

Full Length Research Paper

# Antidiarrhoeal activity of *Ziziphus mauritiana* root extract in rodents

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**Preliminary phytochemical screening of the root extract of *Ziziphus mauritiana* revealed the presence of alkaloids, flavonoids, glycosides, saponins and volatile oil. Intraperitoneal LD<sub>50</sub> of the extract was found to be 447.21 ± 20 mg/kg (bw) in mice. The Antidiarrhoeal effect of the methanolic extract as evaluated exhibited a concentration dependent inhibition of the spontaneous pendular movement of the isolated rabbit jejunum and inhibited acetylcholine induced contraction of rat ileum. A dose dependent decrease of gastrointestinal transit was observed with extracts (25 and 50 mg/kg) which also protected mice against castor oil induced diarrhoea and castor oil induced fluid accumulation, respectively. The presence of some of the phytochemicals in the root extract may be responsible for the observed effects, and also the basis for its use in traditional medicine as antidiarrhoeal drug.**

**Key words:** *Ziziphus mauritiana*, antidiarrhoeal activity, castor oil, rodents.

## INTRODUCTION

Medicinal plants play a key role in the human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant material (WHO, 1993). Scientific studies available on a good number of medicinal plants indicate that promising phytochemicals can be developed for many health problems (Gupta, 1994). Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. These drugs are invariably single plant extracts or fractions thereof or mixtures of fractions/extracts from different plants, which have been carefully standardized for their safety and efficacy (Subramoniam and Pushpangadan, 1999). The use of herbal drugs in the treatment of diarrhoea is a common practice in Africa (Abdullahi et al., 2001).

*Ziziphus mauritiana* Lam belongs to the family *Rhamnaceae*. It is widely grown in mild-temperate, rather dry areas, of both hemispheres and is adapted to warm climates. It is often called merely jujube, Chinese date, Indian plump (Morton, 1987). In northern Nigeria it is called magarya (Hausa) or huya (Kilba). The plant finds

various uses in traditional medicine for instance; the fruits are applied on cuts and ulcers; are employed in pulmonary ailments and fevers; the dried ripe fruit is a mild laxative. The seeds are sedative and are taken sometimes with butter, to halt nausea, vomiting and abdominal pains in pregnancy. Mixed with oil, they are rubbed on rheumatic areas. The leaves are helpful in liver trouble, asthma and fever. The bitter, astringent bark decoction is taken to halt diarrhoea and dysentery and relieve gingivitis. A root decoction is given as a febrifuge, taenicide and emmenagogue, and the powdered root is dusted on wounds. Juice of the root bark is said to alleviate gout and rheumatism (Morton, 1987). The root is also used in the treatment of epilepsy (Msonthi et al., 1983). The dried root is also used to treat diarrhoea in Northern Parts of Nigeria (Mallam Muazzam, Herbarium Department of Medicinal Plant Research and Traditional Medicine (MPRT) NIPRD, Abuja, Nigeria, personal communication).

A search in NIPRALERT database did not yield any information on the pharmacological effect of the methanolic root extract of the plant on castor oil induced diarrhoea, small intestinal propulsion, castor oil induced fluid accumulation, and its effect on gastrointestinal smooth muscle. This research work reports the effect of the methanol extract of the root of *Z. mauritiana* on castor

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oil-induced diarrhoea. Diarrhoea is one of the principal causes of death, especially in the infant population (Zavala et al., 1998). Where diarrhoea is not life threatening, it causes much discomfort affecting the quality of life (Saito et al., 2002). In developing countries, one out of five children dies of diarrhoea before the age of five. Each year more than 5 million people die of diarrhea, 80% of who are children less than one year of age (Nester et al., 1998).

## MATERIALS AND METHODS

### Animals

Adult male wistar rats weighing 150-180 g, albino mice weighing 18-22 g and rabbits weighing 2.3-2.5 kg were used for the studies. The animals were purchased from the National Veterinary Research Institute, Jos, Nigeria and National Institute for Pharmaceutical Research Development, Abuja, Nigeria. The animals were housed in stainless steel cages at room temperature  $28 \pm 2^\circ\text{C}$  and 12/12 h light dark cycle. All animals were fed with standard pellet diet (Vital Feed Ltd. Jos) and water *ad libitum*. Animals were treated in line with the guide and care for laboratory animals.

