ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF BARK OF *ZANTHOXYLUM ARAMATUM* D.C.

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Summary

The purpose of the present study was to investigate the anti-inflammatory properties of ethanolic extract of the stem barks of *Zanthoxylum armatum* D.C. The different anti-inflammatory models used to induce paw oedema are Carrageenan, dextran, histamine, serotonin and Cotton pellet-induced granuloma. The ethanolic extract of *Z. armatum* at dose (250 and 500 mgkg $^{-1}$ p.o) was given to observe % inhibition of paw oedema which were comparable with indomethacin (10 mgkg^{-1} p.o) used as a reference drug.

The ethanolic extract of *Z. armatum* exhibited significant anti-inflammatory activity on the tested experimental animal models. The extract (500 mgkg^{-1} b.w.) exhibited maximum anti-inflammatory effect i.e., 30.01, 21.72, 32.34 and 29.62% ($P < 0.001$) at the end of 4 h with carrageenan, dextran, histamine and serotonin respectively. Administration of the extract of *Z. armatum* (500 mgkg^{-1} b.w.) and indomethacin (10 mgkg^{-1} b.w.) significantly reduced the formation of granuloma tissue induced by cotton pellet method at a rate of 44.81 and 51.64% respectively. The effect produced by the extract was comparable to that of indomethacin a prototype nonsteroidal anti-inflammatory agent.

A significant percentage inhibition of paw oedema by the ethanolic extract of *Z. armatum* as compared to standard drug suggests its usefulness in acute and chronic anti-inflammatory models.

Keywords: Anti-inflammatory activity; *Zanthoxylum aramatum;* oedema; indomethacin

Introduction

Inflammation is a local response of living mammalian tissues to injury. It is a body defence reaction in order to eliminate or limit the spread of injurious agent. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation [1].

Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow [2]. Several experimental models of paw oedema have been described. Carrageenan-induced paw oedema is widely used for determining the acute phase of inflammation. Histamine, serotonin and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation, [3] whereas prostaglandins are detectable in the late phase of inflammation [4]. Plants continue to be major resources for therapeutic compounds. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them [5]. Ethnobotanical and ubiquitous plants serve as a rich resource of natural drugs for research and development. Medicinal plant-based drugs owe the advantage of being simple, effective and exhibit broad spectrum activity. Medicinal plant products when compared to their synthetic counterparts minimize the adverse effects [6]. As a result, a search for other alternatives seems necessary and beneficial. Medicinal plants having a wide variety of chemicals from which novel anti-inflammatory agents could be discovered. Scientific studies are required to judge their efficacy.

For the most part, modern science has neglected botanical source of knowledge and the pharmacological potential of old remedies have not been systematically evaluated. Nevertheless many species used in traditional medicine have the potential to provide pharmacologically active natural products. *Zanthoxylum armatum* an evergreen or sub-deciduous shrub found in India is extensively used in the Indian System of Medicine, as carminative, stomachic and antihelmintic [7]. *Z. armatum* DC [syn. *Zanthoxylum alatum* Roxb] belong to the family Rutaceae. The fruits and seeds of *Z. armatum* are employed as an aromatic tonic in fever, dyspepsia, and for expelling roundworms [8]. Mehta et al. (1981) have reported that the essential oil of fruits of *Z. armatum* DC exhibited good antibacterial, antifungal and anthelmintic activities [9]. Kokate et al. (2001) reported that the petroleum extract of *Z. armatum* DC, shows significant insecticidal activity against *Culex* spp [10]. Recently, larvicidal activities of essential oil from the seeds of *Z. armatum* DC against mosquito vectors has been reported [11]. Ranawat L. et al., (2010) have reported the hepatoprotective activity of *Z. armatum* DC in CCl_4 induced hepatic damage in rats [12]. Various phytopharmaceuticals like berberine, dictamnine, xanthoplanine, armatamid, asarinin and fargesin, alpha- and beta-amyrins and lupeol are present in the plant [8, 13]. Hence, the present study was under taken to evaluate the anti-inflammatory activity of ethanolic extract of stem bark of *Z. aramatum*,DC. (EZA).

