Research Article

Evaluation of analgesic and anti-inflammatory activities of *Ziziphus xylopyrus* stem bark extracts in different experimental animal models

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ABSTRACT

The aim of the present study was to evaluate the analgesic and anti-inflammatory activities of chloroform and methanol extracts of stem barks of *Ziziphus xylopyrus* in different experimental animal models. Hot plate and tail immersion methods in mice were used for study the central analgesic activity; acetic acid writhing method in mice was used for study the peripheral analgesic activity; and carrageenan induced paw edema method in rat was used for study the anti-inflammatory activities. In the hot plate and tail immersion models, both chloroform and methanol extracts of stem barks of *Z. xylopyrus* at doses of 50, 100 and 200 mg/kg showed significantly increase the pain threshold. In the acetic acid writhing test the extracts of *Z. xylopyrus* showed significant inhibition of writhing. And in carrageenan induced paw edema method significant inhibition of paw edema by the stem bark extracts of *Z. xylopyrus* was found, which is comparable with the standard drug. *Z. xylopyrus* bark extracts showed dose-dependent action in all the experimental models. The results of the study indicates the plant *Z. xylopyrus* is having significant analgesic and anti-inflammatory activities and hence scientifically justifies the use in the folklore remedies.

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INTRODUCTION

About seventeen species of Ziziphus are found in India in a wild state and some common species of Ziziphus genus are *Z. jujuba* Mill, Z. *mauritiana* Lam, Z. *nummularia* (Burm.f.), Z. *oenoplia* Mill, Z. *xylopyra* Willd, Z. *glabrata* Heyne and *Z. oxyphylla* Edgew.[1,2] *Ziziphus xylopyrus*, belongs to the family Rhamnaceae, is a medium to large evergreen tree and is commonly found in different parts of India. In Hindi it is known as Kat-ber, gote, ghont; in Marathi it is known as Goti, kantegoti; in Telgu Gotte; in Tamil Kottei; and in Odiya Got, gotoboro, kantabohul.[2]

The various parts of *Z. xylopyrus* have different medicinal properties. The *Z. xylopyrus* possess antidepressant, antimicrobial and anthelmintic activities.[3-5] The fruit powder of *Z. xylopyrus* useful for stomachache and indigestion.[6] The different parts of the plant contains various chemical constituents which are responsible for its medicinal properties.The leavesof *Z. xylopyrus* contains quercetin and quercitrin; the fruits contains catechol-type of tannins and oleanolic acid; the bark contains tannin, d-7, 3', 4'- tri hydroxy flavan- 3, 4-diol and oleanolic acid[2] and the stem wood contains triterpenoids[7], Flavonoids[3] and Alkaloids (xylopyrine- A and B).[8,9] On this basis, the present study was carried out to evaluate the analgesic and anti-inflammatory activities of chloroform and methanol extracts of stem barks of *Z. xylopyrus* by using different animal models.

MATERIALS AND METHODS

Plant material

The stem bark of *Ziziphus xylopyrus* was used for the study and collected from the forest of Similipal Biosphere at Mayurbhanj district of Odisha. The plant material was authenticated at the Central National Herbarium, Botanical Survey of India, Botanical Garden, Howrah, India.

Preparation of extracts

The cleaned, dried and pulverized stem bark of *Ziziphus xylopyrus* (100 g) was extracted by successive extraction with petroleum ether, chloroform and methanol by using soxhlet apparatus. Petroleum ether (60–80°C) was used for defatting the bark. The different extracts were filtered and dried separately by using rotary vacuum evaporator.[5]

Phytochemical analysis

Qualitative phytochemical analysis of different stem bark extracts of *Ziziphus xylopyrus* were carried out for confirming the presence of various chemical constitutes by using conventional protocol.[10, 11]

Animals

Swiss albino mice (20-25g) and wistar albino rats (180-200 g) of either sex were

used for the study. All animals were obtained from the departmental animal house of the institute. They were kept in polypropylene cages and under controlled room temperature (22 ± 2°C) and humidity (55 ±5°C) with 12 : 12 h light and dark cycle. The animals were fed pellet diet and water *ad libitum*. The study was conducted after prior approval from Institutional Animal Ethics Committee of the institute bearing registration number 1018/C/06/CPCSEA.

