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Research Article

Alkaloids from *Zanthoxylum alatum* stem bark with anti inflammatory potential in rats against acute and chronic inflammation in rats

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ABSTRACT

The study was designed to investigate anti-inflammatory active fraction(s) from the aqueous extract of *Zanthoxylum alatum* stem bark in acute and chronic models of inflammation. Wistar rats of either sex were employed. Acute inflammation was induced by subplantar administration of carrageenan (1 %) in rat hind paw. Chronic inflammation was induced by interscapular implantation of a sterile cotton pellet (50 mg). Alkaloid fraction (100 mg/kg b.w) was found to be more effective in comparision to flavonoid (100 mg/kg) in carrageenan induced inflammation model. Hence was selected for further detailed study. Pretreatment with alkaloid fraction (100, 150 mg/kg) have shown significant ($p < 0.05$) check on carrageenan induced paw edema and significantly ($p < 0.05$) decreased granuloma tissue formation, as compared to control. The alkaloid constituents are more potent in prevention of acute and chronic inflammation. Hence, the present study has concluded that the alkaloids fraction is responsible for its anti inflammatory claim.

INTRODUCTION

Inflammation is defined as the local response of living mammalian tissues to any agent. It is a body defense reaction in order to eliminate or limit the spread of injurious agent, pathogens and noxious substances such as chemical or physical injury followed by removal of necrosed cells and tissues[1]. Inflammation is characterized by immune cell invasion, local release of cytokines and chemokines and sometimes accompanied by functional or structural damage of the invaded tissue[2]. The classic signs of inflammation are local redness, swelling, heat, pain and loss of function[3].

Zanthoxylum alatum (ZA) also known as *Zanthoxylum armatum* belongs to family Rutaceae is a perennial shrub or a small tree height upto 6 m with dense glabrous foliage and straight prickles on stem. It is distributed in Himalayas from Kashmir to Bhutan upto 2100 m and in Khasia hills upto 1350 m.

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The bark contains berberine, asarinin, fargesin, dictamine, ɣ-fagarine, 8-hydroxydictamine, armatamine, epieudesmin, eudesmin, (-)-planinin, (+)-sesamin, (-)-sesamin, pluviatide, β-sitosterol, β-D-glucoside, α and β amyrin, β-amyrone, lupeol and vanillic acid[4]. The plant is extensively used in indigenous system of medicine as a carminative, stomachic and anthelmintic[5], in fever, dyspepsia and cholera[6]. Traditionally, the plant is used for abdominal colic, asthma, cancer, diabetes, cough, diarrhea, dysuria, fever, headache, hapatosis, microbial infections, toothache and worms, and useful in improving blood circulation to affected parts. It is also used as cardioprotective, analgesic, anti-inflammatory, pesticide, stomachic and tonic[7,8]. Pharmacological activities so far are anti-inflammatory[9], antioxidant[10], hepatoprotective [11] and larvicidal activities[12]. Since, the plant has been used traditionally for various inflammatory disorders and the aqueous extract of ZA has been already shown to have anti inflammatory activity[13] and aqueous extract has shown the presence of alkaloid and flavonoid in it when phytochemical screening was done. Therefore, with this background, the present study was designed to investigate the anti inflammatory

active fraction (alkaloids or flavonoids) from the stem bark of ZA using acute and chronic models of inflammation in rat.

MATERIALS AND METHODS

Plant material

The stem bark of ZA was collected from the local areas of Tehri (Garwal), Uttrakhand, India and got authentified from NISCAIR, New Delhi (Ref. NISCAIR/RHMD /Consult / 2009-10 / 1324 / 127).

Preparation of extract

Aqueous extract of shade dried defatted stem bark (1.5 Kg) was prepared by Triple maceration. The extract was concentrated *in vacuo*.

Phytochemical screening

The aqueous extract was screened phytochemically for the presence of carbohydrates, glycosides, phenolic compounds, alkaloids, flavonoids, saponins, steroids and terpenoids[14].

Separation of alkaloids and flavonoids fraction

Alkaloids and flavonoids fractions were separated from the aqueous extract. [15],[16] The confirmation of alkaloids fraction was done using chemical tests like Mayer's (Potassium Mercuric Iodide), Wagner's (Iodine in Potassium Iodide), Dragendorff's (solution of Potassium Bismuth Iodide), Hager's (saturated picric acid solution) reagent test[14]. Flavonoids fraction was confirmed using Shinoda's test, alkaline Reagent test and lead acetate test[14,17].

