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Research Article

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Antidiarrheal Effects of Aqueous Leave Extract of *Ziziphus mauritiana* in Wistar Strain Albino Rats

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Abstract In the antidiarrheal activity evaluation, the aqueous leave extract of *Ziziphus mauritiana* was found to be effective against castor oil – induced diarrhea in Wistar strain albino rats at the doses of 200, 400 and 800 mg/kg body weight, the fecal droppings decreased and percentage inhibition was 51.05 %, 54.48 % and 58.10 % respectively which was dose – dependent and statiscally significant at ($p < 0.05$). The control group showed typical diarrheal signs with 0.00 % inhibition while the group given the standard antidiarrheal drug loperamide 5 mg/kg body weight was protected 100 %. In the intestinal transit of charcoal meal method, the percentage inhibition of the extract treated rats were 19.07 %, 06.00 % and 18.94%, the atropine sulphate (3 ml/kg) body weight treated group was inhibited by 41.96 % and the inhibition was not dose dependant. In the intestinal fluid accumulation method, the extract inhibited the intestinal fluid accumulation in rats by 28.04 %, 43.93 % and 54.21 % relative to the control group with 0.00% inhibition. The group given atropine sulphate had 85.05 % inhibition. The results of this study suggest that the aqueous leaves of *Ziziphus mauritiana* has antidiarrheal effect, this might have justified its traditional use in the treatment of diarrhea.

Keywords Albino Rats, Castor oil, Diarrhea, Loperamide and Atropine

Introduction

Folk medicinal practices are very common in Nigeria. Besides, herbal medicine practice is also increasing day by day due to fewer side effects [1]. The use and identification of medicinal plants have been in existence throughout human history. Plants have the ability to synthesis a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators such as insects, fungi and herbivorous mammals[2]. At least 12,000 such compounds have been isolated so far. A number estimated to be less than 10% of the total chemical compounds present in plants [2] carry out their functions on humans through processes identical to those already well understood (i.e. chemical compounds in conventional drugs). Herbal medicine does not differ greatly from conventional drugs in terms of how they work [3].

Alok Nahata of Harisingh Gour University defined medicinal plant as any plant which provides health promoting characteristics, temporary relief from symptomatic problems or has curative properties. In 2001, researchers identified 122 compounds used in modern medicine which were derived from "ethnomedical" plant sources [4]. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies including aspirin, digitalis, quinine and opium [5]. The use of herbs in the treatment of diseases is very common among non-industrialized societies and often more affordable than purchasing expensive modern pharmaceuticals. The World Health Organization (WHO) estimates that 80% of the populations of some Asian and African countries presently use herbal medicine for some aspects of primary health care. Studies in the United States and Europe have shown that their use is less common in clinical settings, but has become increasingly more in recent years as scientific evidence about the effectiveness of herbal medicine has become more widely available [5]. *Ziziphus mauritiana*, also known as Ber, chine Apple, Jujube, Indian plum and masau is a tropical fruit tree species belonging to the family *Rhamnaceae.* It is also called "kusulu" in by the Kanuri people, "Jabe" by the Fulani and "Magarya" by the Hausa People. *Ziziphus mauritiana* which is believed to have originated from Indo- Malaysian region of

South – East, Asia is grown in various parts of the world which includes Nigeria [6]. *Ziziphus mauritiana* is a spiny ever green shrub or small tree up to 15m high with trunk 40cm or more in diameter; spreading crown; stripular spines and many dropping branches. The fruit varies in shape and size. It can be oval, obviate, oblong or round and that can be 1.0-2.5cm long depending on the variety. The fruit is white and crispy with a pleasant aroma [1]. The plant is highly medicinal. The leaves are helpful in liver troubles, asthma, fever and diarrhea [7]. The leaves are applied as poultice and together with catechu are administered when an astringent is needed as on wounds. The bitter bark decoction is taken to halt diarrhea, dysentery and relieve gingivitis. The root is purgative. Juice of the bark is said to alleviate gout and rheumatism. The dried ripe fruit is a mild laxative. The seed are sedative, they cure diarrhea and are poultice on wounds. Diarrhea is described as loose of watery stools that occur more frequently than usual. It could also be defined as the deviation from normal bowel movement *i.e.* greater looseness of stool. The word "diarrhea" originated from the greek word *diarrhoia. Dia* means "through" and *rheo* means "flow". The term "flowing through" was coined by Hippocrates [8]. Diarrhea is the opposite of constipation and can have many causes which may be infectious or non-infectious. In many cases, diarrhea usually last for few days which is usually termed as acute diarrhea. However, in some cases diarrhea may last for weeks. In this situation, the diarrhea is said to be chronic. Generally there are five types of diarrhea which are the motility diarrhea, secretary diarrhea, osmotic diarrhea, inflammatory diarrhea and dysentery. Diarrhea could be caused by bacteria *Vibrio cholera.* It could also be caused by undigested lactose, too much magnesium, vitamin C, it may also be caused as a result of celiac disease or laxatives [9]. Symptoms of diarrhea may include frequent passage of watery stool, stomach ache, abdominal pain, Nausea (fever), head ache, loss of appetite etc. it could be managed/ treated with medications such as loperamide which slows down the bowels movement and increases the guts absorption. Diarrhea could also be treated by drinking a lot of fluid which helps in replacing the fluid loss and reduce the risk of dehydration. Medicinal plants have now generated renewed interest amongst scientists of various cadres covering agriculture, Veterinary medicine, Human medicine Pharmacognosy etc. the current study was done to evaluate the anti-diarrhea property of *Ziziphus mauritiana* leaves in albino rats.

