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RESEARCH ARTICLE

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ANTI DIARRHOEAL ACTIVITY OF *ZIZIPHUS JUJUBA* LEAF EXTRACT IN RATS

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ABSTRACT

The plant *Ziziphus jujuba*(Rhamnaceae), is a small ever green and possesses a number of medicinal properties. The purpose of the present study was to evaluate the anti diarrhoeal activity of the aqueous extract of the leaves of *Ziziphus jujuba*, by using castor oil and MgSo4 (Magnesium sulphate) induced diarrhea model. The aqueous extract of leaves of this plant at graded doses was investigated for anti -diarrhoeal activity in terms of reduction in the rate of defaecation and consistency of faeces in Castor oil, Mgso4 induced diarrhea. Effect was further evaluated on gastrointestinal transit time with charcoal meal. The leaves extract showed significant inhibitory activity against castor oil and Mgso4 induced diarrhea. There was significant reduction in gastro intestinal motility by the charcoal meal test in rats. The results obtained by this study substantiate the anti diarrhoeal effects of the aqueous extract.

KEY WORDS

Anti diarrhoeal activity, *Ziziphus jujuba*, Castor oil, Mgso4, Char coal meal, Loperamide.

INTRODUCTION

Diarrhea is a major health problem especially in children under the age of 5years, and up to 17% of children admitted in the pediatrics ward die of diarrhea. World wide distribution of diarrhea accounts for more than 5-8 million deaths each year in infants and children below 5 years old especially in developing countries[1]. According to WHO estimates for 1998, about 7.1 million deaths were caused by diarrhea [2]. The incidence of diarrhoeal diseases still remains high despite the efforts of many governments and international organizations to cure it. It is therefore important to identify and evaluate available natural drugs as alternatives to currently used anti-diarrhoeal drugs, which are not always readily accessible and free from adverse effects[3]. A range of medicinal plants with anti-diarrhoeal property is widely used. Traditional medicines are used by nearly 60 % of the world's population. These are not only used for primary health care in rural areas but also in developing countries. In rural India, 70% of the population is dependent on the traditional system of medicine[4].

Ziziphus jujuba, commonly called, Red date, belongs to family Rhamnaceae. This family consists of 50 genera and more than 900 species. It is found mainly in subtropical to tropical areas. The species has a wide range of morphologies from shrubs to small or medium sized trees which might be erect, semi-erect or spreading. Height can vary from 3-4 to 10-16 m or more although trees of 20m are rare. The bark has deep longitudinal furrows and it is grayish brown or reddish in color. Leaves are petiole, 1.1-5.8 mm long and stipules are mostly spines, in each pair one hooked, and one straight or both hooked, or more not developed into a spine.

It has been recognized as a useful edible fruit since mythology of Ram and Shabari in India and depicted in Ramayana. There are large numbers of traditional medicinal uses that are not necessarily based on knowledge of the constituents. The bark cures boils and is good for the treatment of dysentery and diarrhea[5]. Presence of Pectin- A in *Z. jujuba* fruit is also reported[6]. Pectin has a number of pharmaceutical properties such as binding bile acid, lowering plasma cholesterol and anti-diarrhoeal properties[7].

Alkaloids are distributed in all parts of plant. Stem bark of *Ziziphus* species contain alkaloids[8]. The alkaloids Coclaurine, Isoboldine, Norisoboldine, Asimilobine, Iusiphine and Iusirine were isolated from *Z. jujuba* leaves [9]. Different parts of *Z. jujuba* that is seeds, leaf and stem contain glycosides. Kurihara et al[10] extracted the saponin, ziziphin from the dried leaves of *Z. jujuba*.

MATERIALS AND METHODS

Collection Of Plant Material:

The plant material was collected from different areas of Mangalagiri, Guntur district, India, taxonomically identified by the botanist in Acharya Nagarjuna University and a specimen has been preserved in our laboratory for further references. The leaves were washed thoroughly to remove dirt particles present on the surface. The leaves were subjected to air-drying in shade for period of three weeks. The dried leaves were powdered in mixer- grinder and passed through a mesh. The extract was prepared by maceration procedure.

Preparation Of Extract:

A 25g of leaf powder was weighed, and macerated with 100ml sterile distilled water in a blender for 10min. The macerate was first filtered

through double layered muslin cloth and then centrifuged at 4000rpm for 30 minutes. The supernant was filtered through Whatman No.1 filter paper and heat sterilized at 100°c for 30 minutes. This served as mother extract. The extract was preserved aseptically in sterile bottles at 5°c until further use [11].

