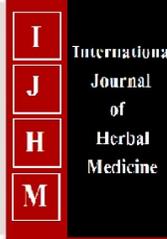




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Antidiarrhoeal evaluation of traditionally used *Ziziphus oenoplia* (L.) Mill root

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Abstract

Ziziphus oenoplia (L.) Mill. (*Z. oenoplia*) (Family-Rhamnaceae) is an annual herb that is used in the traditional medicine of Iran for treating stomach and intestinal disorders. In order to collect ethnobotanical information about antidiarrhoeal plants, we performed inquiries among traditional healers, were designated as having antidiarrhoeal and antidiarrhoeal properties. They were found to contain alkaloids, tannins, saponins, sterols and/or triterpenes and reducing sugars. This study evaluates the potential antidiarrhoeal activity of "methanol extract of *Z. oenoplia* root" (ZOM) in experimental diarrhoea, induced by castor oil and magnesium sulphate in rat at 200 and 400 mg/kg b.w. Both doses were given orally, showed significant antidiarrhoeal activity comparable with that of the standard drug loperamide. On the basis of these findings, it can be assumed that *Z. oenoplia* could be a potential source for novel discovery for antidiarrhoeal. These results may support the fact that this plant is used traditionally to cure diarrhoea.

Keywords: Antidiarrhoeal, *Z. oenoplia*, ZOM, Castor oil induced

1. Introduction

Ziziphus oenoplia (L.) Mill. (*Z. oenoplia*) (Family-Rhamnaceae) is one of the folk herbal plant used in various traditional medicament. Traditionally the roots are used as astringent bitter, anthelmintic, digestive, antiseptic, hyperacidity, *Ascaris* infection, stomachalgia and healing of wounds. Fruits are found to have medicinal properties like blood purifier, febrifuge, abdominal pain. In Siddha medicine the fruits and the seeds are used in fever, retention of urine, poisoning, aphrodisiac, tonic etc [1]. This plants root parts also used by the Chakma tribe in Bangladesh for gastrointestinal disorder [2]. Commonly the root bark decoction used in central zone, Uttar Pradesh (India) in diarrhea & dysentery. *Z. oenoplia* a thorny sprawling bush, commonly known as makai in Hindi and jackal jujube in English, widely found in India, Pakistan, Sri Lanka, Malaysia and Australia. Antimicrobial activity [3], Antiulcer activity of *Z. oenoplia* roots in rats [4], Hepatoprotective activity of *Z. oenoplia* roots [5], wound healing and anthelmintic activity [6, 7], Antiplasmodial activity [8] of *Z. oenoplia*. Chemically the root bark contains cyclopeptide alkaloids (zizyphine-A and zizyphine-B), betulinic acid, d-glucose, d-fructose, sucrose and unidentified polysaccharides. Stem bark contains cyclopeptide alkaloids Zizyphine (A-G) and abyssinine A and B [9-11]. Root also contains four new 13-membered cyclopeptide alkaloids (zizipine N-Q) [8]. Keeping above in view, attempts were made to evaluate the antidiarrhoeal potential of methanol extract of *Z. oenoplia* roots.

Diarrhoea has long been recognized as one of the most important health problems in the developing countries and a major cause of infant mortality and morbidity [12, 13]. Worldwide distribution of diarrhoea accounts for more than 5-8 million deaths each year in infants and small children less than 5 year. According to WHO estimation for the year 1998, there were about 7.1 million deaths due to diarrhoea [14]. Secretory diarrhoea is the most dangerous symptom of gastrointestinal problems [15] and is associated with excessive defecation and stool outputs, the stools being of abnormally loose consistency [16]. So the present study was aimed to evaluate the traditional claim of antidiarrhoeal activity of *Z. oenoplia* root bark in various experimental models.

2. Materials and Methods

2.1 Collection and authentication of plant material:

Roots of *Z. oenoplia* were collected from District Lucknow, Uttar Pradesh, (India) in the month of January 2010 and were authenticated by Dr. Tariq Hussain Scientist, Taxonomy at 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.

2.2 Preparation of the extract

Roots of *Z. oenoplia* were washed with distilled water to remove dirt and soil and shade dried in a ventilated place at room temperature. The dried plant materials were reduced to coarse powder by mechanical grinder, extracted with 80% methanol as solvent in soxhlet extractor for 18 h. The "methanol extract of *Z. oenoplia*" (ZOM) was filtered and concentrated under reduce pressure using rotavapor (Buchi, USA), then freeze-dried (Freezone® 4.5, Labconco, USA) and stored in deep freezer for further use. Solutions of the extracts were prepared freshly for each study.

2.3 Preliminary phytochemical screening

The methanol extract obtained was tested for the presence of various chemical constituents such as saponins, flavonoids, glycosides, alkaloids, tannins and reducing sugar^[17, 18].