Study the central analgesic activity:

Hot-plate method:

The Eddy's hot plate method deals on the principle of the thermal radiation. Eddy's hot-plate is a device used to give heat stimulus to the paws of animal, which consists of an heated surface made up of iron, aluminum, copper or glass. The temperature of the plate was maintained at 50°C ± 1°C by the thermostat. The animals were placed on the heated surface and the different reactions i.e. jumping and licking of the paws were observed. The basal reaction time of mouse is less than 5 sec. A cut off period of 15 sec isfollowed to prevent damage tothe skin of the animal.[12]

The selected mice were divided into 8 different groups containing six animals in each group. Group 1 animals were treated with 10 ml/kg of 1% DMSO (p.o.) and served as control. Group 2 animals were treated with 5mg/kg of morphine sulphate (s.c.) and served as standard. Group 3, 4 and 5 animals were treated with chloroform extract of *Z. xylopyrus* and group 6, 7 and 8 were treated with methanol extracts of *Z. xylopyrus* at a dose of 50mg/kg, 100 mg/kg and 200mg/kg respectively. All the extracts were administered through per oral route. The animals were placed one by one on the heated surface (50°C ± 1.0°C) of the hot plate walled with cylindrical glass and the reaction time was noted. The observations were made before; and 30 min, 60 min, 120 min, and 180 min after administration of above drugs.[13, 14]

Tail immersion method:

Morphine-like central analgesics are prolonged tail-withdrawal reflex in mice or rats when immersing the end of the tail in hot water (55°C). The tail immersion method deals on the principle of the thermal radiation and is based on the above observation. This method is more acceptable because, it is simple, reliable and no sophisticated instrument is required.The basal reaction time of mouse is less than 5 sec. A cut off period of 15 sec is followed to prevent damage to the skin of the animal.[15]

The selected mice were divided into 8 different groups containing six animals in each group. Group 1 animals

were treated with 10 ml/kg of 1% DMSO (p.o.) and served as control. Group 2 animals were treated with 5mg/kg of morphine sulphate (s.c.) and served as standard. Group 3, 4 and 5 animals were treated with chloroform extract of *Z. xylopyrus* and Group 6, 7 and 8 were treated with methanol extract of *Z. xylopyrus* at a dose of 50 mg/kg, 100 mg/kg and 200 mg/kg respectively. All the extracts were administered through par oral route. The tail of the mice were immersed into the warm water at 55°C one by one and the reaction time (RT) was noted. The observations were made before and 30 min, 60 min, 120 min, and 180 min after administration of the above drugs.[13,14]

Study of peripheral analgesic activity:

Writhing Test:

A chemical irritants (such as acetic acid, phenyl quinone, aconite, prostaglandin E_1 or bradykinins) injected intra peritoneally for producing severe pain and irritation to the animal. The writhing or a characteristic stretching reaction in animal (i.e. abdominal constriction, twisting of trunks and extension of hind limbs) is evaluated. The test is used to detect both peripheral and central acting analgesic activity.[15]

The selected mice were divided into 8 different groups containing six animals in each group. Group 1 animals were treated with 10 ml/kg of 1% DMSO (p.o.) and served as control. Group 2 animals were treated with 5mg/kg of diclofenac sodium (p.o.) and served as standard. Group 3, 4 and 5 animals were treated with chloroform extract of *Z. xylopyrus* and Group 6, 7 and 8 were treated with methanol extracts of *Z. xylopyrus* at a dose of 50 mg/kg, 100 mg/kg and 200 mg/kg respectively. All the extracts were administered through par oral route. Thirty min after administration of the above drugs, the mice were injected10 ml/kg of 0.6% acetic acid intra peritoneally. Five min after administration of acetic acid the number of writhing produced in those animals were counted for next 10 min. The percentage of inhibition of writhing in the different groups were calculated as per the following formula.[13-15]

$$\% \text{ inhibition} = \frac{C - T}{C} \times 100$$

Where,

C= Mean writhes in the control group,

T= Mean writhes in the treated group.

Study of Anti-inflammatory activity:

Carrageenan induced paw edema

Carrageenan induced paw edema is one of the commonly used method for screening the anti-inflammatory activity of different compounds. Injection of 0.1ml of 1% w/v carrageenan solution subcutaneously into the plantar surface of the rat hind paw

induces edema. Anti-inflammatory drug like indomethacin treated animals may reduce the inflammation and edema. The edema is measured by plethysmometer, which is used to measure the changes in volume.[15-17]

The rats were divided into 8 different groups containing six animals in each group. Group 1 animals were treated with 10 ml/kg of 1% DMSO (p.o.) and served as control. Group 2 animals were treated with 4 mg/kg of indomethacin (p.o.) and served as standard. Group 3, 4 and 5 animals were treated with chloroform extract of *Z. xylopyrus* and Group 6, 7 and 8 were treated with methanol extracts of *Z. xylopyrus* at a dose of 50mg/kg, 100 mg/kg and 200mg/kg respectively. All the extracts were administered through par oral route.