Experimental Animals

Albino rats of either sex weighing between 180-200 gm were used in this study. The cages of animals placed in a room temperature with controlled cycles of 12 hrs light and 12 hrs of darkness. The relative humidity was maintained at 44-45%. All animals were fed with standard pellet diet (Nutrivet Life Sciences, Pune, India) and water ad libitum. The study protocol was approved by the Institutional animal ethical committee. (Animal house Reg.no.798/03/C/CPCSEA-2003) of NRI Medical College, Chinakakani. The animal bed in the cages was renewing thrice a week to ensure hygienic condition and minimum discomfort to the animals.

Acute Toxicity Study

The acute toxicity study described by Miller et al [12] was employed in the determination of the maximum tolerated dose. Aqueous extract was administered orally at a dose of 62.5, 125, 250, 500, 1000, and 2000mg/kg to a group of 5 animals each. The general signs and symptoms of toxicity, intake of food and water and mortality were recorded for 48 h. The leaves extract of *Ziziphus jujuba* was found to be non – toxic up to the dose of 2000mg/kg body weight.

Castor Oil Induced Diarrhea

Twenty five (25) rats were fasted for 18hours and divided into five groups of five animals each. The plant extract (50, 100, 200 mg/kg body weight) was administered orally to groups 3, 4, and 5 respectively. The first group received normal saline (5ml/kg body weight) and served as control, while second group received the standard drug Loperamide (5mg/kg body weight). One hour later, all the animals received 1ml/rat of castor oil orally by gavage. The animals were kept in separate metabolic cages with a transparent plastic container beneath the cage to collect faeces. The severity of diarrhea was assessed each hour for 4h. The total number of faeces (both diarrhoeal and non diarrhoeal) expelled were compared with the control group. The total score of diarrhoeal faeces for the control group was considered as 100 %. The results were expressed as a percentage of inhibition of diarrhea.[13]

Magnesium Sulphate- Induced Diarrhea

A similar protocol as for castor oil- induced diarrhea was followed. Diarrhea was induced by oral administration of Magnesium sulphate at the dose of 2g/kg to the animals, 30 minutes after pretreatment with normal saline to the control group, Loperamide (5mg/kg) to the positive control group, the aqueous extract at the doses of 50, 100 and 200 mg/kg to the test groups. All the administrations were carried out through oral route [14].

Effect on Gastrointestinal Transit Time

Rats were fasted for 24hr and divided in to five groups of five rats each and each animal was given 1ml of 1% charcoal suspension orally 60 min after an oral dose of the test drug, standard and vehicle. Group I was administered 1 ml distilled water, and Group II received Loperamide 5mg/kg, Group III, IV and V received extract at the dose of 50mg/kg, 100mg/kg and 200mg/kg body weight respectively. The faecal bolus was expelled were collected. Each faecal bolus was pressed on a white sheet of paper examine the presence of char coal particle. The time for the appearance of the 1st faecal bolus with char coal particle was recorded

STATISTICAL ANALYSIS

The data generated was treated statistically using Microsoft Excel application soft ware. The data of total number of faeces were expressed as mean per group ±S.E.M (standard error of mean). The data obtained in the studies were subjected to one way of analysis of variance

(ANOVA) for determining the significant difference. The inter group significance was analyzed using Dunnet's – t test. A p value ≤ 0.05 were considered to be significant. All the values were expressed as Mean ± SEM.

RESULTS AND DISUSSION

In the castor oil-induced diarrhea experiment, the leaf extract of *Ziziphus jujuba* produced a marked anti diarrhoeal effect in the rats, as shown in the Table 1.

Table1

Effect of Aqueous Extract of Leaves of Ziziphus jujuba on Castor oil (1ml) Induced diarrhea in Rats

Groups	Treatment	No of faecal droppings in 4h	%Inhibition of defecation
I Control	Castor oil (1ml p .o)+ Normal saline (1ml/p.o)	12 ± 1.26	0
II Standard	Castor oil (1ml p .o)+ Loperamide 5mg/kg)	1.2 ± 0.37**	90
III	Castor oil (1ml/p.o)+ AEZJL 50mg/kg	9.2 ± 0.37**	23.3
IV	Castor oil (1ml/p.o)+ AEZJL 100mg/kg	7.0 ± 0.44**	41.6
V	Castor oil (1ml/p.o)+ AEZJL 200mg/kg	4.6 ± 0.24**	61.6

Values are presented as Mean ± SEM, (n=5); ** p≤0.05, Dunnet's t-test as compared to Control. AEZJL: Aqueous extract of Ziziphus jujuba leaves.