2.4 Animals

Wistar albino rats of either sex weighing 200 ±25g were kept at departmental animal house at a temperature (25 ± 2)⁰C and 12 h light/dark cycle respectively for one week before and during the experiments and fed with standard diet and water ad libitum. Animal studies were conducted according to the Institute Animal Ethics Committee. All the experiments were performed in the morning according to the current guidelines for the care of laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals

2.5 Drugs and chemicals

Atropine sulphate and Loperamide (Ranbaxy (I) Ltd, castor oil (Galaxo) all other chemicals were of analytical grade.

2.6 Acute toxicity

Acute toxicity study was performed according to OECD guidelines No. 425. Swiss albino mice of either sex were used for acute toxicity of "*Z. oenoplia* methanol extract" (ZOM). It is non toxic at 2000 mg/kg.

2.7 Statistical analysis

The experimental results were expressed as the mean ± standard error of the mean (S.E.M.). Data were evaluated by student's t-test and means were compared using Graph pad prism3 software t- test at p≤0.05.

2.8 Antidiarrhoeal Experiment Models

2.8.1 Castor oil-induced diarrhoea

This was determined according to the method of Amresh *et al.*^[19] modified by Adeyemi *et al.*^[20] was used to assess the antidiarrhoeal activity of the ZOM extract. Wistar albino rats of either sex (200 ±25g) were fasted for 24 h before starting the experiment. The animals were randomly housed in individual cages and divided into four groups (n=5). The first group received 1% CMC (10 ml/kg p.o.) served as the control and group II was received loperamide (3mg/kg) acting as the standard. The last two groups received different doses (200 and 400mg/kg p.o.) of the plant extract. One hour after the treatment, each animal received castor oil (10 ml/kg, p.o.) through a feeding needle. At 4th hour after dosing the castor

oil, the individual mouse cages were inspected for the presence of unformed water fecal pellets; their absence was recorded as a positive result, indicating protection from diarrhea at that time.

2.8.2 Magnesium sulphate-induced diarrhoea

To assess the antidiarrheal activity, a previously described method by Uddin *et al.*^[21] was modified and used to assess the antidiarrhoeal activity of the ZOM extract. A similar protocol as for castor oil-induced diarrhoea was followed. Aimal divided in four group (n=5) and diarrhoea were induced by oral administration of magnesium sulphate at the dose of 2 g/kg b.w. to the animals, one hour after pre treatment with 1% CMC (10 ml/kg p.o.) to the control group, loperamide (3mg/kg p.o.) to the standard group, and the ZOM plant extract at the doses of 200 mg/kg and 400 mg/kg to the remaining groups. During an observation period of 4 h, the total number of faecal output and the number of diarrheic faeces excreted by the animals were recorded.

2.8.3 Castor oil-induced fluid accumulation

The rats fasted for 24 h but with free access to water were randomized and allocated to four groups of six rats each. Group I (control) was administered 1% CMC (10 ml/kg, p.o.), group II was administered castor oil only (2 ml), groups III and IV were administered 200 and 400 mg/kg of ZOM extract, respectively, 1 h prior to castor oil administration. After 30 min, the rats were killed by cervical dislocation and exsanguinated; the small intestine was ligated both at pyloric sphincter and at the ileo-caecal junctions. The entire small intestine was dissected out, its contents were expelled into a graduated measuring cylinder and the volume of the contents was recorded^[19, 20]

3. Results

3.1 Qualitative analysis of plant extracts

The phytochemical analyses of ZOM (80% methanol extract) were revealed the presence of various chemical constituents such as alkaloids, saponins, glycosides, tannins, flavonoids, reducing sugar etc. (Table 1)

Table 1: Qualitative analysis of methanol extract of *Z. oenoplia* root

Test	ZOM
Alkaloids	+++
Steroids/Terpenes	++
Flavonoids	+
Glycosides	+
Saponins	+
Tannins	+
Reducing sugars	++

3.2 Effect on castor oil-induced diarrhoea

In the castor oil-induced diarrhoeal rats, the 80% methanol extract of the ZOM at the doses of 200 & 400 mg/kg, reduced the total number of faeces as well as of diarrheic faeces and the results were statistically significant (Table 2). ZOM were shows dose dependent antidiarrhoeal activity. Both the dose reduced the total number of faeces and total number of wet faeces in test animals.

Table 2: Effect of *Z. oenoplia* on Castor oil induced diarrhea in rats

Treatment	Dose (mg/kg)	Total no of faeces in 4 hr	Total no of wet faeces in 4 hr	Reduction (%)
Control (1%, 10ml/kg CMC) + Castor oil	--	10.4±0.50	9.2±0.37	--
Loperamide + Castor oil	3	1.6±0.28**	0.6±0.24**	93.48
ZOM + Castor oil	200	6.4±0.32*	3.6±0.27*	60.87
ZOM + Castor oil	400	5.4±0.42*	2.6±0.34**	71.74

Values are mean ± SEM (n=5)

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

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

3.3 Effect on magnesium sulphate-induced diarrhoea

In the magnesium sulphate-induced diarrhoeal model in rat the 80% methanol extract of ZOM at the 200 & 400 mg/kg dose levels was found to reduce the severity of diarrhoea in

test animals and the results were statistically significant (Table 3). Both the doses were able to reduce the total number of faeces and wet faeces as compared to the control.

