



ANTI-INFLAMMATORY ACTIVITY OF ZYZIPHUS XYLOPYRUS BY USING CARRAGEENAN INDUCED PAW EDEMA MODEL (ACUTE INFLAMMATION)

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ABSTRACT

Context: Ziziphus xylopyrus, locally known as 'sider' or 'ber' has been used in various condition but scientific evidence are not available.

Aims: to study anti-inflammatory activity of Ziziphus xylopyrus by using Carrageenan induced Paw Edema model (acute inflammation) in albino rats.

Settings and Design: This was a longitudinal study done on 30 no. Wistar rats done in post graduate Department of Pharmacology, VIMSAR, Burla

Methods and Material: the study was done Carrageenan induced paw edema

Statistical analysis used: One way ANOVA followed by Dunnett's test.

Results: On comparison with basal values, EEZX 200 and 400 mg/kg showed significant reduction in paw edema.

Conclusions: These results suggest that EEZX at 200 & 400 mg/kg doses possesses significant anti-inflammatory activities, however more studies are recommended.

KEYWORDS : Carrageenan, inflammation

INTRODUCTION:

Inflammation, pain and fever are very common clinical phenomena and accompany diverse disease conditions. The drugs currently used to treat them are mainly NSAIDs, glucocorticoids and opioids. However, these drugs produce many adverse reactions especially when used chronically.

India has a centuries old tradition of using medicinal plants and herbal medicines for the alleviation of various ailments. There are about 45000 plant species and among them, several thousands have been claimed to possess medicinal properties.

Ziziphus xylopyrus, locally known as 'sider' or 'ber' is one of such versatile tree species used for food, fodder, medicines and desertification control in arid lands. Ziziphus xylopyrus (Family : Rhamnaceae) commonly known as 'Jujab' in English, 'Kantabaula' in Odia and 'Ghunnta' in Hindi is either a large struggling shrub or a small tree armed with spines and found in Odisha, North Western India, UP, Bihar and other parts of India. (1)

As per the ethnomedicinal information, various parts of this plant have been reported to be used traditionally for the treatment of diverse disease conditions like stem bark for stomach-ache and cholera, root bark for bleeding piles, epistaxis, bleeding mouth and skin rash, fruits for diabetes, stomach-ache, urinary spasm, sterility in women and diarrhoea, leaves and flowers for pimples, boils, snake bite and leucoderma, leaves and stem for hysteria, antiseptic and headache, root for asthma, pyorrhoea and seeds for chest pain and diarrhoea. (2,3,4) This plant is also reported to possess antidepressant, antimicrobial, anthelmintic and wound healing activities. (5,6,7) However, there are few scientific studies as regards its anti-inflammatory, analgesic and antipyretic properties.

With this background, the present study was planned to screen the ethanolic extract of stem bark for anti-inflammatory activities as part of exploring the traditional system of medicine for finding useful properties in a scientific manner.

SUBJECTS AND METHODS:

This was a longitudinal study done on 30 no. Wistar rats done in post graduate Department of Pharmacology, VIMSAR,

Burla after obtaining due permission from the Institutional Animal Ethics Committee, VIMSAR, Burla. Evaluation of Anti-inflammatory Activity was done in following way.

a) Carrageenan induced paw edema: model of acute inflammation : in Wistar rats : using indomethacin as standard drug.

Preparation of extracts

The stem bark of Ziziphus xylopyrus plant was collected, cleaned, shade dried and powdered by mechanical grinder. 100 gms of the pulverized stem bark was extracted with petroleum ether and ethanol successively in a soxhlet apparatus. Petroleum ether was used as the initial step of extraction for defatting the plant materials. Then, from this, ethanol extract was separately filtered and concentrated at reduced temperature on a rotatory evaporator. The yield of petroleum ether and ethanol extract was found to be around 3.21 and 15.13 (W/W) respectively. The ethanol extract was found to be soluble in 5% Tween 80.