All the animals were injected 0.1 ml of 1% carrageenan in the sub planter region of left hind paw after 30 min of administrating the above drugs. Paw volume was measured by using plethysmometer before and 30 min, 1, 2, 3 and 4 hr after injecting the carrageenan. Calculated the mean paw volume and mean paw edema in each group of animals. Also calculated the percentage inhibition of paw edema in different groups as per the following formula.[13-15]

Paw edema = final paw volume – Initial paw volume.

$$\% \text{ inhibition} = \frac{C - T}{C} \times 100$$

Where,

C= Mean paw edema of control group,

T= Mean paw edema of test group.

Statistical analysis

The statistical analysis of different treatment group means were assessed by one-way ANOVA (nonparametric), followed by Bonferroni's multiple comparison tests. All values were expressed in Mean ± SEM, n=6.[18]

RESULTS AND DISCUSSION

Phytochemical analysis

The percentage yield (w/w) of petroleum ether, chloroform and methanol extracts of *Z. xylopyrus* bark were 3.21, 5.1 and 15.13% (W/W) respectively. The phytochemical analysis of petroleum ether extract of *Z. xylopyrus* stem bark indicated the presence of proteins, amino acids, steroids and fixed oils;the chloroform extract have alkaloids, proteins, amino acids, flavonoids and steroids; whereas the methanol extract contains the alkaloids, proteins, amino acids, carbohydrates, gums and mucilage, tannins, flavonoids and steroids.

Hot-plate method:

Central analgesic activity of chloroform and methanol extracts of *Z. xylopyrus* bark was carried out by hot plate method. The table 1 and figure 1 indicates that the control group animals

did not have any significant change in basal reaction time; whereas the morphine treated standard group animals showed significant analgesic activity. The different dose of methanolic extract of *Z. xylopyrus* showed highly significant effect at different time as compared with control group. The chloroform extract 200mg/kg also showed a significant activity. As compared to standard drug, the 200mg/kg of methanolic extract of *Z. xylopyrus* bark was found to have no significant differences in basal reaction time at different time periods.

Table 1: Evaluation of central analgesic activity of chloroform and methanol extracts of *Z. xylopyrus* bark by hot plate method

Grp.	Treatment	Dose (mg/kg body wt)	Basal reaction time	Reaction time(in sec) after administration of drugs at different time (minutes)			
				30	60	120	180
1	Control (1% DMSO)	10 ml/kg	4.5±0.115	5.0±0.208	4.6±0.145	4.8±0.152	5.1±0.152
2	Morphine sulphate	5 mg/kg	4.7±0.115	9.7±0.057***	12.8±0.115***	13.9±0.100***	13.4±0.115***
3	ZXCH	50 mg/kg	4.6±0.152	5.1±0.100	5.5±0.152**	5.4±0.173	5.3±0.115
4		100mg/kg	4.8±0.152	5.4±0.173	5.8±0.115***	6.0±0.360*	6.1±0.057***
5		200mg/kg	5.1±0.208	6.1±0.057**	6.3±0.057***	6.5±0.115***	7.7±0.120***
6	ZXME	50 mg/kg	4.±0.100	5.8±0.115*	6.9±0.100***	7.6±0.057***	7.9±0.100***
7		100mg/kg	5.0±0.100	7.9±0.152***	9.4±0.115***	10.8±0.305***	11.0±0.152***
8		200mg/kg	4.9±0.100	#9.0±0.208***	#12.2±0.173***	#13.2±0.185***	#12.9±0.057***

ZXCH = *Ziziphus xylopyrus* chloroform extract, ZXME = *Ziziphus xylopyrus* methanolic extract. Values are mean ± SEM, n=6; ***P< 0.0001, **P< 0.001 and *P< 0.05 when compared with control group; and '#' no significant difference between standard and test group at P< 0.05.