Materials and Methods

Collection of the Plant Sample and Identification

Leaves of *Ziziphus mauritiana* were collected from old GRA Maiduguri. The plant was identified and authenticated by a Botanist from the Department of Biological science, University of Maiduguri.

Preparation of Plant Material

After collection of leaves, it was washed and shade dried at room temperature for 7 days in the Biochemistry Research Laboratory University of Maiduguri. The dried leaves were made coarse by the use of a mortar and pestle. 808 grams of the dried leaves were soaked in 27 liters of distilled water for three days. The soaked leaves were refrigerated and stirred occasionally. The soaked leaves was filtered using muslin cloth. The residue was discarded and the filtrate was evaporated using a hot plate at a temperature range of 40-45 °C. The weight of the extract obtained was 193 grams and the percentage yield was 23.89 % [10].

Experimental Animals

Sixty Albino Rats weighing between 125 to 195 grams of both sexes were used. The animals were kept in the animal house of Department of Biochemistry, University of Maiduguri at room temperature for two weeks for acclimatization they had free access to clean water and food. The experiments reported here complied with ethical procedures of animal ethics [11].

Effect of Aqueous Leaf Extract of *Ziziphus mauritiana* on Castor oil Induced Diarrhea in Wistar Strain Albino Rats

A total of 20 Albino Rats weighing between 135-195 grams were used. The rats were fasted for 18 hours and separated into five groups of four animals each. Group I was treated with (2 ml/kg) body weight of normal saline orally (negative control). Groups II, III, and IV were respectively treated with 200, 400, and 800 mg/kg body weight of aqueous leaf extract of *Ziziphus mauritiana* orally. Group V (positive control) received 5 mg/kg (intraperitoneal) body weight of loperamide (diphenoxylate). After one hour, the rats were placed singly in cage laid with plain white sheet of paper and each rat was treated with 1ml of castor oil orally. The rats were observed for six (6) hours for watery (wet) or unformed faeces. The watery faeces of each rat were counted at the end of six hours [12-13].

Effect of Aqueous Leaf Extract of *Ziziphus mauritiana* on Intestinal Transit Time of Charcoal Meal in Wistar Strain Albino Rats

Twenty Albino Rats weighing between 125 to 160 grams were used. The rats were fasted for 18 hours and placed into five groups of four animals each. Group I was treated with 1ml normal saline orally (negative control). Groups II, III and IV were respectively treated with 200, 400 and 800 mg/kg body weight of Aqueous Leaf Extract of

Ziziphus mauritiana orally. Group V received 3 ml/kg (intraperitoneal) body weight of atropine sulfate. Thirty minutes after drug and extract administration, 1 ml of 5 % activated charcoal suspension in 10 % aqueous solution of acarcia gum powder was given orally to each rat. The charcoal meal was prepared by dissolving 2.5 g of acarcia gum powder and 2.5 g of activated charcoal in 25 ml of normal saline. After thirty (30) minutes, the rats were sacrificed and then the abdomen opened. The distance travelled by charcoal meal from pylorus were measured and expressed as percentage of the total length of the intestine from the pylorus to the caecum [14-15].

Effect of Aqueous Leaf Extract of *Ziziphus mauritiana* on Castor Oil Induced Enteropooling in Wistar Strain Albino Rats

Twenty (20) Albino Rats weighing between 131 to 175 g were used. The rats were fasted for 18 hours and separated into five groups of four rats each. Group I rats were treated with 2 ml/kg body weight of normal saline orally (negative control). Groups II, III, and IV were respectively treated with 200, 400 and 800 mg/kg body weight of aqueous leaf extract *Ziziphus mauritiana* orally. Group V received ml/kg (intraperitoneal) body weight of atropine sulfate. After thirty minutes (30) each rat was treated with 1ml of castor oil. One hour after castor oil treatment, the rats were sacrificed and the small intestine removed, tied on both ends with thread and weighed. The intestinal content was collected by milking and the volume measured. The intestine thereafter weighed and the differences between full and empty intestine were calculated [14, 16].

