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Anticonvulsants and anti-nociceptive effect of ziziphus xylopyrus wild (Rhamnaceae) stem bark ethanolic extract

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Abstract
The available synthetic analgesic and anti-nociceptive drugs possess several health problem during their clinical use. To avoid these ill effect on health, it is necessary to search or develop a more effective drug with fewer side effects. Hence, the present investigation was carried out to test anticonvulsant effect and anti-nociceptive effect *Ziziphus Xylopyrus* (Retz.) Willd bark ethanolic extract. The anticonvulsant effect was done using using Maximum Electo Shock (MES) induced convulsion in rats. The extract (200 mg/kg p.o) significantly increased the threshold of MES-induces convulsion in rat compared with the control group. Which showed 83.34% protection in rat. The anti-nociceptive effect was done using tail flicl method. A maximum effect was 10.9 sec at 120 min post treatment with 200mg/kg p.o. *Ziziphus xylopyrus* (Retz.) Willd is effcetive. Where in the vehicle treat control group the reaction was 4.8 sec. Thus, *Ziziphus Xylopyrus* (Retz.) Willd bark is very useful medicinal plants besides its several economical use. The ethanolic extract is useful for epilepsy and pain as suggested dose.

Keywords: Anticonvulsants, anti-nociceptive effect, ziziphus xylopyrus wild, stem bark ethanolic

Introduction
Pain is one of the most common complaints for which patients seek advice and help from health professionals. It can be appropriately defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage. The etiology of chronic pain is seldom all psychological or all physical. Commonly there is little association between the extent of injury and the amount of pain complains. Excessive pain may produce other effects-sinking sensation, apprehension, sweating, nausea, palpitation, rise or fall in blood pressure (BP) and tachypnoea.

Analgesic is a drug that selectively relieves pain by acting in the CNS (Central Nervous System) or on peripheral pain mechanism, without significantly altering consciousness. Analgesics relieve pain as a symptom, without affecting its cause. They are used when the noxious stimulus (evoking the pain) cannot be removed or as adjuvants to more etiological approach to pain [1].

Epilepsy is a major neurological disorder and up to 5% of the world population develops epilepsy in their Lifetime [2]. The current therapy of epilepsy with modern antiepileptic drugs is associated with side effects, dose-related and chronic toxicity, as well as teratogenic effects, and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy [3, 4]. Traditional systems of medicine are popular in developing countries and up to 80% of the population relies on traditional medicines or folk remedies for their primary health care need [5].

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects [6].Medicinal plants, which form the backbone of traditional medicine, have in the last few decades been the subject of very intense, pharmacological studies [7]. In this connection, higher plants continue to be a rich source of therapeutic agents since they produce hundreds to thousands of diverse chemical compounds as secondary metabolites with different biological activities [8]. The compounds produced by plants are active against plant and human pathogenic microorganisms [9]

As presently available synthetic analgesic and anti-nociceptive drugs possess several health problems during their clinical use. The use of natural products is growing in the world especially in developing countries like India where over 75% of the population relies mainly on plants and plant extracts for healthcare. *Ziziphus Xylopyrus* (family Rhamnaceae) is such a plant which is commonly found in various parts of north-western India, Uttar Pradesh, Bihar and Central and South India. As per the ethnomedicinal information, various parts of this plant

possess several medicinal properties. The fruit powder with pinch ginger powder thrice in a day is useful for stomachache and indigestion [10]. Further, it was reported that this plane also possess antidepressant, antimicrobial and anthelmintic activities [11, 12, 13]. The biochemical constituents present in this plant are quercetin and quercitrin in leaves; catechol-type of tannins (8- 12%), oleanolic acid, l-epicatechin, l-leucocyanidin, 3, 3, 4-tri- O-methyl-ellagic acid in fruits; tannin (7.2%), d-7, 3', 4'- trihydroxyflavan- 3, 4-diol and oleanolic acid in barks [14]. The stem wood of the plant is reported to contain triterpenoid compounds [15], alkaloids (xylopyrine-A & B) [16, 17] and flavonoids [11]. Thus, a specific study now need to undertake to understand the analgesic and anti-nociceptive effects stem barks ethanolic extracts of *Z. xylopyrus*.

2. Materials and Methods

2.1 Plant material: The stem barks of the selected plant were collected from the forest of Similipal Biosphere Reserve, Mayurbhanj, Orissa, India in August 2006. The plant material was identified and authenticated taxonomically at the Central National Herbarium, Botanical Survey of India, Botanical garden, Howrah, West Bengal, India. A voucher specimen of the collected sample was deposited in the institutional herbarium for future reference [18].