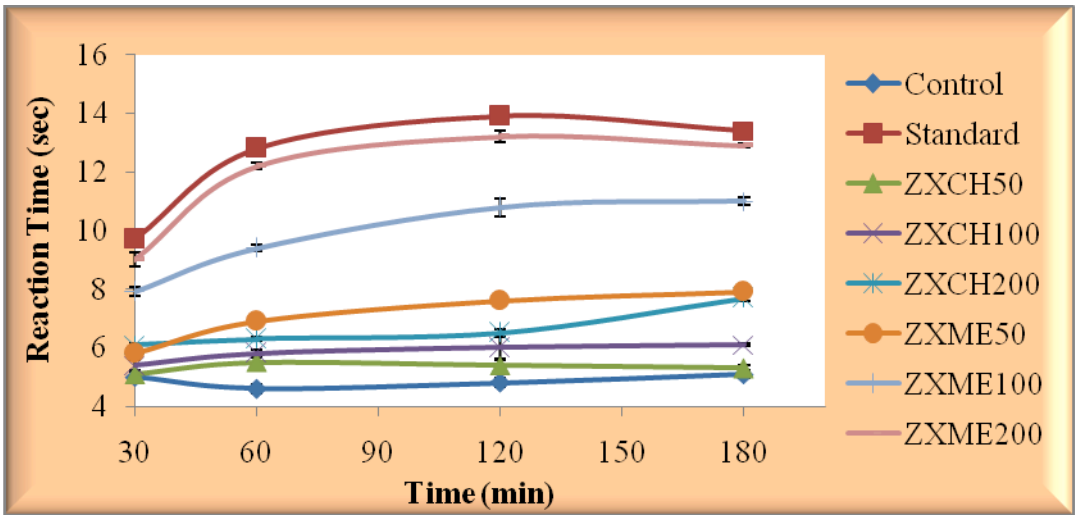

Figure 1: Graph showing central analgesic activity of *Z. xylopyrus* bark extracts by hot plate method

Tail immersion Test:

Central analgesic activity of chloroform and methanol extracts of *Z. xylopyrus* bark was carried out by tail immersion method. The table 2 and graph 2 indicates that the control group animals did not have any significant change in basal reaction time whereas, the morphine sulphate showed highly significant analgesic effect at different time. All doses of methanolic extract of *Z. xylopyrus* bark showed highly significant activity at different time interval as compared to control group. The chloroform extract at 100mg/kg and 200mg/kg showed highly significant activity. There were no significant differences observed between the standard and 200mg/kg methanolic extract.

Table 2: Evaluation of central analgesic activity of chloroform and methanol extracts of *Z. xylopyrus* bark by tail immersion method

Group	Treatment	Dose (mg/kg body wt)	Basal reaction time	Reaction time(in sec) after administration of drugs at different time (minutes)			
				30	60	120	180
1	Control (1%DMSO)	10 ml/kg	5.3±0.115	5.1±0.057	5.3±0.115	4.9±0.208	5.4±0.173
2	Morphine sulphate	5 mg/kg	4.9±0.100	9.4±0.057***	12.5±0.152***	14.4±0.351***	14.2±0.152***
3	ZXCH	50 mg/kg	4.5±0.152	4.9±0.115	5.6±0.057	6.2±0.100**	6.4±0.057**
4		100mg/kg	4.7±0.115	5.5±0.152	6.4±0.057***	7.1±0.152***	7.0±0.115***
5		200mg/kg	4.3±0.152	5.4±0.173	6.8±0.057***	7.7±0.115***	8.2±0.115***
6	ZXME	50 mg/kg	5.1±0.100	5.8±0.115*	6.7±0.152***	7.8±0.115***	7.9±0.100***
7		100mg/kg	4.8±0.100	6.4±0.057***	8.2±0.120***	9.9±0.152***	10.6±0.057***
8		200mg/kg	4.6±0.100	8.4±0.100***	#11.9±0.145***	#13.5±0.133***	#13.6±0.173***

ZXCH = *Ziziphus xylopyrus* chloroform extract, ZXME = *Ziziphus xylopyrus* methanolic extract. Values are mean ± SEM, n=6; ***P< 0.0001, **P< 0.001 and *P< 0.05 when compared with control group; and '#' no significant difference between standard and test group at P< 0.05.