Material and methods

Plant material

Bark of *Z. aramatum* were collected from medicinal garden of Siddhartha Institute of Pharmacy, Dehradun, in the months of October 2009 and the plant was authenticated in the Department of Botany, Siddhartha group of Institution, Dehradun. The stem bark was dried under shade and then powdered with a mechanical grinder and stored in airtight container. 400 g of dried powder material of the bark was defatted with petroleum ether and the marc thus obtained was then extracted with ethanol in a soxhlet extractor for 72 hr. The extract was concentrated to dryness under reduced pressure and controlled temperature (40-50 ˚C). The ethanolic extracts yielded brown and semi-solid residue. The dried ethanolic extract of *Z. aramatum* was suspended in normal saline and used for the present study.

Animals

Studies were carried out using Wistar rats of either sex weighing 180–200 g and Swiss albino mice of either sex weighing 18–22 g. They were obtained from the animal house, Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, India. The animals were grouped in polyacrylic cages (38 cm × 23 cm × 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light circle (12/12 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Mumbai, India) and water *ad libitum*. The rats were acclimatized to laboratory condition for 10 days before commencement of experiment.

Drugs

Carrageenan (S.D. Fine Chemicals Limited, Bombay), 5-hydroxytryptamine hydrochloride (Serotonin), histamine, dextran and indomethacin (Sigma, USA) were used in the study. All other chemicals were of analytical grade and purchased from Merck.

Anti-inflammatory activity

Carrageenan-induced rat paw oedema

The rats were divided into four groups (n = 6). The different groups were treated orally with EZA (250 and 500 $mgkg^{-1}$ b.w.), indomethacin (10 $mgkg^{-1}$ b.w.), and control (0.9% NaCl, 5 $mlkg^{-1}$ b.w.). The administration of extract and drugs was 30 min prior to injection of 0.1 ml of 1% freshly prepared suspension of carrageenan in normal saline in the right hind paw subplantar of each rat [14, 15]. The paw volume was measured initially and then at 1, 2, 3 and 4 h after the carrageenan injection by using plethysmometer.

Percentage inhibition of oedema was calculated as follows:

$$\% \text{ Inhibition} = [1 - (V_T / V_C)] \times 100$$

where V_T and V_C are the paw volume in treated rats and control group of rats respectively.

Dextran-induced rat paw oedema
The animals were treated in a manner similar to that of carrageenan-induced paw oedema models; dextran (0.1 ml, 1% w/v in normal saline) was used in the place of carrageenan [16].

Histamine-induced rat paw oedema
In this model hind paw oedema in the right foot of a rat was induced by subplantar injection of 0.1 ml of 1% freshly prepared histamine in normal saline and the paw oedema was measured as mentioned earlier [17].

Serotonin-induced rat paw oedema
In another model oedema of the right hind paws of the rat was induced by subplantar injection of 0.1 ml of 1% freshly prepared serotonin in normal saline. Group division and treatment of the animals were the same as the carrageenan-induced rat paw oedema model and the paw volume was measured as mentioned in Winter et al., 1962 [14].

Cotton pellet-induced granuloma
The rats were divided into four groups (n = 6). The different groups were treated orally with EZA (250 and 500 $mgkg^{-1}$ b.w.), indomethacin (10 $mgkg^{-1}$ b.w.), and control (0.9% NaCl, 5 $mlkg^{-1}$ b.w.). The administration of extract and drugs was 30 min prior to injection of 0.1 ml of 1% freshly prepared suspension of carrageenan in normal saline in the right hind paw subplantar of each rat. The paw volume was measured initially and then at 1, 2, 3 and 4 h after the carrageenan injection by using plethysmometer [14, 18].

The percentage inhibition increase in the weight of the cotton pellet is calculated.

$$\% \text{ Inhibition} = [1 - (W_T / Wc)] \times 100$$

where W_T and W_C difference in pellet weight of the drug treated group and control group of rats respectively.

Statistical analysis
The values were expressed as mean ± S.D. The statistical significance was determined by using the ANOVA followed by Tukey's. Values of $P < 0.05$ were considered as statistically significant.

Results

In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects, whereas plants still hold their own unique place, by the way of having no side effects. Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation so as to exploit them as herbal anti-inflammatory agents.