### Plant material

Mallam Muazzam, Head of the Herbarium Department of Medicinal Plant Research and Traditional Medicine (MPRT) NIPRD, Abuja, collected the plant root from the Sahel region of Kaduna, Nigeria around April 2003.

### Preparation of plant extract

The root of *Z. mauritiana* was washed with clean water, air-dried, pulverized using pestle and mortar and sieved with 0.3 mm sieve (Endecott Ltd. London). One hundred grams of the coarsely powdered root was extracted with 500 ml of methanol using soxhlet apparatus for 12 h. Methanol was evaporated using rotary evaporator at less than  $40^\circ\text{C}$ . Distilled water was used to reconstitute the solid extract to obtain a desired concentration for the studies.

### Phytochemical screening

Preliminary phytochemical screening for the presence of alkaloids, flavonoids, tannins, glycosides resins, phenols, volatile oils and saponins were carried out using standard test procedures (Trease and Evans, 1996).

### Acute toxicity studies

The acute toxicity studies were carried out based on the method described by Lork (1983). Sterilized extract was administered intraperitoneally to mice, and the dose that killed 50% of the animal population was estimated as the  $\text{LD}_{50}$  (Adzu et al., 2003).

### Effect of extract on intestinal propulsion

The effect of *Z. mauritiana* on intestinal propulsion in rats was tested using the charcoal meal method (Capaso et al., 1976). The rats were fasted for 24 h but allowed free access to water. They were randomized and placed in 3 plastic cages of 4 animals per

cage corresponding to 3 groups of animals. Group 1 was administered distilled water orally by orogastric cannula. Groups 2 and 3 were pretreated with 25 and 50 mg/kg extract of *Z. mauritiana*, respectively. After 30 min each rat was administered 1 ml of 5% activated charcoal suspended in 10% aqueous tragacanth orally. The rats were sacrificed 30 min later by inhalation of chloroform and the abdomen was exposed after dissection; the distance traveled by the charcoal plug from the pylorus to the caecum was then measured (Mukherjee et al., 1998).

### Effect of extract on castor oil induced diarrhoea

The method of Awouter et al. (1978) was adopted in assaying for the effect of the *Z. mauritiana* extract on castor oil induced diarrhoea. Mice were weighed and grouped into 4 groups ( $n=4$ ). Group 1 received distilled water, group 2 and 3 were administered 25 and 50 mg/kg extract orally while group 4 received diphenoxylate (5 mg/kg) orally. Each animal was then given 0.5 ml of castor oil orally after 30 min of treatment and placed in transparent cages to observe for consistency of faecal matter and frequency of defecation for 4 h. Faeces were collected with an absorbent sheet of paper placed beneath the transparent cages (Mukherjee et al., 1998). The wet faeces were read at the end of the experiment by lifting up the upper part of the cage containing the sheet of paper and animals.

### Effect of extract on castor oil induced intestinal fluid accumulation

This was determined as described and modified by Robert et al. (1976) and Dicarlo et al. (1994). Briefly, the rats were fasted for 24 h but allowed free access to water. The rats were randomized and placed in 3 cages of 4 rats per cage each. Group 1 was administered distilled water, while groups 2 and 3 were pre-treated with 25 and 50 mg/kg of the extract, respectively. 30 min later, each rat was administered 2 ml castor oil. The rats were anaesthetized 30 min later by inhalation of chloroform. The small intestine from the pylorus to caecum was dissected out and its content expelled into a measuring cylinder to measure the volume of the fluid (Adzu et al., 2003).

### The effect of extract on isolated rabbit jejunum

Each of the adult rabbits was starved of feed for about 18 h and, sacrificed by a blow on the head. It will be exsanguinated and the abdomen cut open. About 2 cm piece free of mesentery of the ileum was mounted in 20 ml organ bath containing aerated Tyrode solution at  $37^\circ\text{C}$ . A tension of 0.5 g was applied, connected to Ugo-Basil Unirecorder microdynamometer, sensitivity of 1.0 and the speed of 20 mm/min. The effect of 10 min incubation of extract on the tissue responses to acetylcholine was tested (Schlemper et al., 1996).

### Effect of extract on isolated rat ileum

Rats were starved of feed for 18 h but allowed access to water. The rats were exsanguinated and the stomach dissected to expose the abdomen. About 2 cm of the rat ileum was removed, dissected free of adhering mesentery and mounted in 20 ml organ bath containing aerated Tyrode Solution. The effect of acetylcholine, histamine, at a different concentration of *Z. mauritiana* root extract was tested after 10 min of incubation at  $30^\circ\text{C}$ .

