ISSN: 2279 - 0594

Journal of Biomedical and Pharmaceutical Research

Available Online at www.jbpr.in

CODEN: - JBPRAU (Source: - American Chemical Society)

Volume 5, Issue 4: July-August; 2016, 34-40

Research Article

IN - VIVO ANTIPYRETIC AND ANTISTRESS ACTIVITY OF HYDROALCHOHOLIC EXTRACT OF *ZIZIPHUS XYLOPYRUS*

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Received 14 July 2016; Accepted 05 August 2016

ABSTRACT

Ziziphus xylopyrus Willd. (*Z. xylopyrus*) is reported for widely use in diarrhoea, chest pain, analgesic, anti-inflammatory and healing of wounds in folk medicine. The aim of our study is to determine the antipyretic and antistress activity of hydroalcoholic extract of *Z. xylopyrus* fruits. The anti pyretic activity evaluated by milk induced pyrexia and antistress activity by swimming endurance test, writhing test and anoxic tolerance test in Swiss Albino rats. The extract administered orally at different dosages (100 mg/kg and 200 mg/kilogram). The potential of hydroalcoholic extract of fruits at 200 mg/kg dose in both the activity was extremely significant and equivalent to standard. Results obtained from the present work, it can be inferred that the extract of *Z. xylopyrus* fruits contributing to the stated activities.

Keywords: *Ziziphus xylopyrus,* Hydroalcoholic extract, Pyrexia, Antistress

INTRODUCTION:

Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states (2). It is the body's natural function to create an environment where infectious agents or damaged tissues cannot survive (2). Normally the infected or damaged tissue initiates the enhanced formation of proinflammatory mediators (cytokines, such as interleukin 1 β, α β, and TNF- α), which increase the synthesis of prostaglandin E 2(PgE2) near hypothalamic area and thereby trigger the hypothalamus to elevate the body temperature (13). When body temperature becomes high, the temperature regulatory system, which is governed by a nervous feedback mechanism, dilates the blood vessels and increases sweating to reduce the temperature. When the body temperature becomes low, hypothalamus protects the internal temperature by vasoconstriction. High fever often increases faster disease progression by increasing tissue catabolism, dehydration, and existing complaints, as found in HIV (14).

Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PgE2 biosynthesis (4). These synthetic agents irreversibly inhibit COX-2 with a high selectivity and are toxic to the hepatic cells, glomeruli, cortex of brain, and heart muscles. Natural COX-2 inhibitors have lower selectivity with fewer side effects (3). A natural antipyretic agent with reduced or no toxicity is therefore, essential. Stress is a common phenomenon that is experienced by every individual. When stress becomes extreme, it is harmful for the body and, hence, needs to be treated. Stress is involved in the pathogenesis of a variety of diseases that includes psychiatric disorders such as depression and anxiety, immuosuppression, endocrine disorders including diabetes mellitus, male impotence, cognitive dysfunction, peptic ulcer, hypertension and ulcerative colitiss (1).

As *Z. xylopyrus* fruits are an old traditional medicament used in fever, diarrhoea, chest pain, analgesic, anti-inflammatory and healing of wounds, it is hoped that fruits of this plant will provide a cost effective alternative antipyretic and antistress agent. Hence, the present study was designed to determine the antipyretic and antistress effect of hydroalcoholic extract of *Z. xylopyrus* fruits.

MATERIALS AND METHODS:

Preparation of extract:

The fruit were crushed and air dried at room temperature. The dried Fruit were coarsely powdered and successfully extracted with ethanol (80%) using Soxhlet extractor at a temperature of 55-60 ^{0}C for a period of 72 hrs. The solvents was

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distilled off at lower temperature under reduced pressure and concentrated to dryness (crude extract). The dried extract was weighed and then stored in a freezer. The crude extract was used for the experiments.

Phytochemical Studies:

The extracts were subjected to phytochemical screening tests for the detection of various constituents using conventional protocol (7, 9).

Animals:

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Chemicals:

Aspirin (CDH, Delhi) were used in present antipyretic study. Diazepam (Ranbaxy, India) was used as the standard drug (positive control) in various stress models.