Carrageenan-induced rat paw oedema
The anti-inflammatory activity of EZA was measured at the dose of 250 and 500 $mgkg^{-1}$ b.w. against acute paw oedema induced by carrageenan EZA exhibited 22.62 and 30.01% of inhibition at the dose of 250 and 500 $mgkg^{-1}$ b.w. respectively in carrageenan-induced

rat paw oedema (Table 1). The effect of oral administration of ethanolic extract of Z. *aramatum* on carrageenan induced paw edema in rats is summarized in table 2. The subplantar injection of carrageenan caused a time-dependent paw oedema in the rat. In carrageenan-induced paw oedema in rats, oral administration of EZA (250 and 500 $mgkg^{-1}$ p.o.) inhibited paw swelling dose-dependently at 1, 2, 3, and 4 hr after carrageenan injection ($P < 0.05$) which was comparable with the indomethacin treated group.

Table 1: Percentage inhibition of inflammation by standard Indomethacin, ethanol extract of *Zanthoxylum aramatum* (250, 500 $mgkg^{-1}$ b.w.) in different inflammation models.

	PERCENTAGE INHIBITION (%)			
	CARAGEENAN	DEXTRAN	HISTAMIN	SEROTONIN
INDOMETHNACIN	40.95	29.85	41.46	46.86
EZA 250	22.62	18.61	20.71	24.28
EZA 500	30.01	21.72	32.34	29.62

EZA 250: Ethanolic extract of *Zanthoxylum aramatum* at a dose of 250$mgkg^{-1}$
EZA 500: Ethanolic extract of *Zanthoxylum aramatum* at a dose of 500$mgkg^{-1}$

Table 2: Effect of oral administration of ethanol extract of *Zanthoxylum aramatum* on carrageenan induced paw oedema in rats.

	PAW VOLUME (in ml)			
	CONTROL	INDOMETHACIN	EZA 250	EZA 500
1 hr	0.58±0.014	0.48±0.022*	0.55±0.012†#	0.53±0.096**#
2hr	0.63±0.013	0.41±0.009*	0.53±0.011*#	0.48±0.001*#
3 hr	0.63±0.013	0.35±0.020*	0.49±0.016*#	0.40±0.009*#
4hr	0.62±0.009	0.37±0.022*	0.48±0.008*#	0.43±0.010*#

Values are presented as mean±S.D., n=6 in each group.
EZA 250: Ethanolic extract of *Zanthoxylum aramatum* at a dose of 250$mgkg^{-1}$
EZA 500: Ethanolic extract of *Zanthoxylum aramatum* at a dose of 500$mgkg^{-1}$
$P<0.001$ *; $P<0.01$ **; $P<0.05$ † significantly different from control.
$P<0.001$ $^{\#}$; $P<0.01$ $^{\#\#}$; $P<0.05$ ¶ significantly different from standard (Indomethacin).

Dextran-induced paw oedema

The differences in the paw volume after the administration of EZA and standard drug indomethacin were presented in table 3. The extract produced significant anti-inflammatory activity and the results were comparable to that of the standard drug indomethacin. EZA exhibited 18.62 and 21.72% of inhibition at the dose of 250 and 500 $mgkg^{-1}$ b.w. respectively in dextran-induced paw oedema in rats (Table 1).

Table 3: Effect of oral administration of ethanol extract of *Zanthoxylum aramatum* on dextran induced paw oedema in rats.

	PAW VOLUME (in ml)			
	CONTROL	INDOMETHACIN	EZA 250	EZA 500
1 hr	0.47±0.014	0.33±0.010*	0.47±0.018#	0.37±0.005*#
2 hr	0.46±0.012	0.29±0.015*	0.39±0.016*#	0.34±0.015*#
3 hr	0.52±0.016	0.26±0.017*	0.36±0.009*#	0.28±0.023*
4hr	0.34±0.016	0.24±0.013*	0.28±0.013*##	0.27±0.019*¶

Values are presented as mean±S.D., n=6 in each group.
EZA 250: Ethanolic extract of *Zanthoxylum aramatum* at a dose of 250mgkg^{-1}
EZA 500: Ethanolic extract of *Zanthoxylum aramatum* at a dose of 500mgkg^{-1}
P<0.001 *; P<0.01 **; P<0.05 † significantly different from control.
P<0.001 #; P<0.01 ##; P<0.05 ¶ significantly different from standard (Indomethacin).