Experimental Animals

Wistar albino rats of either sex weighing 180-220 g were procured from CPCSEA approved breeder (IIIM, Jammu). The animals were kept in polypropylene cages (3 rats in each cage) and maintained at temperature 25±2˚C, 55-65 % relative humidity and 12-12 h light and dark cycle in animal house of ISF College of Pharmacy, Moga. The rats were fed at commercially available chow diet procured from Aashirwad Industries Ltd., Ropar, Punjab, and water *ad libitum*. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) (Reg. No. 816/04/C/ CPCSEA).

Drugs and Chemicals/Equipments

Carrageenan and diclofenac sodium were procured from Sigma Chemical Co., St. Louis, MO, USA and Novartis, India respectively. All other chemicals and reagents were of analytical grade. Plethysmograph was used to estimate paw volume. Test drugs (alkaloid and flavonoid fractions), reference standard (diclofenac sodium) were suspended in vehicle 1% CMC and administered orally.

Acute anti inflammatory study

Carrageenan induced rat paw edema

The acute anti-inflammatory effect was evaluated by the carrageenan-induced paw edema method. Acute edema was induced by subplantar administration of 0.1 ml of carrageenan (1% w/v) in rats. The test drugs were administrated orally 1 h before injection of carrageenan. Paw volume was measured prior to injection of carrageenan (0 h) and then at predetermined intervals from 1 hr up to 5 hr using plethysmograph. The change in paw volume was measured using the formula: % paw volume = final – initial/initial x100[18].

Preliminary screening for selection of anti inflammatory active fraction

Alkaloid and flavonoid fraction (100mg/kg) were used for preliminary study. The selected fraction was studied at three doses (50, 100, 150 mg/kg) in acute and chronic animal study (100 mg/kg, 150 mg/kg).

Grouping of animals for acute model:

(i) For Preliminary study

The rats were divided into four groups (n=6) for preliminary study and in five groups (n=6) for selected (alkaloid) fraction.

Group 1 Carrageenan control; vehicle only
Group 2 Diclofenac sodium (20 mg/kg)
Group 3 Flavonoid fraction (100 mg/kg)
Group 4 Alkaloid fraction (100 mg/kg)

(ii) For Alkaloid fraction at different doses

Group 1 Carrageenan control; vehicle only
Group 2 Diclofenac sodium (20 mg/kg)
Group 3 Alkaloid fraction (50 mg/kg)
Group 4 Alkaloid fraction (100 mg/kg)
Group 5 Alkaloid fraction (150 mg/kg)

Table 1: Effect of alkaloid fractions and diclofenac sodium on cotton-pellet-induced granuloma

Treatment	Dose (mg/kg)	Granuloma wt. (mg)	% inhibition
Control		290.4±8.7	-
Alkaloid fraction	100	155.0±6.5[a]	46.62
Alkaloid fraction	150	114.6±9.2[ab]	60.74
Diclofenac sodium	50	116.7±7.9[a]	63.25

* Results: mean ± S.D; a= p < 0.05 vs. control; b= p< 0.05 vs. diclofenac sodium (50 mg/kg)