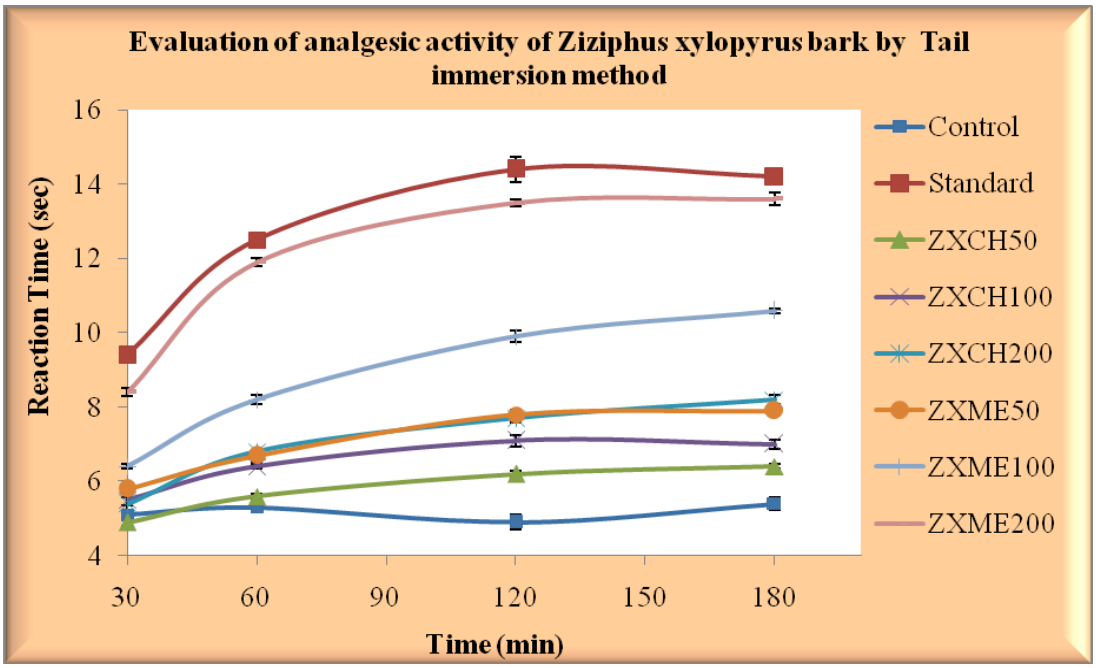

Figure 2: Graph showingcentralanalgesic activity of *Z. xylopyrus* extracts by tail immersion method

Writhing Test

Peripheral analgesic activity of chloroform and methanol extracts of *Z. xylopyrus* bark were carried out by acetic acid induced writhing method. Table 3, and figure 3 and 4 showed the control group did not have any significant decrease in average numbers of writhes whereas, the diclofenac sodium treated standard group showed highly significant analgesic activity in acetic acid induced writhing method in swiss albino mice. Diclofenac sodium at the dose of 5mg/kg body weight showed significant inhibition of writhing in mice. *Z. xylopyrus* methanolic extract at all dose showed significant activity as compared to control group. The chloroform extract at 200mg/kg showed significant activity. There was no significant differences found between standard and methanolic extract at 200mg/kg dose level.

Table 3: Evaluation of peripheral analgesic activity of chloroform and methanol extracts of *Z. xylopyrus* bark by acetic acid induced writhing method

Groups	Treatment	Dose	Avg. no. of Writhing	% Inhibition
1	Control (1% DMSO)	10ml/Kg	76.66±0.594	-
2	Diclofenac sodium	5mg/kg	28.57±1.225***	62.73%
3	ZXCH	50 mg/kg	75.32±0.614	1.74%
4		100mg/kg	69.45±1.644*	9.40%

5		200mg/kg	61.25±0.797***	20.10%
6		50 mg/kg	68.29±1.087**	10.91%
7	ZXME	100mg/kg	49.86±1.942***	34.95%
8		200mg/kg	#32.49±0.701***	57.61%

ZXCH = *Ziziphus xylopyrus* chloroform extract, ZXME = *Ziziphus xylopyrus* methanolic extract. Values are mean ± SEM, n=6; ***P< 0.0001, **P< 0.001 and *P< 0.05 when compared with control group; and '#' no significant difference between standard and test group at P< 0.05.