Experimental animals:-

The healthy adult albino rats weighing between 150-250 gms and Swiss albino mice weighing between 20-30 gms were selected for the study. They were kept in polypropylene cages in temperature regulated rooms with air cooling and 12: 12 hours light and dark cycle, provided with standard laboratory diet and had free access to water. The animals were allowed to get acclimatized to environmental conditions for at least five days before the start of experiment. Food was withdrawn 12 hours before and also during the experimental hours.

Evaluation of Anti-inflammatory activity of Ziziphus Xylopyrus Carrageenan induced rat paw Edema (8,9,10)

Drugs/chemicals were used as follows-

Drug administration plan in Carrageenan induced rat paw edema method

Group	Treatment	Dose (mg/kg)	Route of administration
1	0.5% Tween 80	10 ml/kg	Per oral
2	Indomethacin	10	Per oral
3	EEZX	100	Per oral
4	EEZX	200	Per oral
5	EEZX	400	Per oral

Procedure :-

The acute inflammation was induced in rats employing the method of Winter et al., (1962). The animals were fasted overnight and divided into control, standard and three test groups each consisting of six animals. EEZX was administered to the animals of the three test groups in the doses of 100, 200 and 400 mg/kg body weight respectively by oral route. These doses were determined based on the results of preliminary or pilot studies. Animals in the standard group received indomethacin at the dose of 10 mg/kg by oral route. Control group animals received 0.5% Tween 80 in distilled water at the dose of 10 ml/kg body weight. Thirty minutes after administration of respective agents, the animals were injected with 0.1 ml of 1% carrageenan in the sub plantar region of left hind paw. Paw volume was measured by using digital plethysmometer immediately before administration of carrageenan and at 30 min, 1hr, 2 hr , 3 hr and 4 hr intervals. The efficacy of the extract was tested on their ability to inhibit paw edema as compared to control group.

Volume of edema = Final paw edema – Initial paw edema
 The percentage inhibition of paw edema was calculated by the formula % inhibition of Paw edema = $(V_c - V_t) / V_c \times 100$
 Where V_c = Paw edema of control group and

Table No. 1 Effects of Ziziphus xylopyrus on Carrageenan induced Paw edema in rats

Gr	Drug	Dose and route	Paw Volume-(in cc) MEAN ± SEM				
			0 hr	1 hr	2 hr	3 hr	4 hr
I	0.5% Tween 80	10 ml/kg Per oral	0.68 ± 0.02	1.15 ± 0.03	1.31 ± 0.01	1.34 ± 0.00	1.35 ± 0.00
II	Indomethacin	10 mg/kg Per oral	0.67 ± 0.03	0.85 ± 0.02*	0.82 ± 0.03*	0.81 ± 0.02*	0.80 ± 0.02*
III	EEZX	100 mg/kg Per oral	0.67 ± 0.02	1.15 ± 0.03	1.31 ± 0.01	1.34 ± 0.00	1.35 ± 0.01
IV	EEZX	200 mg/kg Per oral	0.95 ± 0.02	1.23 ± 0.02	1.20 ± 0.02	1.17 ± 0.02	1.15 ± 0.02*
V	EEZX	400 mg/kg Per oral	0.83 ± 0.02	1.24 ± 0.02	1.04 ± 0.03*	1.02 ± 0.03*	0.99 ± 0.02*

Data were analysed by one way ANOVA followed by Dunnett's Test. Each value is expressed as Mean ± SEM. n=6. * is p value <0.001 compared to control

Table No. 2 Percentage Inhibition of paw edema volume

Group	Drug	Dose and route	Mean Paw Volume (in cc) ± SEM		Size of edema at 4 hr	% Inhibition
			0 hr	4hr		
I	0.5% Tween 80	10 ml/kg Per oral	0.68 ± 0.02	1.35 ± 0.00	0.67	
II	Indomethacin	10 mg/kg Per oral	0.67 ± 0.03	0.80 ± 0.02	0.13*#@	80.59
III	EEZX	100 mg/kg Per oral	0.67 ± 0.02	1.35 ± 0.01	0.68	
IV	EEZX	200 mg/kg Per oral	0.95 ± 0.02	1.15 ± 0.02	0.20*	70.14
V	EEZX	200 mg/kg Per oral	0.83 ± 0.02	0.99 ± 0.02	0.16*#	76.11

Data were analysed by one way ANOVA followed by Dunnett's Test. Each value is expressed as Mean ± SEM. n=6. * is p value <0.001 compared to control, # is p value <0.05 compared to EEZX200, @ is p value <0.05 compared to EEZX 400.

