



**ANTISPASMODIC EFFECT OF METHANOLIC EXTRACT OF *ZIZIPHUS OENOPLIA*
(L.) MILL. ROOT BARK**

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ABSTRACT

Ziziphus oenoplia (L.) Mill. (Family- Rhamnaceae) is a shrub that is used in the traditional medicine of India for diarrhea, dysentery and spasm. The antispasmodic activity of 80% methanolic extract of *Ziziphus oenoplia* was assessed on contraction of isolated rat ileum, induced by acetylcholine and compared with effect of loperamide. The methanolic extract of *Ziziphus oenoplia* composed of alkaloid, flavonoid, and tannins rich components. The extract has exhibited an inhibitory effect on dose response response curve induced by acetylcholine (Ach.) on rat ileum and significantly reduced the maximal response in a concentration dependent manner. The present study demonstrate that the 80% methanolic extract of *Ziziphus oenoplia* exerts antispasmodic activity on rat ileum and thus provides sound mechanistic basis for some there folkloric use.

KEYWORD: *Ziziphus oenoplia*, 80% methanolic extract, antispasmodic.

INTRODUCTION

Ziziphus oenoplia (L.) Mill. (*Z. oenoplia*) (Family- Rhamnaceae) is commonly known as Makai in Hindi and Jaykal jube in English, is a straggling shrub distributed all over hotter part of India and as well as Asia.^[1] It is one of the folk herbal medicine has some major pharmacological properties as anthelmintic activity^[2] angiogenic potential^[3], antiulcer activity^[4], hepatoprotective^[5], antimicrobial activity^[6], wound healing activity^[7], antiplasmodial activity.^[8] The root bark of *Z. oenoplia* decoction was frequently used to cure of diarrhoeal and spasm as well as other gastrointestinal disorder by rural people of Uttar Pradesh India. The plants was previously evaluated for anti diarrhoeal^[9] and antibacterial activity, gives significant results of *Z. oenoplia* root bark extract. Therefore the present study designed to evaluate the antispasmodic activity of 80% methanolic extract of *Z. oenoplia* against acetylcholine (Ach.) induced contraction in rat ileum.

MATERIALS AND METHODS

Plant material

The root of *Z. oenoplia* were collected from Jaunpur District of Uttar Pradesh, India in month of January 2010 was authenticated by taxonomic department of National Botanical Research Institute (NBRI) Lucknow, Uttar Pradesh India. A voucher specimen (NBRI/CIF/145/2010) has been deposited in the institute for further reference.

Preparation of extract

The root of *Z. oenoplia* was collected and washed with water to removed dust and soil then shade dried in a ventilated place at room temperature. The dried plant material was cut in small part and pulverized in coarse powder. The coarse powder was extracted with 80% methanol, using soxhlate apparatus. The 80% methanolic extract was filtered and concentrated using rotavapor then freeze dried and stored in deep freezer for further use. Solutions of the extract were prepared freshly for study.^[10, 11]

Preliminary phytochemical study

The 80% methanolic extract was subjected to preliminary phytochemical screening according to recommended standard procedure^[12, 13] by performing different qualitative chemical tests.

Animals

Wistar albino rats either sex weighing 200 ± 25 gm were used for all experiments in present study. Animals were collected at random from Animal house of PBRI, Bhopal. All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal (Reg No.-1283/PO/c/09/CPCSEA). Animals were housed in a group of four in separate cages under controlled conditions of temperature (22 ± 2°C). All animals were given standard diet (Golden Feed, New Delhi) and water ad libitum. Animals were further divided in three groups with five animals in each group.

Chemicals

Acetylcholine (Sigma Aldrich, Germany) Sodium chloride, Sodium bicarbonate, Glucose, Sodium dihydrogen phosphate, Potassium chloride, Magnesium chloride, Calcium chloride (Merck India) etc.

Preparation of physiological salt solution

Physiological salt solution (tyrode) consisted of sodium chloride (8 g), sodium bicarbonate (1 g), glucose (1 g), sodium dihydrogen phosphate (0.05 g), potassium chloride (0.2 g), magnesium chloride (0.1 g), calcium chloride (0.2 g), and the volume was made upto 1 litre with distilled water (pH 7.4). Sodium chloride maintains isotonicity and along with potassium chloride it also maintains the membrane potential. Sodium bicarbonate maintains alkaline pH and sodium dihydrogen phosphate acts as a buffer. Magnesium chloride relaxes smooth muscles, calcium chloride causes contraction of the smooth muscles and glucose provides energy to the cells.

Assessment of antispasmodic activity

Wistar albino rats either sex (200± 25) were sacrificed by cervical dislocation and a segment (2 cm) was dissected from the terminal ileum and mounted in an organ bath containing Tyrode's solution at 37°C and bubbled with oxygen, with a thread under 1 g initial tension. The lower part was fixed at the bottom of the organ bath and upper one was connected to the lever and the responses were taken on a kymograph paper placed on Sherrington revolving drum. The equilibrium period was 60 min and the bath solution was refreshed every 15 min. The responses of spasmogens such as acetylcholine (10µg/ml) were seen and the effect of the *Z. oenoplia*, 200mg/ml, 400mg/ml and Loperamide 5mg/ml were seen in cumulatively.^[14-16]

Statistical analysis

The result of experiment was reported as mean±sd. these result was further analysed by using student's t-test to calculate significance of the result. The p-value less than .05 was considered as statistically significant.