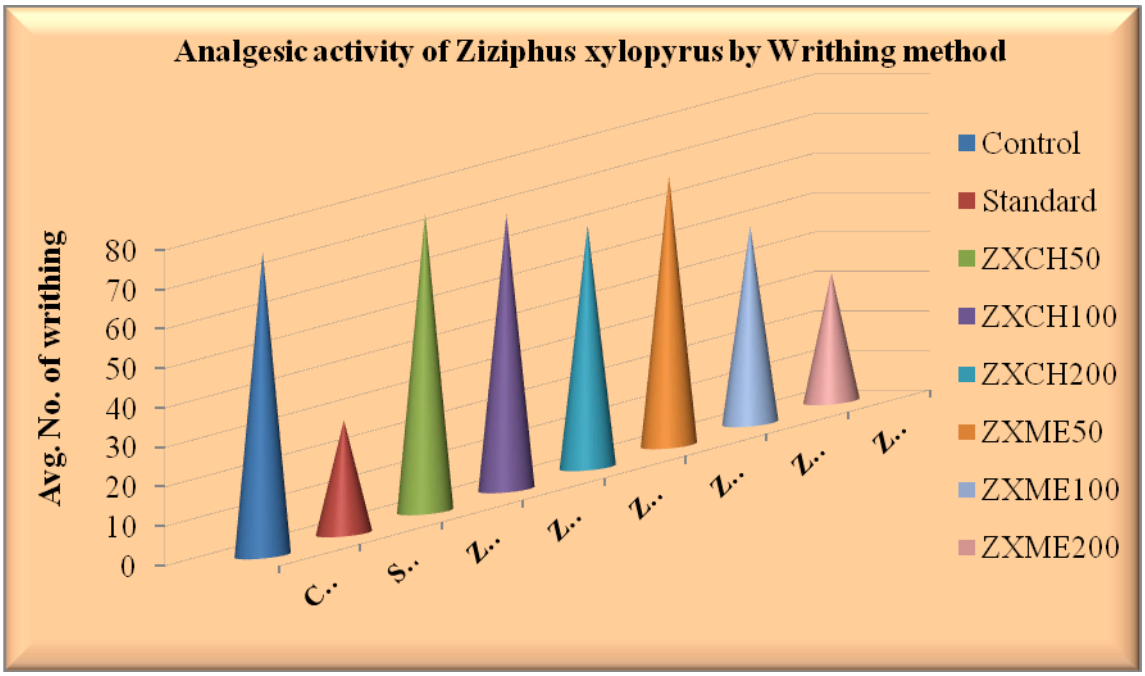

Figure 3: Histogram showing average no. of writhing produced by *Z. xylopyrus* extracts by acetic acid induced writhing method.

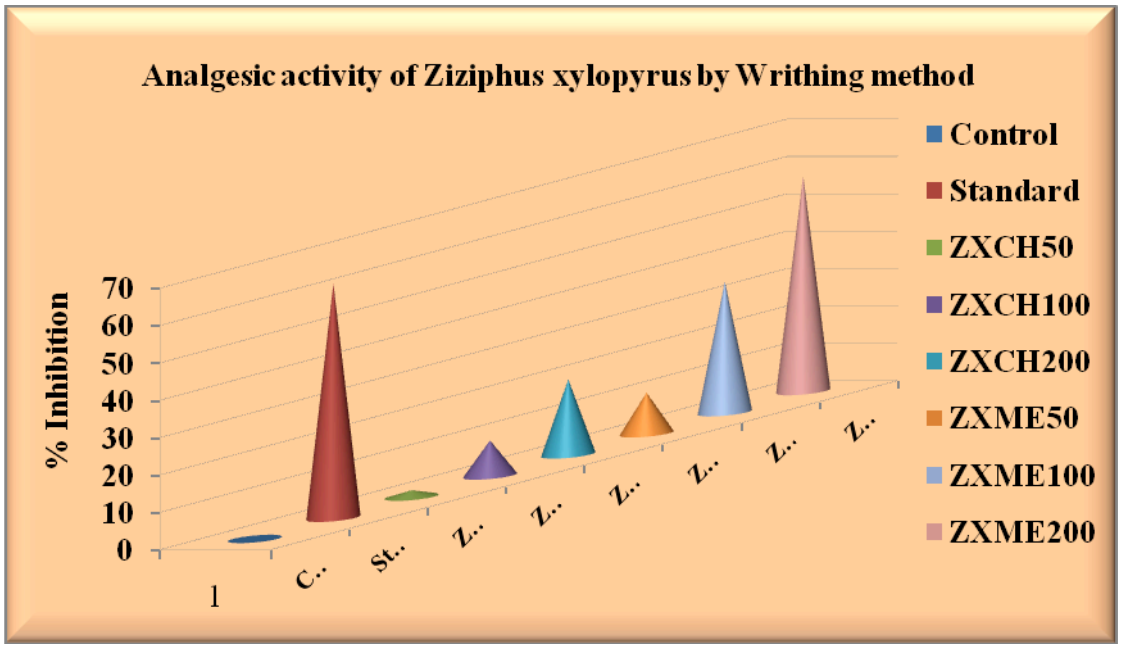

Figure 4: Histogram showing percentage inhibition of *Z. xylopyrus* extracts by acetic acid induced writhing method

Carrageenan induced paw edema

Anti-inflammatory activity of chloroform and methanol extracts of *Z. xylopyrus* bark was carried out by using carrageenan induced paw edema (as per table 4, 5, 6 and figure 5, 6). From the investigation it was found that a gradual increase in paw volume in control group animals after injected with carrageen. The indomethacin treated standard group showed significant activity as compared to control group. *Z. xylopyrus* methanolic extract at different dose level showed significant anti-inflammatory activity as compared to control group. There were no significant differences found in paw edema between standard group and 200 mg/kg of methanolic extract administered group.

Table 4: Determination of paw volume of rats for evaluation of anti-inflammatory activity of chloroform and methanol extracts of *Z. xylopyrus* bark by carrageenan induced paw edema method

Groups	Initial paw volume	Paw volume at different time interval (in ml)				
		0.5hr	1hr	2hr	3hr	4hr
Control(1%DMSO)	1.22±0.182	1.50±0.122	1.63±0.109	1.89±0.370	2.08±0.106	2.01±0.185
Indomethacin (4mg/kg)	1.25±0.303	1.40±0.101	1.48±0.158	1.61±0.110	1.70±0.121	1.64±0.135
ZXCH (50 mg/kg)	1.27±0.101	1.55±0.100	1.67±0.121	1.94±0.143	2.10±0.221	2.07±0.070
ZXCH (100 mg/kg)	1.23±0.110	1.50±0.092	1.63±0.102	1.91±0.143	2.09±0.271	2.02±0.043
ZXCH (200 mg/kg)	1.25±0.124	1.50±0.094	1.64±0.111	1.88±0.106	2.04±0.269	1.98±0.064