2.2 Preparation of extracts

The said plant parts were cleaned, dried under shade and powdered by a mechanical grinder as per standard procedure. Hundred grams of the pulverized stem bark was extracted with the solvent of ethanol using Soxhlet apparatus, then subjected to condensation and kept at 4°C prior to testing. The percentage yield of the ethanolic extract of the stem bark of Z. *xylopyrus* Willd was 7.79 % w/w [19, 20].

2.3 Pharmacological evaluation

2.3.1 Anti-convulsent activity study

2.3.1.1 Animals and Drugs

Swiss albino rats of Wistar strain (180-250g) of either sex were procured from the animal house of V.S.S. Medical College, Burla, Odisha, Animals were housed at temperature of 24±2°C and relative humidity of 30-70%. A 12:12 light: day cycle was followed. All the animals were allowed to free access to water and feed with standard commercial pellet [2].Experimental protocols and procedures were approved by the Institutional Animal Ethics Committee CPCSEA/IAEC (5/1/2014). The drugs Phenytoin (PHY) (Eptoin®, Sun Pharma Ltd, India) used in present study all other reagents were purchased from Novel Chemicals, Berhampur, Odisha, India.

2.3.1.2 Maximum electro shock induce convulsion

MES induced convulsion in rat, sixty male rat were allotted into five groups of twelve animals each and treated as described below:

a. **Group 1 :** Distilled water (0.5 ml p.o.) - Control condtion
b. **Group 2 :** Phenytoin (30 mg/kg i.p.).
c. **Group 3 :** Ethanolic extract (100 mg/ kg body weight) in 0.5% Tween 80
d. **Group 4 :** Ethanolic extract (200mg/kg body weight)in 0.5% Tween80
e. **Group 5 :** Ethanolic extract (300mg/kg body weight) in 0.5% Tween 80was administered

After a pre-treatment time of 60 minutes, a CFP stimulator (model-8048) was used to deliver a stimulus of 50 Hertz at 20 volts via ear electrodes to the different groups. The animals were observed for 2 minutes. The onset of tonic hind limb extension and number of animals protected was recorded. [21]

2.3.2 Anti-nociceptive activity study

2.3.2.1 Animals and Drugs

Wistar rats (120-130 g) were used for the study. The animals had free access to food and water. They were fasted overnight before the experiment. They were housed in animal room, with alternating light-dark cycle of 12 h each. The animals were acclimatized to the laboratory conditions for at least five days prior to the experiments. All experiments were conducted between 0900 h and 1800 h [22]. Diclofenac potassium (Delta-K 50mg), Ranbaxy Laboratories Ltd. India, purchase from market was used for the study. Distilled water was used as vehicle. All the chemicals and solvents were of analytical grade.

2.3.2.2 Tail flick response

In this model sixty male rat were allotted into five groups of twelve animals each were treated as described below:

a. **Group 1 :** Distilled water (0.5 ml p.o.)- Vehicle - Control condition
b. **Group 2 :** Diclofenac potassium (10 mg/kg body weight)- Standard
c. **Group 3 :** Ethanolic extract (50 mg/kg b.w) in Tween80
d. **Group 4 :** Ethanolic extract (100mg/kg b.w)in Tween 80
e. **Group 5 :** Ethanolic extract (200mg/kg b.w) in 0.5% Tween 80

The subject were assessed by observing the reaction time in the treated groups. [21]. As per the administration of drugs, the reaction time was noted at 0.30, 60, 90, 120 and 150 min. [22]

2.4 Statistical analysis

The data were analysis using Microsoft Excel 2010 for mean, standard error of mean (SEM), One-Way Analysis of Variance (ANOVA), and Chi square test. Where Values of $p < 0.05$ were considered significant.

3. Result and Discussion

3.1. Preliminary phytochemical analysis

A small amount of coarsely powdered drug was spread on a white tile and physically examined for general appearance i.e colour, taste, texture etc. The powdered of the bark of *Ziziphus xylopyrus* (Retz) Willd. was yellowish brown in colour with aromatic odour and rough texture. The powder was slightly bitter in taste. Approximately 2g of powdered drug sample was used for the evaluation. Phytochemical evaluation of *Ziziphus xylopyrus* (Retz.) Willd. Bark showed the presence of Flavonoids, Sterols, Triterpenoids, Tannins, Carbohydrates and Glycosides.

3.2. Acute oral toxicity

There was no mortality and noticeable behavioral changes in all the groups tested. The extract was found to be safe up to a dose level of 300 mg/kg body weight.

3.1 anticonvulsant effect

The anticonvulsant effect of ethanolic extract of *Ziziphus xylopyrus* (Retz.) Willd bark was studied using maximum electro shock (MES) induced convulsion in rat. The extract (200 mg/kg bw) significantly ($p < 0.05$) increased the threshold of MES-induced convulsions in rat compared with

the control group and Phenytoin. A dose of 200 mg/kg p.o. the extract showed highest onset of convulsion (9.98 ± 0.71) and highest protection (83.34%) in rat, followed by dose of 100 mg/kg p.o. (8.50 ± 0.95 and 75%), and 300 mg/kg p.o. (8.34 ± 0.63 and 66.67%). Similarly, the number of convulsed rat were lowest in 200 mg/kg p.o. followed by dose of 100 mg/kg p.o. and 300 mg/kg p.o. (Table 1).