RESULTS AND DISCUSSION

Extraction of plant material

The amount of 80% methanolic extract was found to be sticky, brown color, 14.5% yield of *Z. oenoplia* root bark. The extractive was mixture of various phytoconstituents.

Preliminary phytochemical analysis

The plant extract was tested for presence of different group of phytoconstituents and it was found to contain flavonoid, alkaloids, tannins terpenoids and phenolic compounds.

Antispasmodic activity

The result showed that an inhibitory action of 80% methanol extract of the plants *Ziziphus oenoplia* on dose- response curves induced by ACh (10µg/ml) on rat ileum. The rat ileum suspended in tyrode's solution under 1g tension after 15 min had a stable tension. ACh caused a rapid contraction, reaching their maximum point within 30 sec of contact. The extract significantly reduced the maximal response of ACh in a concentration-dependent manner (Fig. 1). The inhibitory effects were long-lasting but completely reversible by intermittent washing of the ileum.

The 80% methanolic extract of *Z. oenoplia* produced a concentration dependent spasmolytic activity in-vitro. Control of tension in gastrointestinal smooth muscle is dependent on the intracellular Ca²⁺ concentration. The ACh act via specific receptors can produce changes in tension, without necessarily affecting membrane potential. ACh has functional role in natural contraction of gastrointestinal tract. ACh is a neurotransmitter at prostaganglionic neurons that innervate the gut. The response to ACh is mediated by activation by two types of muscarinic (M2 and M3) receptors. Activation of these receptors results in an increase intracellular Ca²⁺ an effect mediated by inositol triphosphate acting on internal calcium stores.^[15, 17]

Table 1: Antispasmodic evaluation of ACh induced contraction in rat ileum

Treatment	Dose (mg/ml)	Response (contraction)	% Contraction	% Relaxation
Control	--	39.12±4.425	100	--
<i>Z. oenoplia</i>	200	19.4±2.701**	49.59±6.522*	49.55±7.352*
<i>Z. oenoplia</i>	400	13.0±2.345*	33.23±5.435*	64.61±8.273*
Slandered (Loperamide)	5	9.4±1.516*	24.02±3.762*	78.63±3.00*

Values are mean ± Sd (n=5)

* = p < .0001 vs Control student's t-test

** = p < .001 vs Control student's t-test

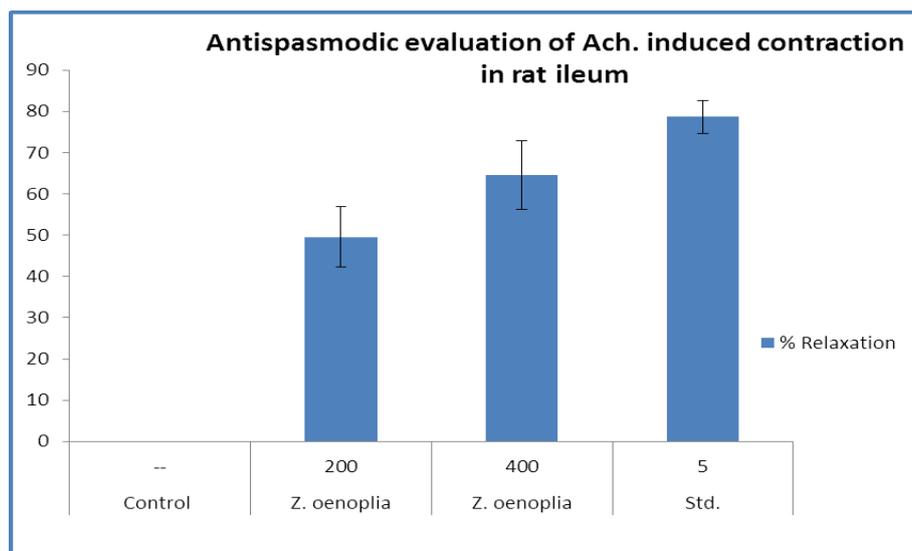


Fig.1: Antispasmodic response of 80% methanolic extract of *Z. oenoplia* root bark

CONCLUSION

As a conclusion our result support that the 80% methanolic extract of *Z. oenoplia* exhibit antispasmodic activity on rat ileum probably by inhibiting Ca^{2+} influx on dose dependent, and it can inhibit the ileum contraction induced by Ach spasmogens. It is well known that some flavonoids can act as spasmolytic agents by relaxing smooth muscles.^[18] The *Z. oenoplia* contains flavonoids might be responsible for this activity. Further studies must be conducted in order to clarify which constituent of the extract is responsible for antispasmodic action in gastrointestinal disorder.

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