Acute oral toxicity study:

To determine acute oral toxicity studies were carried out on normal healthy rats, the method of acute oral toxicity at fixed doses was used (1). The extract of *Ziziphus xylopyrus* (50,100,150,200,300 mg/kg/day) was administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible antiulcer effect.

Antipyretic Activity:

Effect of *Ziziphus xylopyrus* extract on Milk induced pyrexia in rats:

Experimental Design:

Group I: 2% v/v aqueous Tween 80 solutions (5 ml/kg body wt., p.o) [Control group]
Group II: Aspirin (150 mg/kg body wt., p.o) [Standard group]
Group III: 100 mg/kg *Ziziphus xylopyrus* extract (ZRE), p.o.
Group IV: 200 mg/kg *Ziziphus xylopyrus* extract (ZRE), p.o.

Before experiment rectal temperature of rat were recovered by inserting a bulb of digital thermometer in the rectum. Care was taken to insert it to the same depth each time. Milk was collected from local cow been boiled. When temperature of the boiled milk equilibrates to room temperature then rats were injected boiled milk at the dose of 2ml/kg (intraperitoneal route) body weight to induce pyrexia. Induction of fever was taken about in one to two hours (11). The *Ziziphus xylopyrus* extract (100 & 200mg/kg, p.o.) was given on experimental group; standard antipyretic agent Aspirin (150mg/kg, intraperitoneal route) was taken as positive control. Finally rectal temperatures were recorded for 4 hrs at consecutive time intervals.

Evaluation of parameters:

Antipyretic activity was evaluated by comparing initial rectal temperature (°C) before treatment of boiled milk, with rectal temperature (°C) after treatment at different time intervals.

Antistress Activity:

Experimental Design:

Group I: Control group
Group II: Diazepam (2 mg / kg, i.p.) [Standard group]
Group III: 100 mg/kg *Ziziphus xylopyrus* extract (ZRE), p.o.
Group IV: 200 mg/kg *Ziziphus xylopyrus* extract (ZRE), p.o.

Swimming endurance test:

The rats were randomly divided into four groups of six animals each. The treatment groups were pretreated with ZRE (100 mg / kg, 200 mg / kg, p.o.) for seven days. The control group was pretreated with normal saline (10 ml / kg, p.o.), while the positive control group received diazepam (2 mg / kg, i.p.) for seven days. The swimming test was carried out on the seventh day, after one hour of oral and 30 minutes of intraperitoneal administration of the drug, using a polypropylene vessel (45 × 40 × 30 cm) with a

water level of 20 cm, and the immobility time was recorded for 30 minutes (5).

Anoxic tolerance test:

The rats were randomly divided into four groups of six animals each. The treatment groups were pretreated with ZRE (100 mg / kg, 200 mg / kg, p.o.) for seven days. The control group was pretreated with normal saline (10 ml/ kg), while the positive control group received diazepam (2 mg / kg, i.p.) for seven days. On the seventh day, the rats were subjected to anoxic stress by keeping them in a confined airtight 250 ml glass jar. The time taken for the rats to exhibit the first clonic convulsion was taken as the end point. The animals were removed immediately from the vessel for recovery and resuscitated if needed (8).

Writhing test:

The mice were randomly divided into four groups of six animals each. The treatment groups were pretreated with ZRE (100 mg / kg, 200 mg / kg, p.o.) for seven days. The control group was pretreated with normal saline (10 ml / kg, p.o.), while the positive control group received diazepam (2 mg / kg, i.p.) for seven days. At the end of the seventh day, writhing was induced one hour after oral and 30 minutes after intraperitoneal administration of the drug by giving 0.1 ml of 0.4% (0.4 ml / 20 mg, i.p.) glacial acetic acid. The numbers of writhing responses produced were recorded for 20 minutes [5].

Statistical analysis:

The results are expressed as mean value ± S.E.M. one way ANOVA followed by Tukey test was applied to the results. Mean values were considered significantly different when $P < 0.05$.

RESULT AND DISCUSSION:

Preliminary phytochemical analysis of hydroalcoholic extracts of fruit of the plant indicated the presence of alkaloids, tannins, flavonoids and resins in extract.