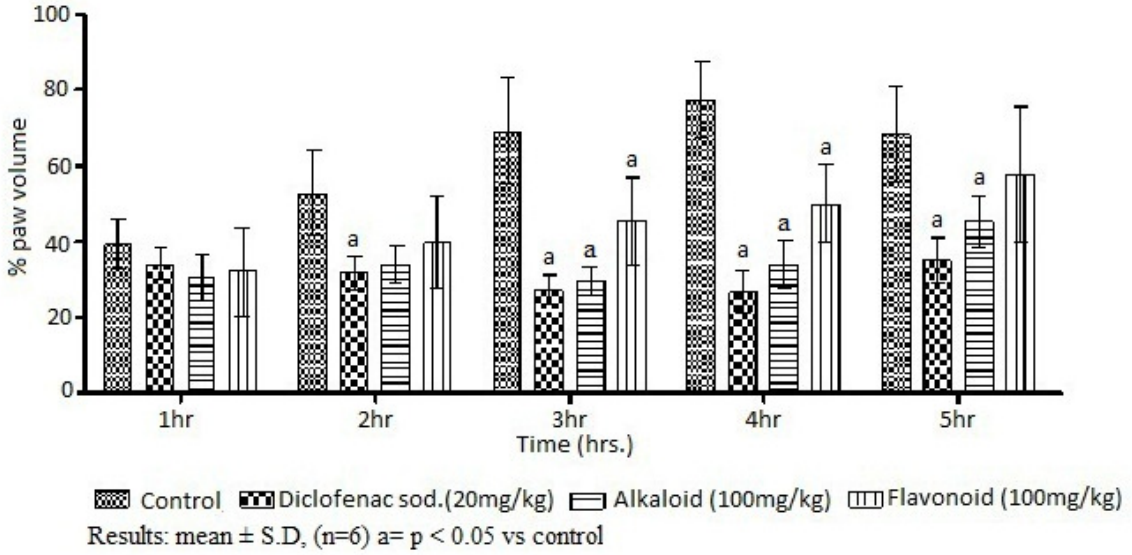

Figure 1: Effect of alkaloid and flavonoid fraction (100 mg/kg) on carrageenan induced paw edema model.

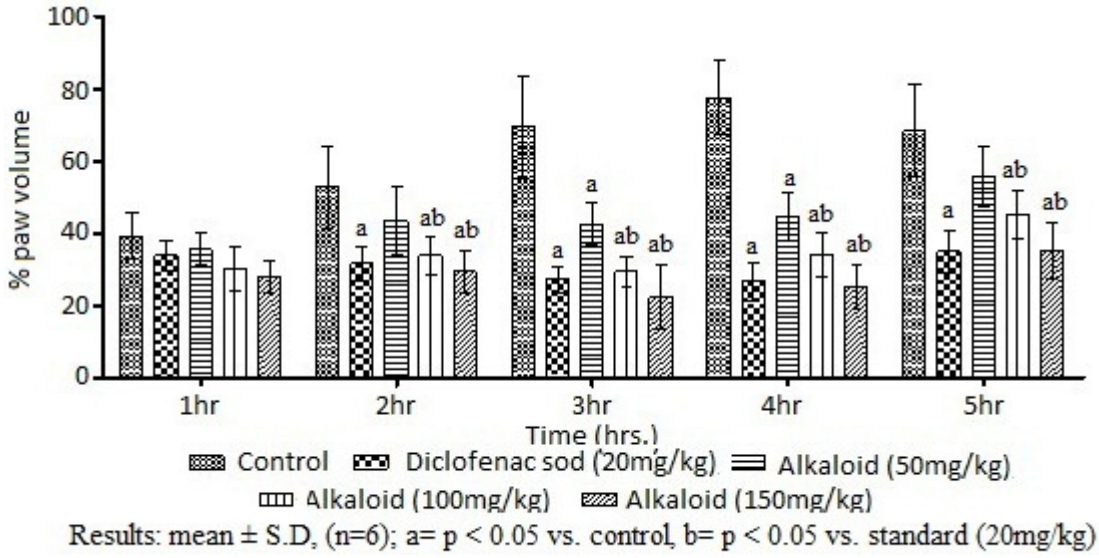

Figure 2: Effect of alkaloid fraction (50, 100, 150 mg/kg) on carrageenan induced rat paw edema model.

Chronic anti-inflammatory study

Cotton pellet induced granuloma tissue formation in rats

The rats were anaesthesized with thiopental sodium (40 mg/kg, i.p.). After shaving the fur, 50 mg sterile cotton pellets were surgically implanted into both interscapular regions. The vehicle, test drugs (100 mg/kg, 150 mg/kg) and standard drug (50 mg/kg) were given for 7 consecutive days from the day of cotton pellet implantation. On 8th day the rats were sacrificed; cotton pellets were surgically removed and dried at 60˚C to constant weight. The weight of the dried pellet over 50 mg was taken as the measure of granuloma formation. [19],[20] Change in weight of cotton pellet after drug treatment was taken as index of chronic anti-inflammatory activity[21].

Grouping of animals for chronic model

Group 1 Vehicle Control
Group 2 Diclofenac sodium (50 mg/kg)
Group 3 Alkaloid fraction (100 mg/kg)
Group 4 Alkaloid fraction (150 mg/kg)

Stastical analysis

Results were expressed as mean ± Standard deviation (SD) and were analyzed using one way and two way analyses of variance (ANOVA) tests followed by Bonferroni post tests $p < 0.05$ was considered to be statistically significant.

TLC profile of alkaloid fraction

Analytical thin layer chromatography of alkaloid fraction was performed on the precoated silica gel 60 F_{254} aluminium sheets plate (E-Merck) using solvent system (Ethyl acetate : Toluene, 70:30). The Dragendorff's reagent was used as detecting agent.