**Table 1.** Phytochemical screening of *Ziziphus mauritiana* root extract.

Test	Alkaloids	Flavonoids	Glycosides	Phenols	Resins	Saponins	Tannins	Volatile oil
Inference	+	+	+	-	-	+	-	+

+ present, - absent.

**Table 2.** Effect of methanolic root extract of *Ziziphus mauritiana* on gastrointestinal transit in rats.

Treatment	Dose(mg/kg)	Distance traveled by charcoal (cm)	% Inhibition
Control (saline)	10	68.16 ± 2.07	-
Extract	25	51.67 ± 5.29*	24.19
Extract	50	45.86 ± 1.75*	32.72
Yohimbine	3	28.72 ± 2.12*	57.87

Results are mean ± SEM, n = 4

\*Significant relative to control (p<0.05)

**Table 3.** Effect of *Ziziphus mauritiana* extract on castor oil induced diarrhoea in mice.

Treatment	Dose (mg/kg)	No. of watery diarrhoea	% Inhibition
Control (castor oil)	2	34 ± 3.27	-
Extract	25	21 ± 2.31*	38.23
Extract	50	19 ± 1.60*	44.11
Diphenoxylate	5.0	10 ± 1.21*	70.59

Results are mean ± SEM, n = 4;

\*Significant relative to control (p<0.05)

**Table 4.** Effect of *Ziziphus mauritiana* root extract on castor oil induced intestinal fluid accumulation.

Treatment	Dose (mg/kg)	Fluid volume (ml)	% inhibition
Control(castor oil)	2	2.23 ± 0.43	-
Extract	25	1.67 ± 0.16*	25.11
Extract	50	1.33 ± 0.6*	40.35
Diphenoxylate	2.5	0.51 ± 0.12	77.13

Results are mean ± SEM, n = 4

\*Significance relative to control (p<0.05)

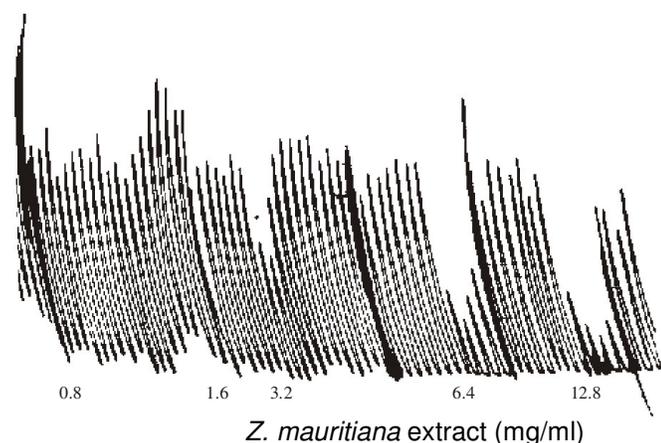
### Statistical analysis

Results are presented as means ± S.E.M and simple percentages. The student 't' test was used to determine the significant difference between two groups (p<0.05).

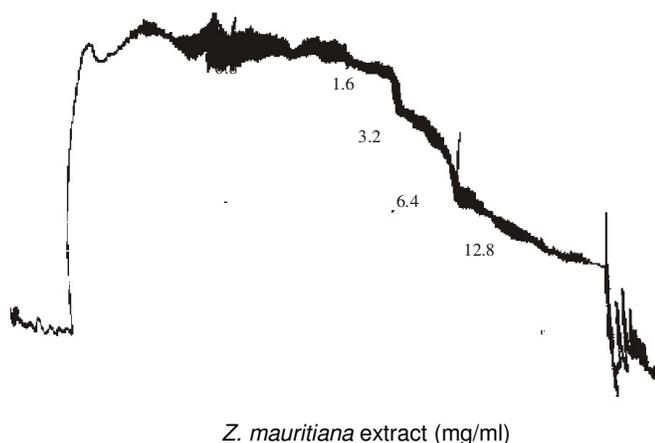
## RESULTS

The extract represents 22.26% plant material. The result of the phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, saponins and volatile oil (Table 1). The LD<sub>50</sub> of the extract was estimated to be 447.21 mg/kg (ip) in mice. The effect of the methanolic extract of the root of *Z. mauritiana* on intestinal propulsion (Table 2) revealed that 25 mg/kg of the extract decrease the intestinal propulsion by 24.19% while 50 mg/kg of the