No adverse effect or mortality was detected in albino rat s up to 200mg/kg, p.o. of all the extracts of *Z. xylopyrus* during the 24h observation period basing on which the respective doses are selected for further study.

Antipyretic activity:

In the present investigation hydroalcoholic extract of *Z. xylopyrus* has been evaluated for the antipyretic activity against Milk induced pyrexia in rats the resultant effects extract of *Z. xylopyrus* on boiled milk induced pyrexia in rats are depicted in Table 1. At a dose of 100mg/kg, p.o body weight, fractions reduced 37.40 ± 0.10 of elevated rectal temperature compared to aspirin (37.15 ± 0.29 % after 4 h). At a dose of 200mg/kg, p.o body weight, reduced 37.00 ± 0.20, of elevated rectal temperature compared to aspirin (37.15 ± 0.29 % after 4 h). Thus hydro alcoholic fractions produced significant ($P < 0.05$) antipyretic effect. It was also observed that the solvent have no effect on the reduction of pyrexia of rat.

Table 1: Antipyretic effect of *Ziziphus xylopyrus* extract.

Treatment/Dose	Initial rectal Temp. in °C	Rectal temp. in °C Temperature after Treatment (Mean±Sem)				
		0 hr	1 hr	2 hr	3 hr	4 hr
Group I(2% aqueous Tween 80 solution, 5ml/kg, p.o)	38.00 ± 0.0	39.80 ± 0.13	39.80 ± 0.17	39.82 ± 0.17	39.90 ± 0.15	39.98 ± 0.10
Group II (Aspirin, 150mg/kg, p.o)	37.50 ± 0.4	39.15 ± 0.12	38.35 ± 0.10 [a]	38.30 ± 0.14 [a]	37.17 ± 0.20 [a]	37.15 ± 0.29 [a]
Group III (ZRE, 100mg/kg, p.o)	37.10 ± 0.2	39.18 ± 0.05	39.45 ± 0.15 [b]	39.14 ± 0.07 [b]	38.13 ± 0.02 [a]	37.40 ± 0.10 [a]
Group IV (ZRE, 200mg/kg, p.o)	36.50 ± 0.5	39.80 ± 0.05	39.20 ± 0.06 [a]	38.40 ± 0.06 [a]	37.70 ± 0.20 [a]	37.00 ± 0.20 [a]

Expressed as mean± SEM (n = 6), one way ANOVA followed by Tukey test; * P < 0.05, P < 0.001 when compared with control group**

Antistress (adaptogenic) activity

Antistress (adaptogenic) activity against different types of stresses viz. Anoxia, swimming endurance and writhingmodels. Diazepam, benzodiazepine anxiolytics was used as standard. Diazepam is reported to possess a non-specific anti-stress activity involving the mesocortical dopamine system and the norepinephrine and 5HT levels of whole brain and hypothalamus. It is proposed that this effect is producedimmobilisation models. Diazepam, benzodiazepine anxiolytics was used for the comparison. Diazepam is reported to possess a non-specific anti-stress activity involving the mesocortical dopamine system and the norepinephrine and 5HT levels of whole brain and hypothalamus. It is proposed that this effect is produced through an enhancement of GABAergic neurotransmission (6).

In case of swimming endurance test hydroalcoholic extract of *Z. xylopyrus* exhibited significant antistress activity as indicated by increase in swimming endurance time (Table 1). There are reports that plasma levels of adrenaline and noradrenaline are enhanced during stress induced by swimming endurance test. In addition, monoamine oxidase (MAO) levels in the brain are reportedly decreased during stress (4). The swim endurance test results indicate clearly that the hydroalcoholic extract of *Z. xylopyrus* has the properties whereby it increases the physical endurance as well as the overall performance in rats and possessed significant anti-stress activity. It may be possibly normalizing the plasma level of catecholamine and MAO.

In anoxia stress tolerance model, depletion of oxygen in hermetic vessel leads to convulsions in animals and pretreatment with hydroalcoholic extract of *Z. xylopyrus* had increased the duration of stress tolerance indicating their anti-stress activity (Table 2). This effect may be due to that during stress, the hydroalcoholic extract of *Z. xylopyrus* was capable of increasing succinate dehydrogenase (SDH) in the brain. This enzyme is responsible for utilization and conservation of energy in the cellular system of the organism, which helps adaptive processes during stress. Adaptogens producing beneficial effects in stress are believed to act by increasing non-specific resistanc (12).