Statistical Analysis

All values were expressed as mean standard deviation, while analysis of variance (ANOVA) was used to analyze the extent of variation between groups and P values equal to or less than 0.05 were considered statistically significantly. Graphpad instat 3.0 for R windows USA computer software used to analyze the data [17].

Results

Table 1: Effect of Aqueous Leaf Extract of *Ziziphus mauritiana* on Castor Oil – Induced Diarrhea in Wistar Strain Albino Rats

Treatment Dose	Fecal Droppings	% Inhibition
Normal Saline (control) 2ml/kg	19.25± 2.06	-
Extract 200mg/kg	9.25 ± 1.26***	51.05
Extract 400mg/kg	8.75 ± 1.26***	54.48
Extract 800mg/kg	7.75 ± 0.50***	58.10
Control drug (loperamide) 5ml/kg	0.00 ± 0.00***	100.00

Mean ± SD, n=4 ***P<0.001 highly significant compared with the control

The effect of aqueous leaf extract of *Ziziphus mauritiana* on castor oil induced diarrhea was presented on table 1. The result showed that at 200, 400 and 800 mg/kg, the fecal droppings decreased and the percentage inhibition was 51.05 %, 54.48 % and 58.10 % respectively. The effect of aqueous extract was dose dependent. The control drug loperamide (diphydroxylate) inhibited the castor oil induced diarrhea by 100 %.

Table 2: Effect of Aqueous Leaf Extract of *Ziziphus mauritiana* on Castor Oil Induced Enteropooling in Wistar Strain Albino Rats

Treatment Dose	Weight of full Intestine (g)	Weight of Empty Intestine (g)	Volume of Intestinal content (ml)	% Inhibition
Normal Saline 2 ml/kg	7.2 ± 1.39	4.33± 0.72	2.53± 0.53	-
Extract 200mg/kg	6.25 ± 0.66	4.18± 0.26	1.90 ± 0.47	28.04
Extract 400mg/kg	10.98±1.50***	6.93± 1.29**	3.98± 0.42***	43.93
Extract 800mg/kg	4.83 ± 1.12*	3.65± 0.30	1.23± 0.67**	54.21
Control drug Atropine 3ml/kg	4.00±0.37**	3.40± 2.94	0.40± 0.16***	85.05

Mean ± Standard deviation, n=4

* P < 0.05, slightly significant compared with the control

** P < 0.01 moderately significant compared with the control

*** P < 0.001 highly significant compared with the control

There was a statistically significant difference (P<0.05) in the weight of full intestinal contents of rats in the groups treated with various Doses and that of the control group. However, there was a significant difference (P<0.05) in the

weight of intestinal contents of rats treated with 400 and 800mg/kg of extract compared to that of the control group (Table 2). There was also a significant (P< 0.05) marked inhibition of the intestinal fluid volume in the rats treated with 400 and 800mg/kg. There percentage inhibition at 200, 400 and 800mg/kg was 28.04, 43.93 and 54.21% respectively. While in the atropine sulfate treated (positive control drug), there was marked inhibition of 85 % intestinal fluid volume at 3 ml/kg.

Table 3: Effect of Aqueous Leaf Extract of *Ziziphus mauritiana* on Gastrointestinal Transit of Charcoal Meal in Wistar Strain Albino Rats

Treatment Dose	Distance Travelled by Charcoal Meal (cm)	Total Length of Intestine (cm)	% Inhibition
Normal Saline 2ml/kg	91.88± 6.42	106.50± 7.14	-
Extract 200mg/kg	74.25± 6.50**	106.38+- 9.54	19.07
Extract 400mg/kg	86.25± 8.02	112.13± 8.23	6.00
Extract 800mg/kg	74.38± 1.49**	106.25± 3.66	18.94
Control Drug Atropine 3ml/kg	53.25± 8.66***	109.13 ± 2.66	41.96

Mean ± standard deviation, n=4

**P<0.01 moderately significant compared with the control

***P<0.001 highly significant compared with the control.

The distance travelled by charcoal meal in Group I (positive control) rats was longer compared to those treated with various Doses (200, 400 and 800mg/kg) of the extract. The charcoal travelled more rapidly along the small intestine in the control group than those treated. The rate was markedly reduced by 19.07%, 6.00% and 18.94%. While the atropine sulfate 3ml/kg by 41.96% (Table 3).