Histamine and serotonin-induced paw oedema

The anti-inflammatory effect of EZA against acute pedal oedema induced by phlogistic agent's histamine and serotonin has been shown in table 4 and table 5. The extract (500 mgkg^{-1}) showed a maximum 32.34% inhibition in histamine and 29.62% inhibition in serotonin-induced rat paw oedema (Table 1).

Table 4: Effect of oral administration of ethanol extract of *Zanthoxylum aramatum* on histamine induced paw oedema in rats.

	PAW VOLUME (in ml)			
	CONTROL	INDOMETHACIN	EZA 250	EZA 500
1 hr	0.53±0.012	0.40±0.016*	0.48±0.012*#	0.45±0.021*#
2 hr	0.56±0.010	0.36±0.015*	0.48±0.015*#	0.42±0.012*#
3 hr	0.59±0.018	0.35±0.013*	0.44±0.021*#	0.38±0.015*¶
4 hr	0.56±0.010	0.32±0.020*	0.44±0.022*#	0.38±0.016*#

Values are presented as mean±S.D., n=6 in each group.
EZA 250: Ethanol extract of *Zanthoxylum aramatum* at a dose of 250mgkg^{-1}
EZA 500: Ethanol extract of *Zanthoxylum aramatum* at a dose of 500mgkg^{-1}
P<0.001 *; P<0.01 **; P<0.05 † significantly different from control.
P<0.001 #; P<0.01 ##; P<0.05 ¶ significantly different from standard (Indomethacin).

Table 5: Effect of oral administration of ethanol extract of *Zanthoxylum aramatum* on serotonin induced paw oedema in rats.

	PAW VOLUME (in ml)			
	CONTROL	INDOMETHACIN	EZA 250	EZA 500
1 hr	0.38±0.017	0.30±0.015*	0.36±0.019#	0.34±0.016**##
2 hr	0.42±0.018	0.28±0.016*	0.34±0.018*#	0.31±0.017*¶
3 hr	0.41±0.014	0.24±0.014*	0.33±0.016*#	0.31±0.019*#
4 hr	0.42±0.015	0.22±0.015*	0.32±0.019*#	0.29±0.013*#

Values are presented as mean±S.D., n=6 in each group.
EZA 250: Ethanol extract of *Zanthoxylum aramatum* at a dose of 250mgkg^{-1}
EZA 500: Ethanol extract of *Zanthoxylum aramatum* at a dose of 500mgkg^{-1}
P<0.001 *; P<0.01 **; P<0.05 † significantly different from control.
P<0.001 #; P<0.01 ##; P<0.05 ¶ significantly different from standard (Indomethacin).

Cotton pellet-induced granuloma

In the cotton pellet induced inflammation studies in rats, higher dose of EZA i.e., 500 mgkg^{-1} and the standard drug indomethacin showed significant decrease in wet weight of granuloma tissue formation (P<0.05) (Table 6). Further, EZA in the higher dose of 500mgkg^{-1} also significantly decreased the dry weight of granuloma tissue formation, which was comparable than indomethacin (P<0.05).

Table 6: Effect of oral administration of ethanolic extract of *Zanthoxylum aramatum* on cotton pellet induced granuloma in rats.

	DOSE (mgml^{-1})	Wt. of Cotton Pellet mg (Wet)	% INHIBITION	Wt. of Cotton Pellet mg (Dry)	% INHIBITION
CONTROL	5ml (0.9% NaCl)	183.75±10.56	-	46.23±2.52	-
INDOMETHACIN	10	77.38±9.17*	57.88	22.35±1.90*	51.65
EZA 250	250	106.66±10.13*#	41.95	32.18±2.39*#	30.38
EZA 500	500	81.1±5.35*	55.86	25.51±3.53*	44.81

Values are presented as mean±S.D., n=6 in each group.
EZA 250: Ethanolic extract of *Zanthoxylum aramatum* at a dose of 250mgkg^{-1}
EZA 500: Ethanolic extract of *Zanthoxylum aramatum* at a dose of 500mgkg^{-1}
P<0.001 *; P<0.01 **; P<0.05 † significantly different from control.
P<0.001 #; P<0.01 ##; P<0.05 ¶ significantly different from standard (Indomethacin).