On comparison with basal values, EEZX 200 and 400 mg/kg showed significant reduction in paw edema from 1 hour to 4 hour of observation. Indomethacin 10 mg/kg showed similar effect.

1. The effect shown by the test drugs i.e. EEZX 400 mg/kg was comparable with that of indomethacin 10 mg/kg..
2. The effect shown by EEZX 400 mg/kg was significantly higher than EEZX 200 mg/kg implying in a dose dependent response.
3. EEZX 100 mg/kg did not bring about any significant change in paw edema volume.

DISCUSSION:

Anti-inflammatory activity was tested in Carrageenan induced rat hind paw edema (acute inflammation) and cotton pellet induced granuloma (sub-acute inflammation). Carrageenan, a sulphated mucopolysaccharide derived from Irish sea moss, has been widely used for the production of experimental model of inflammation by various workers.

Carrageenan induced paw edema an in vivo model of

V_t = Paw edema of treated group

Statistical Analysis :

The mean and standard error of mean of increase/decrease in weight of granuloma was calculated and analysed statistically by using One way ANOVA followed by Dunnett's test.

RESULTS:

are discussed under two subheading.

Carrageenan induced paw edema

The anti-inflammatory effect of Ziziphus xylopyrus was evaluated in a model of acute inflammation i.e. Carrageenan induced paw edema in rats. The increase or decrease in paw volume was calculated by subtracting the initial paw volume from final paw volume. The increase or decrease in volume of paw edema were recorded for EEZX in all three doses and were compared with that of control (0.5% Tween 80) and standard (Indomethacin).

The results of anti-inflammatory activity by Carrageenan induced paw edema test are given in the Table 1

inflammation has been frequently used to assess the anti-edematous effect of the contribution of mediators involved in vascular changes associated with acute inflammation. Edema formation in the carrageenan induced paw edema model is a biphasic response. In the early hyperaemia, 0-2 hrs after carrageenan injection, there is release of histamine, serotonin and bradykinin on vascular permeability. The inflammatory edema reaches its maximum level at 1 hr and after that it starts declining. The late phase of the inflammatory response has been shown to be due to potentiating effect of bradykinin on mediator release and prostaglandins, vasodilator and is also involved in carrageenan induced paw edema, which may be related to its ability to increase vascular permeability and edema through changes in blood flow. (8,9,10)

In Carrageenan induced paw edema test (acute inflammation), EEZX 200 and 400mg/kg brought about significant decrease in paw volume as compared to control and that with peak effect at 4 hours. The percentage inhibition of paw edema seen with EEZX 200 and 400mg/kg were 70.14 and 76.11 respectively which is comparable with that of indomethacin which is 80.59 during the peak effect of 4 hours. Dose dependent anti-inflammatory activity of EEZX was observed in this study. However EEZX at 100 mg/kg dose did not show significant anti-inflammatory activity. These effects corroborate with the finding of Jena BK et al, 2012. (1)

So our study shows that EEZX possess anti-inflammatory activity in both acute and sub-acute models of inflammation.

These results suggest that EEZX at 200 & 400 mg/kg doses possesses significant anti-inflammatory activities. However, these positive results have to be seen in the context of limitations of the study, which are: the study was conducted in only in single animal model each of acute and sub-acute models of inflammation, single animal model of pain and single animal model of pyrexia. The study has to be carried out in other animal as well as in in-vitro models of inflammation, pain and fever before these activities can be documented beyond any doubt.

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