Table 3: Effect of *Z. oenoplia* on Magnesium sulphate induced diarrhea in rats

Treatment	Dose (mg/kg)	Total no of faeces in 4 hr	Total no of wet faeces in 4 hr	Reduction (%)
Control (1%, 10ml/kg CMC) + MgSO ₄	--	12.64±0.42	8.72±0.37	--
Loperamide + MgSO ₄	3	2.16±0.36**	0.4±0.41*	95.41
ZOM + MgSO ₄	200	6.47±0.36*	4.2±0.32**	51.83
ZOM + MgSO ₄	400	5.6±0.24*	3.2±0.36**	63.30

Values are mean ± SEM (n=5)

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

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

3.4 Effect on castor oil-induced fluid accumulation

In the assessment of castor oil induced fluid accumulation there was a dose dependent decrease in intestinal fluid

accumulation. The ZOM extract of 200 & 400 mg/kg reduced the intestinal fluid accumulation relative to control (Table 4).

Table 4: Effect of *Z. oenoplia* on Castor oil induced fluid accumulation in rats

Treatment	Dose (mg/kg)	Fluid accumulation (ml)	Weight of accumulated fluid (g)	(%) inhibition
Control (1%, 10ml/kg CMC)	--	0.95±0.01	0.98±0.04	--
Castor oil (2ml)	--	2.94±0.12	3.22±.17	00
ZOM + Castor oil	200	2.12±0.09**	2.24±.22*	27.89
ZOM + Castor oil	400	1.54±0.04*	1.72±0.06**	47.62

Values are mean ± SEM (n=5)

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

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

4. Discussion

It is well known that the traditional uses of plants and their effects are due to the presence of secondary metabolites. These metabolites may be alkaloids, glycosides, flavonoids, tannins, triterpenes etc. The medicinal value of plants depends on the presence of these metabolites qualitatively & quantitatively. Number of factors, such as infective, immunological and nutritional has been involved in the perpetuation of the diarrhoeal syndrome [13]. Many plants conveniently available in India are used in traditional folk medicine for the treatment of diarrhoea and dysentery [22], of the indigenous plants used, *Andrographis paniculata*, *Asparagus racemosus*, *Butea monosperma*, *Cassia auriculata*, and others are mentioned [23]. Several studies have shown that prior administration with some plant extracts had a protective effect on the intestinal tract [24-26]. In the present study, the newer plant has been used by tribals and rural have not been studied so far, was evaluated for its anti-diarrhoeal potential against castor oil induced diarrhoea, in Wistar albino rats.

It is widely known that castor oil or its active component ricinoleic acid induces permeability changes in mucosal fluid and electrolyte transport that results in a hypersecretory response and diarrhoea. Ricinoleic acid markedly increased the PGE₂ content in the gut lumen and also caused an increase in the net secretion of the water and electrolytes into the small intestine. The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the

intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion [27, 28]. The mechanism involved has been associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport (decreasing Na⁺ and K⁺ absorption) across the intestinal mucosa [28]. These conditions tend to suggest that the extracts of *Z. oenoplia* reduced diarrhoea by increasing reabsorption of electrolytes and water or by inhibiting induced intestinal accumulation of fluid just as loperamide. Loperamide acts by decreasing the transit velocity and increasing the capacity of the intestines to retain their fluids [29]. So the dose of 200 and 400 mg/kg reduced diarrhoea by inhibiting castor oil induced intestinal accumulation of fluid.

On the other hand, magnesium sulphate has been reported to induce diarrhoea by increasing the volume of intestinal content through prevention of reabsorption of water. It has also been demonstrated that it promotes the liberation of cholecystokinin from the duodenal mucosa, which increases the secretion and motility of small intestine and thereby prevents the reabsorption of sodium chloride and water [30, 31]. The methanol extract was found to alleviate the diarrhoeic condition in this model. The ZOM extract may have increased the absorption of water and electrolyte from the gastrointestinal tract, since it delayed the gastrointestinal transit in rat as compared to the control.

Previous reports have demonstrated the anti-diarrhoeal activity of alkaloids [32], tannin [33], flavonoids [30], saponins, reducing

sugars and sterols and/or terpenes [34] containing plant extracts. The phytochemical analysis of the extract showed the presence of alkaloids, saponins, sterols/or terpenes and sugars. Therefore, these constituents might be responsible for the antidiarrhoeal activity of *Z. oenoplia* methanol extract. The results of the present study justify the traditional claims of *Z. oenoplia* extract being an antidiarrhoeal drug. Moreover, the active constituents responsible for the antidiarrhoeal activity remain to be identified.

5. Conclusion

The 80% methanol extract of selected plant materials showed antidiarrhoeal activity in primarily evaluation of diarrhetic conditions in test animals. The obtained results thus give the experimental basis to understand the use of selected traditional medicine, as an antidiarrhoeal agent. However, further bioassay guided phytochemical and pharmacological studies are required to identify the active principle(s) and exact mechanism(s) of action.

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