ZXME (50 mg/kg)	1.24±0.105	1.47±0.096	1.63±0.144	1.92±0.106	2.09±0.206	2.02±0.072
ZXME (100 mg/kg)	1.25±0.117	1.50±0.133	1.61±0.079	1.79±0.060	1.93±0.015	1.85±0.015
ZXME (200 mg/kg)	1.24±0.106	1.46±0.115	1.53±0.045	1.69±0.058	1.83±0.146	1.75±0.076

ZXCH = *Ziziphus xylopyrus* chloroform extract, ZXME = *Ziziphus xylopyrus* methanolic extract. Values are mean ± SEM, n=6; ***P< 0.0001, **P< 0.001 and *P< 0.05 when compared with control group; and '#' no significant difference between standard and test group at P< 0.05.

Table 5: Determination of paw edema of rats for evaluation of anti-inflammatory activity of chloroform and methanol extracts of *Z. xylopyrus* bark

Groups	Paw edema at different time interval (in ml)				
	0.5hr	**1hr**	**2hr**	**3hr**	**4hr**
Control(1%DMSO)	0.28±0.025	0.41±0.020	0.67±0.011	0.86±0.020	0.79±0.030
Indomethacin (4mg/kg)	0.15±0.005**	0.23±0.020*	0.36±0.026***	0.45±0.025***	0.39±0.040***
ZXCH (50 mg/kg)	0.28±0.017	0.40±0.026	0.67±0.025	0.83±0.015	0.80±0.011
ZXCH (100 mg/kg)	0.27±0.020	0.40±0.035	0.68±0.020	0.86±0.020	0.79±0.020
ZXCH (200 mg/kg)	0.25±0.020	0.39±0.020	0.63±0.030	0.79±0.030	0.73±0.020
ZXME (50 mg/kg)	0.27±0.020	0.39±0.040	0.68±0.020	0.85±0.020	0.78±0.025
ZXME(100 mg/kg)	0.25±0.011	#0.36±0.041	0.54±0.023*	0.68±0.040**	0.60±0.036*
ZXME(200 mg/kg)	#0.22±0.017	#0.29±0.020	#0.45±0.011***	0.59±0.005***	#0.51±0.005***

ZXCH = *Ziziphus xylopyrus* chloroform extract, ZXME = *Ziziphus xylopyrus* methanolic extract. Values are mean ± SEM, n=6; ***P< 0.0001, **P< 0.001 and *P< 0.05 when compared with control group;and '#' no significant difference between standard and test group at P< 0.05.

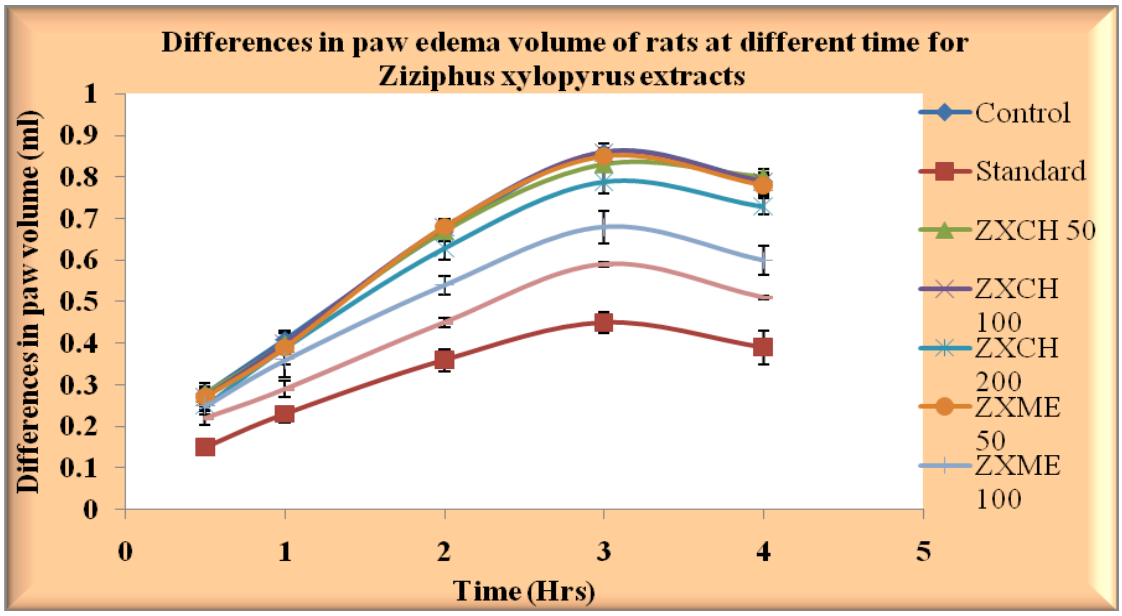

Figure 5: Graph showingdifferences in paw edema volume of rats at different time for *Z. xylopyrus* extracts