Table 1: Effect of ethanolic extract of *Ziziphus xylopyrus* on MES-induced seizures in rat

Treatment	Group	Dose	Onset Convulsion	Number convulsed/number used	Effectiveness (%)
Control(distilled water)	1	0.5ml/kg	10.35±0.45	12/12	0
Phenytoin	2	30mg/kg	-	0/12	100
Ethanolic extract of *Ziziphus xylopyrus*	3	100mg/kg	8.50±0.95	3/12	75
	4	200mg/kg	9.98±0.71	2/12	83.34
	5	300mg/kg	8.34±0.63	4/12	66.67

Value are expressed in terms of mean±SEM, n=12 in each group $p<0.05$ statistically significant as compared with control group

3.2 anti-nociceptive effect

The anti-nociceptive effect of ethanolic extract of *Ziziphus xylopyrus* (Retz.) Willd bark was studied using tail flick method. The maximum effect was observed for dose of 200mg/kg bw 10.9 ± 0.192 sec at 120 min post treatment, whereas in the vehicle treated (10 ml/kg bw) control group the reaction time was 4.8 sec. only (Table 2).

Table 2: Evaluation of anti-nociceptive activity of ethanolic extract of *Z. xylopyrus* stem bark by tail flick method

Treatment	Group	Dose	Basal reaction time	Reaction time (in sec) after administration of drug at different time(min)				
				30	60	90	120	150
Control (0.5% Tween 80)	1	10ml/kg b.w	4±0.021	4.2±0.062	4.4±0.145	4.6±0.134	4.8±0.152	5.1±0.162
Diclofenac potassium	2	10mg/kg b.w	4.06±0.198	11.84±0.123	11.75±0.171	11.69±0.132	11.65±0.153	11.58±0.111
Ethanolic extract of *Z. xylopyrus*	3	100mg/kgb.w	4.5±0.208	5.8±0.152	6.8±0.012	7.4±0.057	7.7±0.015	7.6±0.059
	4	200mg/kg b.w	5±0.014	7.8±0.041	9.5±0.014	10.7±0.141	10.9±0.192	10.7±0.185
	5	300mg/kg b.w	4.8±0.218	7.4±0.152	8.4±0.131	9.1±0.032	9.5±0.041	9.4±0.018

4. Discussion

Utilization of herbal medicine is a preferable and ancient process in India. A lot of plant species being characterized for many clinical study. Among these *Ziziphus xylopyrus* is one of the important herbs where sub-species also under characterization. It has been used for range of medical treatment by using various plant parts. But, a systematic study was undertaken using the bark in the present investigation. Pain is a condition which is regularly dealt with in daily clinical practice. Hence, any attempt to contribute an easily available analgesic drug from the especially from flora is always accepted without any reluctance.

The standard drug used for the study of anti-convulsant activity in mice is Phenytoin, is believed to protect against seizures by causing voltage-dependent block of voltage gated sodium channels. The block sustained high frequency repetitive firing of action potential. For anti-nociceptive effect, the drug Diclofenac potassium was used, which has properties for inhibition of prostaglandin synthesis by inhibition of the transiently expressed prostaglandin endoperoxide synthase-2 also known qs Cycloxygenase-2.

On the basis of the outcome of the present study, it is concluded that the selected plant Z. *xylopyrus* is endowed with potential analgesic and anti-nociceptive activities and the results of the study further scientifically justifies the use in the folklore remedies as analgesic and anti-inflammatory agent since ancient times. The stem bark of the plant possessing both analgesic and anti-inflammatory properties, suggested the presence of non-steriodal anti-inflammatory property, which may be mediated through the prostaglandin inhibition in the living system. To study the mechanism of the action in depth requires further studies and conformations. The phytochemical study of biologically active ethanolic extract of the plant showed the presence Flavonoids, Sterols, Triterpenoids, Tannins, Carbohydrates and Glycosides. Which have been reported to be promising analgesic agents in animal models as per the literature research. [21, 24, 25]. However, the exact active constituent(s) responsible for the analgesic and anti-inflammatory actions may further be isolated and characterized as future work.

5. Conclusion

The results of this study show that the ethanolic extract of bark of *Ziziphus xylopyrus* possess anti-nociceptive and anti-nociceptive properties. These results suggest that the bark of *Ziziphus xylopyrus* will be beneficial in the management of epilepsy and pain. Further studies on the isolation of the active constituents and exact mechanism of action are needed.

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