extract caused a decreased of 32.72% of intestinal propulsion. The effect of the extract on castor oil induced diarrhoea in mice (Table 3) showed a dose dependent decrease in the number of faecal matter passed by the animals. At 25 and 50 mg/kg extract, a significant (p<0.05) reduction in diarrhoea was observed representing 38.23 and 44.11% inhibition, respectively. Diphenoxylate inhibited the castor oil induced diarrhoea by 70.59%. Studies on intestinal fluid accumulation (Table 4) revealed that both 25 and 50 mg/kg of the extract significantly inhibited fluid accumulation at the levels of 25.11 and 40.35% respectively. The methanolic extract of the root of *Z. mauritiana* exhibited no effect on the isolated rabbit jejunum and rat ileum at the lower doses used (0.1- 0.8 mg/ml). However, at higher doses (1.6 – 12.8 mg/ml) a dose dependent relaxation of the tis-



**Figure 1.** Inhibitory effect of *Ziziphus mauritiana* root extract on histamine-induced spontaneous contraction of the rabbit jejunum.



**Figure 2.** Effect of *Ziziphus mauritiana* root extract on acetylcholine-induced contraction of rat ileum.

sue was observed. The contraction caused by histamine (0.8 mg/ml) and acetylcholine (0.4 mg/ml) on the tissues was reversibly inhibited by 3.2 mg/ml of the extract (Figures 1 and 2, respectively).

## DISCUSSION

Phytochemical screening of the methanol root extract of *Z. mauritiana* reveals the presence of alkaloids, glycosides, saponins and volatile oils. The LD<sub>50</sub> value of the methanol extract of *Z. mauritiana* root was found to be 447.21 mg/kg (ip) in mice. Above this dose, the animals exhibited tonic signs, which indicated that the extract might not be relatively safe. Values greater than 1 g had been reported to be generally safe for all practical purposes in the laboratory (Lork, 1983). Castor oil is

made up of 90% ricinoleate (Mekeon et al., 1999) which when metabolized is responsible for the observed effects of the oil. The active metabolite ricinoleic acid is responsible for its diarrhoea inducing property, which diminishes Na<sup>+</sup> and Cl<sup>-</sup> permeability in the intestine (Gaginella and Philips, 1975); it is also associated with endogenous stimulation of prostaglandins release (Zavala et al., 1998). Methanol extract of the root of *Z. mauritiana* was found to inhibit the severity of diarrhoea induced by castor oil. It is possible that the extract was able to inhibit electrolyte permeability in the intestine due to castor oil and or through the inhibition of prostaglandins release (Adzu et al., 2003). Suppression of the intestinal fluid accumulation by the extract might also suggest inhibition of gastrointestinal function (Nwafor et al., 2000). Inhibitions of gastrointestinal transit by yohimbine suggest that the effect was mediated by α<sub>2</sub>-adrenoceptor activation. The above speculation was further supported by the inhibitory action of the extract on intestinal charcoal meal motility. Agents with such property have been reported as possessing antidiarrhoeal activity (Dicarlo et al., 1994).

The inhibition of acetylcholine and histamine by higher doses of the extract (1.6 – 12.8 mg/ml) on rat ileum and rabbit jejunum respectively, suggest that the extract probably contain substances that act through cholinergic and histaminergic mechanisms. Some of the secondary metabolites present in the root extract have been implicated as having antidiarrhoeal activity. The inhibitory activity of flavonoids on intestinal motility in a dose related manner was earlier reported (Dicarlo et al., 1994 and Meli et al., 1990). Saponins have also been reported to inhibit histamine release *in vitro* (Rao and Gurfinkel, 2000). We suggest that saponins and or flavonoids present in the root extract might be responsible for its antidiarrhoeal activity.

From this study, the use of the root of *Z. mauritiana* in traditional medicine as a non-specific antidiarrhoeal agent has been justified. Further studies is, however, needed to establish the safety of the extract and to possibly isolate the active principle responsible for the observed effects. We are currently looking into that aspect.