RESULTS

Percentage yield

The % age yield of alkaloid and flavonoid fractions was found to be 4.35 and 1.71% w/w respectively from aqueous extract.

Phytochemical screening

The qualitative phytochemical study of aqueous extract of ZA exhibited the presence of alkaloids, flavonoids and carbohydrates.

Acute anti-inflammatory study

Carrageenan induced rat paw edema

In the acute inflammatory model, the administration of carrageenan caused a progressive increase in rat paw volume that peaked at 4 h, compared to normal paw volume. Preliminary screening for anti inflammatory activity with alkaloid and flavonoid fraction (100 mg/kg) showed that alkaloid fraction significantly ($p < 0.05$) decreased the paw volume, as compared to carrageenan control. The % availability of paw volume in case of alkaloid fraction (100 mg/kg) was at 3rd and 4th hr. was 29.0, 34.09 %. So, alkaloid fraction studied further on two different doses (50 and 150 mg/kg) and effect produced in decreasing paw volume was dose dependent. The percentage increase in paw volume at alkaloid (50mg/kg) was 42.61 %, 44.43 % whereas at 150mg/kg was only 22.40%, 25.24% and diclofenac sodium (20mg/kg) was 28.65%, 26.73% at 3rd and 4th hr (Fig. 1).

Cotton pellet induced granuloma tissue formation

In chronic granuloma model, the alkaloid fraction (100, 150 mg/kg) and diclofenac sod. (50 mg/kg) significantly ($p<0.05$) inhibited granuloma formation as compared to control. The % age inhibition of granuloma formation in rats treated with alkaloid fraction (100 and 150mg/kg) and diclofenac sodium (50 mg/kg) was 46.62, 60.74 and 63.25 respectively. (Table 1)

TLC Profile of alkaloid fraction

TLC profile of the alkaloid fraction has shown five spots with R_f values 0.07, 0.30, 0.32, 0.51, and 0.70.

DISCUSSION

In living animal tissue, inflammation caused by infection and auto immune stimuli. Several classes of compound such as plasma protein, tissue digestive enzyme and biologically derived oxidant are all associated with inflammation [22,23] and also inflammatory process involve the release of several mediators, including prostaglandin, histamine, cytokines, proteinase, and so as well as that regulate adhesion of molecules and the process of cell migration, activation and degranulation[24]. The number of medicinal plants belonging to family rutaceae has been reported to have anti inflammatory activity like *Ruta graveolens* Linn. [25], *Pleiospermium alatum* [26]. Also many phyoconstituents like alkaloids [27,28], flavonoids [29], lignans [30], triterpnoids [31] etc. has been reported as anti inflammatory agents. In the present study the anti inflammatory activity of alkaloid and flavonoid fractions from ZA stem

bark was evaluated against carrageenan induced rat paw edema model and cotton pellet induced chronic granuloma in rats. Carrageenan-induced paw edema is a conventional model for acute inflammatory study[18]. Inflammation induced by carrageenan involves three distinct phases of the release of the mediator, including serotonin and histamine in the first phase (0 – 2 h), kinins in the second phase (3 h), and prostaglandin in the third phase [32] (>4 h). The alkaloid fraction of ZA significantly inhibited paw edema induced by carrageenan in the second and third phase, suggesting an inhibitory effect on the release of kinins and prostaglandins. The result of carrageenan model indicates that the alkaloid fraction in respective doses shows significant ($p < 0.05$) anti inflammatory potential upto 5th hr. and acts in a dose dependent manner (Fig. 2). Therefore, it can be inferred that the mechanism of action may be by inhibition of kinins or prostaglandin synthesis. The cotton-pellet-induced granuloma model is widely employed to assess the transudative, exudative and proliferative events during the processes of chronic inflammation[33], as evidenced by marked granuloma formation in control group in the present study. Monocyte and neutrophil infiltration, fibroblast proliferation and exudation are reported to take place during chronic inflammation[34]. In this study, the significant ($p < 0.05$) check on the rise in weight of cotton pellet, in alkaloid fraction pretreated animal as compared to control group, confirm the anti inflammatory activity in the chronic treatment. (Table 1) This is due to the ability of ZA alkaloidal constituents to reduce the number of fibroblasts and the synthesis of collagen and mucopolysaccharide, which are natural proliferative markers of granulation tissue formation[35].

CONCLUSION

The present results demonstrated that the alkaloidal fraction of ZA stem bark has dose dependently anti-inflammatory potential.

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