Vol. 7 no. 1, pp. 10-13 (March 2010)

http://ajol.info/index.php/jpb

Journal of
PHARMACY AND BIORESOURCES

Comparative acute toxicity study of the pulp, seed and stem bark (aqueous) extracts of *Zizyphus spina-christi* L.(Kurna)

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Received 29th November 2009; Accepted 31st January 2010

Abstract

The toxicological potentials of the pulp, seed and stem bark (aqueous) extracts of *Zizyphus spina-christi L.* was investigated in rats. Acute toxicity was conducted following intraperitoneal administration of graded doses of the plant extract. LD_{50} of the pulp, seed and stem bark (aqueous) extracts were 6500mg/kg, 6250mg/kg and 6000mg/kg body weight respectively. Mortality which was dose dependent occurred at high doses of 6000mg and 8000mg/kg for the pulp, seed and stem bark extracts. Phytochemical analysis of the aqueous extracts (pulp, seed, stem bark) indicated the presence of flavonoids, alkaloids , carbohydrates, tannins and saponins. The results show that all parts of the plant can be useful for medicinal purposes.

Keywords: *Zizyphus spina-christi;* Acute toxicity, Aqueous extract, Active principles.

Introduction

The plant *Zizyphus spina-christi* is of the family Rhamnaceae. It is a deciduous tree found in different parts of Nigeria and is known as Kurna in Hausa and Kanuri language (Adzu *et al*. 200). Different parts of the plant are used for various medicinal purposes among the local population. The drupes are eaten fresh, pickled, dried or made into confectionery and the juice can be made into drinks (Glew, 1998). *Zizyphus spina-christi* has been reported to have activity against bacteria, fungal and other pathogens that are normally quite resistant (Nazif *et al*.2002). Plant leaves of *Zizyphus* are used in Iranian folk medicine as an antiseptic, antifungal and anti-inflammatory agent and for healing skin diseases such as atopic dermatitis (Amin, 1991). Despite the wide spread use of the plant, for a variety of diseases by traditional healers, adequate information on its toxicity in man and animals is unvailable. Therefore, the objectives of this study were; to evaluate the acute toxicity and also identify the phytochemical constituents in the pulp, seed and stem bark extracts of *Z. spina-christi.*

Experimental

Plant Material. Fresh samples of the ripe fruit and stem bark of *Z. spina-christi* were collected in March, 2007 from Jiddari, polo area of Maiduguri, Borno state. The plant specimen was identified by Prof. S .S. Sanusi

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ISSN 0189-8442

of the Department of Biological Science, University of Maiduguri. A voucher specimen was deposited at the herbarium.

Preparation of plant extract. The Fruit was separated into Pulp and Seed. Both were air-dried along with the Stem bark for two weeks. The dried Pulp, Seed and Stem bark were pulverized with mortar and pestle. Eight hundred grams of the powdered samples were placed each in a thimble and extracted with two liters of distilled water. The aqueous layer was concentrated *in vacuo*, a dark brown solid mass was obtained for the pulp (18.36%w/w), seed (5.31%w/w) and stem bark (4.18%w/w). The extracts were labeled and stored at 4°c for further analysis.

Animal treatment. Forty (40) albino Wistar strain rats of both sexes weighing between 120 and 160g were purchased for the study. They were divided into five (5) groups of eight (8) rats each, allowed free access to drinking water and standard diet (Nutri feed Nigeria Ltd). The animals were administered doses ranging from 1000, 2000, 4000, 6000 and 8000mg/kg of aqueous extracts respectively with a separate group serving as control which was administered orally a single dose of normal saline (0.9%Nacl). The rats were observed for a period of 24 hours for clinical signs of toxicity and death. The arithmetic method of Kaber as modified by Aliu and Nwude (1982) was used to calculate the $LD_{50.}$

The methods of Evans (2002), Harborne (1973) and Sofowora (1993) were used to screen for phytochemical constituents.

Results

Rats in the control group were not affected throughout the 24 hours of the acute toxicity study. There was no death of rats in group 1, 2 and 3 given pulp, seed and stem bark aqueous extract at dose of 1000, 2000 and 4000mg/kg body weight. However, at doses of 6000 and 8000mg/kg some death was recorded dose dependently. Signs and symptoms of toxicity observed in the affected treated rats in order of severity include depression, drowsiness, hind limb paralysis, difficulty in breathing and death.