Discussion, Conclusion and Recommendation

Discussion

In this study, the evaluation of the aqueous leaf extract of antidiarrheal effect of *Ziziphus mauritiana* comprised evaluation of its effects on castor oil-induced diarrhea, its effects on gastrointestinal transit of charcoal meal and castor oil-induced enteropooling was also investigated with reference to actions of drugs like atropine sulfate in reducing gastrointestinal transit and fluid accumulation and also drugs like loperamide which reduces secretory diarrhea.

Castor oil, a very effective laxative is hydrolyzed in the upper small intestine to ricinoleic acid, which can stimulate fluid secretion, inhibit water and electrolyte absorption, reduce active sodium and potassium absorption and decrease Na, K-ATPase in the small intestine and colon. Castor oil also increases the peristaltic activity and produces permeability changes in the intestinal mucosa membrane to electrolytes and water. Furthermore, ricinoleic acid can also lead to the release of endogenous prostaglandins, which play an important role in the modulation of GIT, stimulate motility, secretion and cause diarrhea [18]. In this study, the results showed that aqueous extract of *Ziziphus mauritiana* leaf could in a dose – dependent manner, reduce castor oil-induced diarrhea as well as the number of diarrheal feces and total weight of feces, which could be taken as antidiarrheal activities. Loperamide is one of the most efficacious and widely used antidiarrheal drugs. Loperamide effectively antagonized the diarrhea induced by castor oil. The therapeutic effect of loperamide is believed to be due to its antimotility and antisecretory activity, atropine produced a significant reduction in both the intestinal fluid accumulation and transit time because it is a competitive antagonist of acetylcholine. It acts by blocking the muscarinic receptors of the acetylcholine causing muscle relaxation thereby treating diarrhea [19].

Loperamide (a standard antidiarrheal drug) is a synthetic opiate analogue developed specifically for use in diarrhea. All opiate agonists have effects on intestinal smooth muscle. Loperamide regulates the gastrointestinal tract by inhibiting the propulsive motor activities, predominantly in the jejunum and this effect is partially inhibited by opiate antagonists. Other effects on intestinal motility may be mediated through inhibition of prostaglandin stimulation of gut motility and/or through calcium antagonist action [20].

Antidiarrheal properties of medicinal plants were found to be due to tannins, flavonoids, alkaloids, saponins, reducing sugars, sterol, terpenes and glycosides. Hence, tannins, reducing sugars, flavonoids and saponins may be responsible for mechanism of antidiarrheal activity of Aqueous Leaf Extract of *Ziziphus mauritiana.* These provide a scientific basis for the potential use of *Ziziphus mauritiana* leaf in gastrointestinal disorders such as diarrhea [21].

The Aqueous Leaf Extract of *Ziziphus mauritiana* exhibited significant antidiarrheal activity on gastrointestinal transit of charcoal meal in rats. Hyper motility characterizes forms of diarrhea where the secretory components are not the causative factor. The aqueous leaf extract of *Ziziphus mauritiana* suppressed the propulsive movement of

gastrointestinal transit of charcoal meal which clearly indicates that extract may be capable of reducing the frequency of stools in diarrheal conditions [20].

Aqueous leaf extract of *Ziziphus mauritiana* was found to possess an anti enteropooling in castor oil-induced diarrhea in albino rats by reducing both weight and volume of intestinal content. These effects are direct consequences of reduced water and electrolytes secretion in small intestine, suggesting that extract may enhance water and electrolyte absorption from intestinal lumen [18].

Conclusion

At a dose of 200,400 and 800 mg/kg body weight the fecal dropping was decreased and the % inhibition was 51.05 %, 54.48 % and 58.10 % respectively. The control drug loperamide inhibited the Castor Oil Induced Diarrhea by 100 % at 5 mg/kg body weight. In castor oil induced enteropooling, the percentage inhibition at 200, 400, and 800 mg/kg was 28.04, 43.93 and 54.21 respectively. While the control drug atropine sulphate, there was marked inhibition of 85.05 % intestinal volume at 3 ml/kg. The distance travelled by charcoal meal was markedly reduced by 19.07 %, 6.00 % and 18.94 % at a dose of 200, 400, and 800 mg/kg body weight. While the atropine sulphate revealed 41.9 6 % inhibition at a dose of 3 ml/kg.

In conclusion, the result obtained in this study suggests that the aqueous leaf extract of *Ziziphus mauritiana* has antidiarrheal activity, this may justify its traditional use in the treatment of diarrhea.

Recommendation

Further studies are needed to identify the exact mechanisms and chemical component(s) that are responsible for the antidiarrheal effect.

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