Table 6: Percentageinhibition of rat paw edema in different groups

Groups	% Inhibition of rat paw edema at different time				
	0.5hr	**1hr**	**2hr**	**3hr**	**4hr**
Indomethacin (4mg/kg)	46.42	43.90	46.26	47.67	50.63
ZXCH (50 mg/kg)	-	2.43	-	3.48	-
ZXCH (100 mg/kg)	3.57	2.43	-	-	-
ZXCH (200 mg/kg)	10.71	4.87	5.97	8.13	7.59
ZXME (50 mg/kg)	3.57	4.87	-	1.16	1.26
ZXME (100 mg/kg)	10.71	12.19	19.40	20.93	24.05
ZXME (200 mg/kg)	21.42	29.26	32.83	31.39	35.42

ZXCH = *Ziziphus xylopyrus* chloroform extract, ZXME = *Ziziphus xylopyrus* methanol extract

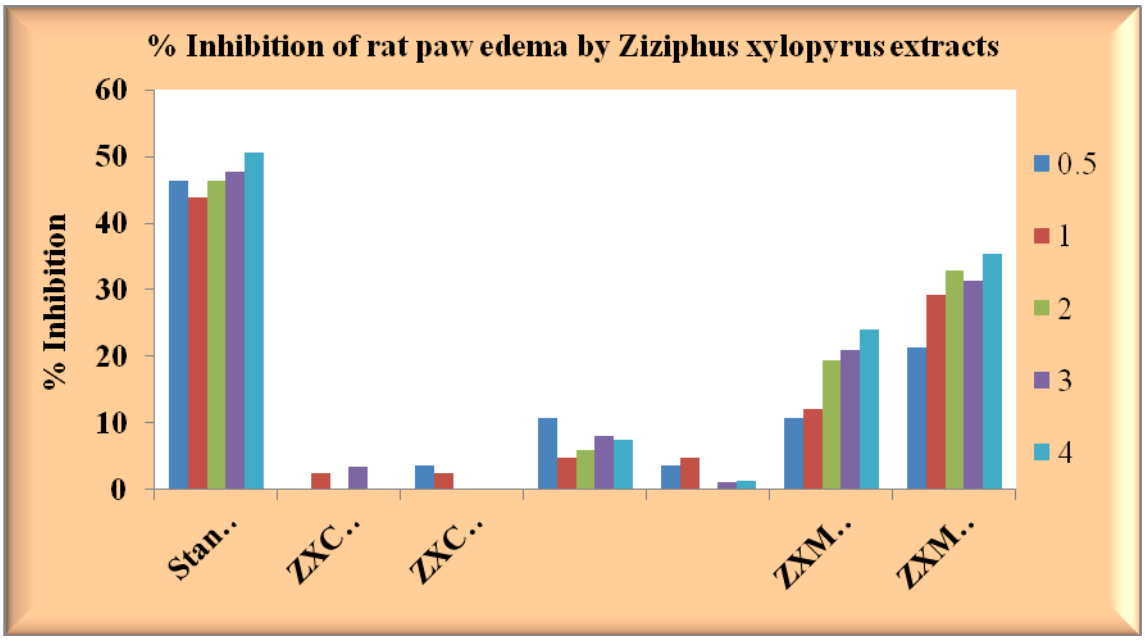

Figure 6: Histogram showing percentage inhibition of rat paw edema by *Z. xylopyrus* extracts

CONCLUSIONS

On the basis of the above results, it is concluded that the selected plant *Ziziphus xylopyrus* is endowed with potential analgesic and anti-inflammatory activities. The observations of the study further scientifically justifies the use in the folklore remedies as analgesic and anti-inflammatory agent since ancient times. The plant *Ziziphus xylopyrus* stem bark possessing both analgesic and anti-inflammatory properties, suggested the presence of non-steroidal anti-inflammatory property, which may be mediated through the prostaglandin inhibition in the living system.

The phytochemical study of biologically active methanolic extract of the plant showed the presence of alkaloids, tannins, flavonoids and steroids respectively.As per the literature research afforded that majority of the active constituents, were identified as alkaloids, flavonoids and rarely xanthones and sterols have been reported to be promising anti-inflammatory agents in animal models. However, the active constituent(s) responsible for the analgesic and anti-inflammatory actions may further be isolated and characterized as future work.

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