoxylum armatum DC [syn. Z. alatum Roxb.] (Rutaceae) is extensively used in the Indian system of medicines as a carminative , stomachic , and anthelmintic. The bark is pungent , and sticks prepared from it are used for preventing toothache. The fruits and seeds are employed as an aromatic tonic in fever, dyspepsia, and expelling roundworms (Wealth of India, 1976). Phytochemical examinations of Z. armatum have afforded volatile oil consisting mainly linalool (Ramidi, 1998). Mono terpentriol-3, 7-dimethyl 1-octane 3,6,7-triol, trans cinnemic acid, nevadensin umbelliferone, β-sitosterol and its glucoside(Talapatra, et al.,1989), 3,5, dihydroxy-7,8,4'trimethoxyflavone (tamblin) and tambulatin (Nair et al., 1982), 3-methoxy-11-hydroxy-6,8-dimethylcarboxylate biphenyl, 3,5,6,7-tetrahydroxy-3',4'-dimethoxyflavone-5-β-d-xylopyranoside(Akhtar et al., 2009), aramatamide, lignans, asarinin and fragesin, α and β-amyrins lupeol, and β -sitosterol β-Dglycoside(Kalia, et al., 1999) have been reported from the plant previously. Antihelmentic (Mehata et al., 1981), antiprolifative (Kumar et al., 1999], antifungal(Dikshit et al.,1984) and anti-insecticidal activities(Tiwary et al.,2007) have also been studied with different plant parts.

II. Materials and Methods

a) Chemicals

Butylated hydroxyl toluene (BHT), 2, 2 diphenyl-1-picrylhydrazyl and carageenin were purchased from HiMedia Lab. Pvt. Ltd. Mumbai, India. All other chemicals and reagents used were of analytical grade.

b) Animals

Male wistar rats (130-160g) kept the animal house of the IIIM Jammu. The animal were housed under standard environmrntal conditions. All experiment were carried out after getting the approval from the committee for the purpose of control and supervision of experimental animals (CPCSEA) having the registration number is 67/CPCSEA/99.

c) *Effect of Z. armatum extract on carrageenin induced rat paw edema*:

Screening for anti-inflammatory activity of Z. armatum extract was done with a carrageenin induced paw edema model (Winter et al., 1962). Administration of carrageenin in the sub-plantar region of rat's hind paw leads to the formation of edema in situ due to localized inflammation. About half an hour prior to the administration of carrageenin solution, experimental animals received test materials and standard anti-inflammatory drug at appropriate doses. The volume of rat paw was measured each hour up to four hours by means of mercury displacement method in traveling

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microscope assembly (Roy et al., 1980). The average percent increase in paw volume with time was calculated and compared against the control group. Percent inhibition was calculated using the formula

$$\% \text{ inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where Vc and Vt represent average paw volume of control and treated animals respectively.

Nine experimental animals were randomly selected and divided into three groups denoted as Group I, Group II and Group III, consisting of 3 rats in each group. Each group received a particular treatment i.e. control, standard drug and the dose of the extract. Prior to any treatment, each rat was weighed properly and the doses of the test samples and control materials were adjusted accordingly. Group I received the crude extract orally at the doses of 250 mg/kg of body weight respectively. Group II received intraperitoneal administration of ibropfen as standard anti-inflammatory drug at a dose of 10mg/kg body weight while Group I was kept as control giving 1% tween 80 in normal saline water. After one hour of drug administration, 0.1 ml of 1% (w/v) carrageenin solution in sterile saline solution was injected through 26-gauge needle into the sub-planter surface of the right hind paw of each rat of every group. Paw volumes were measured up to a fixed mark by mercury displacement as viewed by traveling microscope at 1, 2, 3 and 4 hours after the administration of the standard drug and test extracts.

III. DPPH free Radical Method

In order to measure antioxidant activity DPPH free radical scavenging assay was used. This assay measures the free radical scavenging capacity of the extract under investigation.

DPPH is a molecule containing a stable free radical. In the presence of an antioxidant, which can donate an electron to DPPH, the purple color which is typical for free radical decays and the absorbance was measured at 517nm using a double beam UV-VIS spectrophotometer (Brand et al., 1995). The extract was dissolved in ethanol and various concentrations (10, 20, 50 and 100 µg/ml) of extract were used. The assay mixture contained in total volume of 1 ml, 500µl of extract, 125µl prepared DPPH and 375 µl solvent (ethanol). After 30 min of incubation at 250C, the decrease in absorbance was measured. The radical scavenging activity (RSA) was calculated as a percentage of DPPH using a discoloration using then equation

$$\% \text{ RSA} = [(A0 - As) / A0] \times 100$$

Where A0 and As are the absorbance of control and test sample respectively

IV. Plant Material and Extract

Stem bark of Zanthoxylum armatum were collected from, Singoli Tehri Garhwal Uttarakhand, India and identified from the Plant Identification Laboratory, Department of Botany, H.N.B. Garhwal University Srinagar. A voucher specimen (GUH 3802) was deposited in the Department for future records. The bark was dried under shade and make to powder.

The 2 kg dried powdered bark of plant was exhaustively extracted with ethanol for 72 hour. The solvent were evaporated under reduced pressure in a rotary vacuum evaporator and dried in vacuum. The dried extract obtained was used directly for the assessments of anti inflammatory and antioxidant activities.

V. Results and Discussion

The anti-inflammatory activity of extract Z. armatum was evaluated by carrageenininduced paw edema method in wistar specie of rats. The plant extract at dose 250mg/kg caused inhibition of paw edema by 19.12%, 4 hours after carageenin administration hour (Table I).The 1st, 2nd and 3rd hours results were not significant so we take only 4th hour reading. The carrageenin-induced paw edema in rats is believed to be biphasic (Vinegar et al., 1969). The first phase is due to the release of histamine or serotonin, and the second phase is caused by the release of bradykinin, protease, prostaglandin, and lysosome (Crunkhorn and Meacock, 1971). Therefore, it can be assumed that the inhibitory effect of the extract of plant on carrageenan-induced inflammation could be due to the inhibition of the enzyme cyclooxygenase, leading to the inhibition of prostaglandin synthesis (Biswa Nath Das et al., 2009).

Table 1: Anti-inflammatory activity of Zanthoxylum armatun stems bark

Treatment	Edema volume (ml)* 4th hour	% inhibition
Control	1.26 ± 0.120	
ZA (250/kg)	1.02 ± 0.120	19.02
Ibrofen (5mg/kg)	0.76 ± 0.066	39.68
* value are mean ± SE, n=3, P>0.01, ZA-Z.armatum		

The ethanolic extract of plant showed an effective free radical scavenging in DPPH (2, 2 diphenyl-1-picryl hydrazyl) assay (Table-2). The extract of the plant exhibit a remarkable antioxidant effect at low concentration. When the extract of the plant was tested for DPPH radical scavenging activity, it was found that 50µg/ml and 100µg/ml of the extract lowered the DPPH radical levels above 57% and 94% respectively. Inhabitation of DPPH radicals 50% considered as significant antioxidant properties of any compound (Sanchez-Moreno et al., 1998).

Table 2 : Antioxidant activity of Zanthoxylum armatum stem bark

Concentration ε (g/ml)	DPPH Free radical Scavenging
50	7.06
100	14.22
200	27.33
500	64.58

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