Glacial acetic acid-induced writhings and a chemical-induced stress test caused hyperalgesic effects on the pain pathway (10). The results of glacial acetic acid–induced writhings indicate that the hydroalcoholic extract play a significant role in the inhibition of pain and inflammatory processes.

Table 2:- Effect of hydroalcoholic extract of *Ziziphus xylopyrus*(ZRE) on immobility time in the swimming endurance test

Group	Treatment	Immobility time (sec)
I	Control	1217 ± 44.10
II	Diazepam (2 mg / kg, i.p.) [Standard]	633.3 ± 44.10***
III	*Ziziphus xylopyrus*extract (ZRE-100 mg/kg, p.o.)	700.7 ± 57.74 ***
IV	*Ziziphus xylopyrus*extract (ZRE-200 mg/kg, p.o.)	583.3 ± 44.104***

Expressed as mean ± SEM (n = 6), one way ANOVA followed by Tukey test; * P < 0.05,
**P < 0.001 when compared with the control group

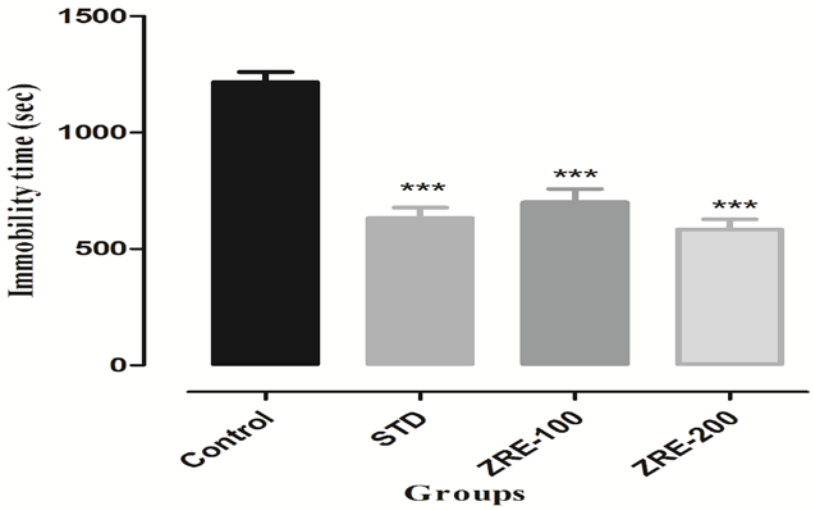

Figure 1: Effect of hydroalcoholic extract of *Ziziphus xylopyrus*(ZRE) on immobility time in the swimming endurance test; Expressed as mean ± SEM (n = 6), one way ANOVA followed by Tukey test; * P < 0.05,P < 0.001 when compared with the control group**

Table 3:- Effect of hydroalcoholic extract of *Ziziphus xylopyrus*(ZRE) on the latency of convulsion in the anoxic tolerance test

Group	Treatment	latency of convulsion (min)
I	Control	60.00 ± 2.88
II	Diazepam (2 mg / kg, i.p.) [Standard]	91.67 ± 4.41**
III	*Ziziphus xylopyrus*extract (ZRE-100 mg/kg, p.o.)	80.00 ± 2.88*
IV	*Ziziphus xylopyrus*extract (ZRE-200 mg/kg, p.o.)	80.00 ± 5.77*

Expressed as mean ± SEM (n = 6), one way ANOVA followed by Tukey test; * P < 0.05, **P < 0.001 when compared with the control group