Table 1: Mortality rate of rats given pulp, seed and stem bark (aqueous) extracts of *Z. spina-christi*

M.R of Pulp				M.R of Seed				M.R of Stem bark			
Grp	Dose of Extract (mg/kg)	No. of Death	Mortality (%)	Grp	Dose of Extract (mg/kg)	No. of Death	Mortality (%)	Grp	Dose of Extract (mg/kg)	No. of Death	Mortality (%)
1	1000	0	0	1	1000	0	0	1	1000	0	0
2	2000	0	0	2	2000	0	0	2	2000	0	0
3	4000	0	0	3	4000	0	0	3	4000	0	0
4	6000	3	37.5	4	6000	4	50	4	6000	4	50
5	8000	6	75	5	8000	6	75	5	8000	8	100

Key : M.R – Mortality rate Grp- Group The LD_{50} of the pulp, seed and stem bark (aqueous) extracts were calculated to be 6500mg/kg, 6250mg/kg and 6000mg/kg.

Table 2: Calculation of LD_{50} of the Pulp aqueous Extract of *Zizyphus spina-christi*

Group N = 5	*Plant Extract Dose Mg/kg body wt*	*Dose Difference Dd (mg)*	*Dead Difference*	*DEAD Mean dead*	*X.md*
1	1000	0			
2	2000	1000	-		
3	4000	2000	0	0	
4	6000	2000	3	1.5	3000
5	8000	2000	6	4.5	9000

$$LD_{50} = \frac{LD_{8000} - DVm.d}{\text{No of rats}} = 8000 - 12000/8 = 6500\text{mg/kg}$$

Table 3: Calculation of LD_{50} of the Seed aqueous Extract of *Zizyphus spina-christi*

Group N = 5	*Plant Extract Dose Mg/kg body wt*	*Dose Difference Dd (mg)*	*Dead Difference*	*DEAD Mean dead*	*X.md*
1	1000	0			
2	2000	1000	-		
3	4000	2000	0	0	0
4	6000	2000	3	2.0	4000
5	8000	2000	7	5.0	10000
LD_{50}=	$\frac{LD_{8000}\text{-DVm.d}}{\text{No of rats}}$ =	8000-14000/8=	6250mg/kg		

Table 4: Calculation of LD_{50} of the Stem bark aqueous Extract of *Zizyphus spina-christi*

Group N = 5	*Plant Extract Dose Mg/kg body wt*	*Dose Difference Dd (mg)*	*Dead Difference*	*DEAD Mean dead*	*X.md*
1	1000	0			
2	2000	1000	-		
3	4000	2000	0	0	0
4	6000	2000	4	2.0	4000
5	8000	2000	8	6.0	12000
LD_{50}=	$\frac{LD_{8000}\text{-DVm.d}}{\text{No of rats}}$ =	8000-16000/8=	6000mg/kg		

Table 5: Phytochemical composition of the Pulp, Seed and stem bark extracts of *Zizyphus spina-christi.*

S/N	Constituents/Test	Pulp (aqueous)	Seed (aqueous)	Stem bark (aqueous)
1.	Flavonoids: Lead acetate test	+	+	+
	NaOH	+	+	+
	Iron (ii)chloride	+	+	+
2.	Alkaloids: Drangendorff's	+	+	+
	Mayer's	+	+	+
	Wagner's	+	−	+
3.	Saponins: Frothing Test	+	+	+
4.	Carbohydrates: Molisch's Test	+	+	+
	Barfoed's Test	+	+	+
	Fehling Test	+	+	+
5.	Tannins: iron (iii) chloride Test	+	+	+
	Lead acetate Test	+	+	+
6.	Steroid nucleus: Salkowski	+	−	+
	Liebermann	+	+	+

Key: + = Present, - = absent

Discussion

The intraperitoneal acute toxicity study carried out produced LD_{50} between 6000mg/kg and 6500mg/kg. This shows that extracts from the pulp, seed and stem bark are of low toxicity which agrees with the work of Clarke and Clarke (1977) that substances with LD_{50} of 1000mg/kg and above are regarded as being safe or of low toxicity. However, the pulp extracts was found to possess a higher LD_{50} value which could be attributed to the presence of saponins at lower concentration and possibly other less toxic principles. The result is in agreement with previous studies carried out which showed the oral LD_{50} of the extract of *Z. spina-christi* to be greater than 6400mg/ml (Islam *et al*.2001).

Acknowledgement

The authors are grateful to Dr. U.K Sandabe, Department of Veterinary Physiology, Pharmacology and biochemistry for his innumerable contributions and Fine Akawo, Department of Chemistry and Justice Jibril, Department of clinical pharmacology, U.M.T.H. Maiduguri.

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