## REFERENCES

- Abdullahi AI, Agho MO, Amos S, Gamaniel KS, Wambebe C (2001). Antidiarrhoeal Activity of the Aqueous Extract of *Terminalia avicenioides* Roots. *Phytother Res*; 15: 431-434.
- Adzu BS, Amos S, Amizan MB, Gamaniel KS, (2003). Evaluation of the antidiarrhoeal effects of *Z. Spina-christi* Sterm bark in rats *Acta Tropica* (1); 6: 1-5.
- Awouter F, Neimegeers CJE, Lenaert FM, Janssen PAJ (1978). Delay of castor oil diarrhoea in rats; A new way to evaluate inhibitors of prostaglandin's biosynthesis. *J. Pharm Pharmacol.* 30: 41-45.
- Capasso F, De-Ruggiero G, Derosa M, Sorrenteno L (1976). *Recerche Farmacologiche Sulla-4-amino-5-chloro-4-(2 di etil amino etil) 2-methossibenzenamide.* *Bollettino Chimico Farmaceutico.* 115: 649-657.
- Dicarlo GD, Mascolo N, Izzo AA, Capasso F (1994). Effect of quereetine on the gastrointestinal tract in rats and mice. *Phytother Res.* 8: 42-45.

- Gaginella TS, Philips SF (1975). Ricinoleic acid; Current view of an ancient oil. *Digestive Dis and Sci.* 23: 1171-1177.
- Gupta SS (1994). Prospects and perspectives of natural plant products in medicine. *Indian J Pharmacol.* 26: 1-12.
- Lork D (1983) A new approach to practical acute testing. *Arch Toxicol* 54: 275-287.
- Mekeon TA, Lin JJ, Stafford AE (1999). Biosynthesis of ricinoleate in castor oil. *Adv. Exp. Med. Biol.* 464:37-46447.
- Meli R, Autore G, Dicarlo G, Capasso F (1990). Inhibitory action of *quereetin* on intestinal transit in mice. *Phytother Res* 4: 201-202
- Morton J (1987). Indian Jujube. In: *Fruits of Warm Climates*. Morton JF (ed), Miami, Florida. 272-275. Last updated: 23/4/2004. Accessed on 5/5/2004 at [http://www. IndianJujube.htm](http://www.IndianJujube.htm)
- Msonthi JD, Magombo D, Hamdard (1983). Medicinal Herbs in Malawi and their Uses *Hamdard Medicus*: 26: 2. 94-100.
- Mukherjee PK, Saha K, Murugesan T, Mandal SC, Pal M, Saha BP, (1998). Screening of antidiarrhoeal profile of some plant extracts of a specific region of West Bengal. *Indian J. Ethnopharmacol*; 60: 85-89.
- Nester WE, Robbert CE, Nancy NP, Danise GA, Martha TN (1998): *Microbiology: A Human Perspective* 2<sup>nd</sup> ed. Mc Graw-Hill Companies USA: 571-573.
- Nwafor PA, Okwuasaba FK, Binda LG (2000). Antidiarrhoeal and antiulcerogenic effects of methanolic extract of asparagus pubescent root in rats. *J Ethnopharmacol.* 72:421-427.
- Rao AV, Gurfinkel DM (2000): Bioactivity of saponins: Triterpenoids and Steroidal Glycosides. Freund Publishing House Ltd. Canada. 211-235
- Robert A, Nezarmis JE, Lancaster C, Henchar AI, Kleppre MS (1976): Enteropooling Assay: A test for diarrhoea produced by prostaglandins. *Prostaglandins.* 11: 809-814.
- Saito T, Mizutani F, Iwanga Y, Morikawa K, Kato H (2002). Laxative and Antidiarrhoeal Activity of Polycarbophil in Mice and Rats. *Jpn J. Pharmacol*; 89: 133-141.
- Schlemper VA, Ribas M, Nicolau, Cechinel GV (1996): Antispasmodic effects of hydroalcoholic extract of *Marrubium vulgare* on isolated tissue. *J. Phytomed.* (3); 2: 211-216.
- Subramoniam P, Pushpangadan P (1999). Development of phytomedicines for liver disease. *Indian J. Pharmacol.* 31: 166-175.
- Trease GE, Evans WC (1996): *Pharmacognosy*. Bailliere Tindall, London; 89-122.
- WHO (1993). Regional Office for Western Pacific, research guidelines for evaluating the safety and efficacy of herbal medicines, Manila.
- Zavala MA, Perez S, Perez C, Vargal R, Perez RM (1998). Antidiarrhoeal activity of *Waltheria americana*, *Commelins cuelestris* and *Alternanthera repens*; *J. Ethnopharmacol* 61: 41-47.