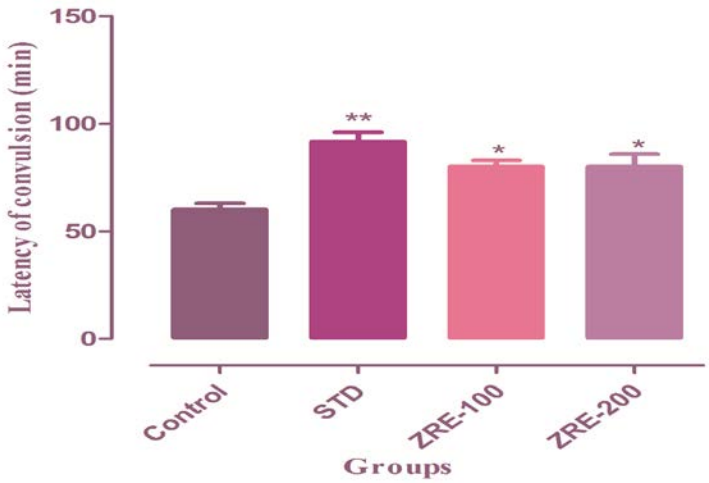

Figure 2: Effect of hydroalcoholic extract of *Ziziphus xylopyrus*(ZRE) on the latency of convulsion in the anoxic tolerance test; Expressed as mean ± SEM (n = 6), one way ANOVA followed by Tukey test; *P < 0.05, ** P < 0.001 when compared with control group

Table 4: Effect of hydroalcoholic extract of *Ziziphus xylopyrus*(ZRE) on the number of writhings in the writhing test

Group	Treatment	Number of writhings
I	Control	58.33 ± 4.41
II	Diazepam (2 mg / kg, i.p.) [Standard]	33.33 ± 2.02**
III	*Ziziphus xylopyrus*extract (ZRE-100 mg/kg, p.o.)	39.00 ± 2.08*
IV	*Ziziphus xylopyrus*extract (ZRE-200 mg/kg, p.o.)	36.67 ± 4.41**

Expressed as mean ± SEM (n = 6), one way ANOVA followed by Tukey test; * P < 0.05, P < 0.001 when compared with control group**

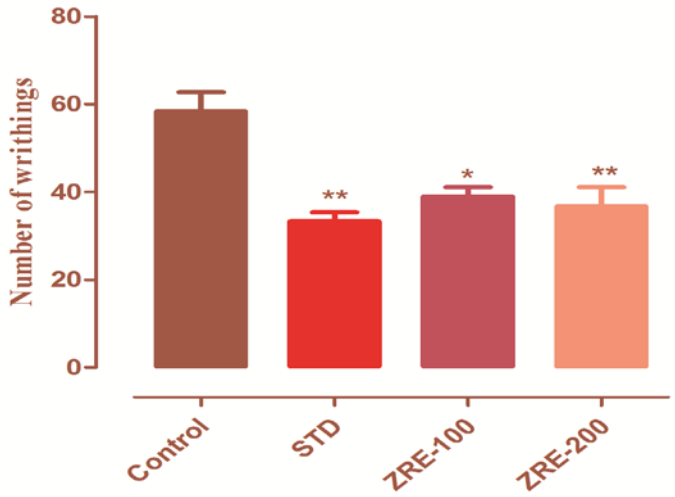

Figure 3: Effect of hydroalcoholic extract of *Ziziphus xylopyrus* (ZRE) on the number of writhings in the writhing test; Expressed as mean ± SEM (n = 6), one way ANOVA followed

CONCLUSIONS

The results obtained demonstrate the significant antipyretic and antistress activity of the hydroalcoholic extract of *Ziziphus xylopyrus*. Inhibition of the synthesis and/or release of inflammatory mediators may be its main mechanism(s) of action. These results also suggest that the presence of certain bioactive molecules may partly be responsible for the reported antipyretic activity of *Ziziphus xylopyrus*, the isolation of which could help to obtain improved antipyretic drugs with specific mechanism of action. Further experimentation is under way in our laboratory to isolate the active molecules from *Ziziphus xylopyrus* and to establish the exact mechanism of action of the extract. our results provide evidence that the seven day treatment with the hydroalcoholic extract of *Ziziphus xylopyrus* shows antistress (adaptogenic) activity in various acute stress models. The observed antistress activity maybe due to the prevention of desensitization of both the peripheral and central components of the hypothalamic pituitary-adrenal axis (HPA) and due to the non-specifically increased resistance produced by the hydroalcoholic extract of *Ziziphus xylopyrus*.This study provides significant evidence of the medicinal and traditional uses of hydroalcoholic extract of *Ziziphus xylopyrus* stress disorders.

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