At doses of 50, 100, 200 mg/kg, the extract significantly decreased (p≤0.05) the total number of faeces produced upon administration of castor oil (23.3% at 50mg/kg, 41.6% at 100mg/kg, and 61.6% at 200mg/kg) compared to the control group. Similarly, the extract 50, 100, 200 mg/kg dose level significantly (p≤0.05) reduced the extent of diarrhea (18.36 at 50mg/kg, 36.7% at 100mg/kg and 51.02% at 200mg/kg) in test animals in Magnesium sulphate - induced diarrhea (Table2).

Table2

Effect of Aqueous Extract of Leaves of *Ziziphus jujuba* on Mgso4 Induced diarrhea in Rats

Groups	Treatment	No of faecal droppings in 4h	%Inhibition of defecation
I Control	Mgso4 (2g/kg p .o)+ Normal saline (1ml/p.o)	9.8 ± 0.37	0
II Standard	Mgso4 (2g/kg p .o)+ Loperamide 5mg/kg)	0.4 ± 0.24**	95.91
III	Mgso4 (2g/kg p .o)+ AEZJL 50mg/kg	8.0 ± 0.31**	18.36
IV	Mgso4 (2g/kg p .o)+ AEZJL 100mg/kg	6.2 ± 0.37**	36.7
V	Mgso4 (2g/kg p .o)+ AEZJL 200mg/kg	4.8 ± 0.37**	51.02

Values are presented as Mean ± SEM, (n=5); ** p≤0.05, Dunnet's t-test as compared to Control. AEZJL: Aqueous extract of Ziziphus jujuba leaves

However, the doses were shown to reduce the total number of faeces when compared to control. In the gastro intestinal transit test, the extract at the doses of 50, 100 and 200 mg/kg retarded the gastro intestinal transit of charcoal meal in rats where a significant ($p \leq 0.05$) retardation of intestinal transit was observed at the doses of 50, 100 and 200 mg/kg dose when compared to control (Table3).

Table 3
Effect of Aqueous extract of leaves of *Ziziphus jujuba* on Char coal particle stimulated gastrointestinal transit time.

Groups	Treatment	Time(minutes) for the appearance of 1st faecal bolus with Charcoal meal
I Control	Distilled water(1ml)	47.0 ± 2.54
II Standard	Loperamide (5mg/kg)	147.8 ± 2.51**
III	AEZJL 50mg/kg	54.4 ± 1.69
IV	AEZJL 100mg/kg	86.0± 4.72**
V	AEZJL 200mg/kg	110.8± 5.46**

Values are presented as mean ± SEM, (n=5); ** p≤0.05, Dunnet's t-test as compared to Control. AEPGL: Aqueous extract of Ziziphus jujuba leaves

Diarrhea results from an imbalance between the absorptive and secretary mechanisms in the intestinal tract accompanied by an excess loss of fluid in faeces. In some diarrhea the secretary component predominates while other diarrhea is characterized by hyper motility. Castor oil causes diarrhea due to its active metabolite, ricinolic acid [15, 16], which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action stimulates the release of endogenous prostaglandins [17]. In this study aqueous extract of leaves of *Ziziphus jujuba* exhibited a significant anti-diarrhoeal activity. Aqueous extract of *Ziziphus jujuba* significantly reduced intestinal transit as observed by the decrease in the intestinal motility of char coal meal. Earlier studies showed that anti dysenteric and anti-diarrhoeal properties of medicinal plants were due to presence of tannins, alkaloids, saponins, flavonoids, sterol and/or tri terpenes and reducing sugars [18-20]. Hence tannins, reducing sugars, sterol and/or tri terpenes may be responsible for the mechanism of action of anti-diarrhoeal activity. This can be due to the fact that the extract increased the reabsorption of water by decreasing intestinal motility as observed in the decrease in the intestinal transit by char coal meal.

CONCLUSION

The results of this investigation revealed that aqueous extract contains pharmacologically active substances with anti-diarrhoeal properties. Further research is to be carried out to fractionate and purify the extract, in order to find out the molecule responsible for the anti-diarrhoeal activity observed.

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