Discussion

EZA showed significant anti-inflammatory effects in various animal models. Our results revealed that administration of ethanolic extract inhibited the oedema starting from the first hour and during all phases of inflammation, which is probably inhibition of different aspects and chemical mediators of inflammation.

For evaluating the anti-inflammatory activity most effective and widely used model for inflammation is carrageenan-induced paw edema. Carrageenan is a mixture of polysaccharides composed of sulfated galactose units and is derived from Irish Sea moss, *Chondrous crispus*. Its use as an endemogen was introduced by Winter et.al. [14]. Carrageenin-induced oedema falls in the category of acute inflammation, which involves the synthesis or release of inflammatory mediators at the injured site which further cause pain and fever [19, 20]. On the other hand, the proliferative phase or chronic inflammation is measured by methods for testing granuloma formation such as cotton pellet granuloma [21, 22]. EZA was effective in both cotton pellet granuloma as well as carrageenin-induced paw oedema.

In the first phase (during the first 2 h after carrageenan injection), chemical mediators such as histamine and serotonin play role, while in second phase (3-4 h after carrageenan injection) kinin and prostaglandins. It can be assumed that EZA is effective in all the phases of inflammation i.e., acute, subacute and proliferative phases.

EZA showed 18.61 and 21.72 % inhibition against dextran induced oedema. In dextran induced aseptic arthritis test in rats (table 4), EZA showed significant inhibition of oedema within 60 min.

In Histamine induced paw edema, histamine causes vasodialation and increase in vascular permeability followed by oedema which is one of the phases of inflammation. Histamine induced paw edema is said to occur in earlier stage in mounting of vascular of the vascular reaction in the chemically induced inflammation. In this, swelling occurs primarily due to action of histamine. Generally histamine is released following the mast cell degranulation by number of inflammatory mediators including substances P interleukin-1. This is likely to evoke the release of neuropeptide as well as release of prostaglandins and monohydroxy eicosatetranoic-acid from endothelial cell leading to hyperalgesia and other pro-inflammatory effects. Both doses i.e., 250 and 500 $mgkg^{-1}$ of EZA and 10 $mgkg^{-1}$ doses of indomethacin showed 20.71, 32.34 and 41.46 % inhibition, respectively against histamine produced oedema (Table 1).

It was reported that the capillary permeability increased in serotonin induced inflammation in rats [23]. EZA inhibited inflammation is almost such as indomethacin. EZA showed 24.28 and 29.62 % inhibition of the inflammation produced by serotonin with doses of 250 and 500 $mgkg^{-1}$ respectively.

The cotton-pellet model is based on the foreign body granuloma which is provoked in rats by subcutaneous implantation of pellets of compressed cotton. The cotton-pellet granuloma is widely used to evaluate the transudative and proliferative components of the chronic inflammation. The moist weight of the pellets correlates with transuda, the dry weight of the pellet correlates with the amount of granulumatous tissues. Chronic inflammation occurs by means of the development of proliferate cells. These cells can be either spread or in granuloma form. Non-steroidal anti-inflammatory drugs decrease the size of granuloma which results from cellular reaction by inhibiting granulocyte

infiltration, preventing generation of collagen fibers and suppressing mucopolysaccharides.
The inhibition of subacute cotton-pellet induced granuloma by EZA was similar to the effect of actual anti-inflammatory agents. In the developing arthritis test the extract showed significant inhibitory activity of the paw oedema in rats.

Conclusion

Thus, in the present investigation, ethanolic extract of *Zanthoxylum armatum* showed that it possesses potent anti-inflammatory activities. Further studies involving the purification of the chemical constituents of the plant and the investigations in the biochemical pathways may result in the development of a potent anti-inflammatory agent with low toxicity and better therapeutic index.

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