African Journal of Biotechnology Vol. 7 (10), pp. 1600-1603, 16 May, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Acute toxicity studies of aqueous stem bark extract of *Ximenia americana*

Maikai, V. A.[1]*, Kobo, P. I.[2] and Adaudi, A. O.[2]

[1]College of Agriculture and Animal Science, Ahmadu Bello University, P.M.B.2134, Mando, Kaduna, Nigeria.
[2]Veterinary Physiology and Pharmacology, Ahmadu Bello University Samaru Zaria, Nigeria.

Accepted 18 April, 2008

Increasing interest in medicinal plants has increased scientific scrutiny of their therapeutic potentials and safety thereby providing physicians with data to help patients make wise decision on their usage. The stem bark of *Ximenia americana* was evaluated for its phytochemical constituents and acute toxicity effect on thirty Swiss albino mice. The extracts were administered intraperitoneally/orally at doses of 10, 100, and 1000 mg/kg body weight. The results revealed no death with doses up to 5000 mg/kg body weight. However, the initial reactions included excitement, restlessness, lack of appetite and later reduced activity during the first 24 h of extract administration. The symptoms were dose dependent with signs noticeable with increasing dosage. Post mortem, hematological and histopathological examination did not show any significant (P<0.05) damage as a result of the extract administration. However, there were significant (P<0.05) weight changes. Phytochemical screening of the aqueous stem bark extract revealed the presence of cardiac glycosides, flavonoids, saponins, and tannin. The results suggest that the aqueous extract is not acutely toxic to the mice.

Key words: *Ximenia americana*, toxicity, phytochemicals.

INTRODUCTION

Medicinal plants play a very significant role in health care needs of rural populations in African and other third world countries especially in treatment of diseases. *Ximenia americana* "Wild plum or Plum" in English or locally called "Tsada" in Hausa and "Chabbuli " in Fulani is a medicinal plant that is bushy and spiny shrub, 4 – 5 m high with an open crown. The fruits are green but turn golden yellow or red when ripe. The fruit when eaten is refreshing and has an almond acid taste. The plant is found from Senegal to Cameroon including Northern parts of Nigeria (Arbonnier, 2004). The plant is used in traditional medicine for treatment of malaria, fever, leprotic ulcers and skin infections of mixed origin in Northern parts of Nigeria (Ogunleye and Ibitoye, 2003). Arbonnier (2004) reported the medicinal uses of *X. americana* to include treatment of fever, stiffness, onchocerciasis, sore throat, asthma, and headaches. Maikai et al. (2007) reported that the stem bark has trypanocidal activity, while Onyekwelu et al. (2000) also reported that the roots have transient antitrypanosomal activity. The roots are used for treating abdominal pains, dysentery, inflamed joints and mouth ulcers (Ake and Guinko, 1991). Phytochemical screening of the leaves revealed the presence of saponins, cyanogenic glycosides, flavonoids, and tannins (Ogunleye and Ibitoye, 2003). Arbonnier (2004) reports that the fruits contain hydrocyanic acid which is toxic. Considering the medicinal value of this plant, the acute toxicity and histopathology of the aqueous extract of the stem bark was evaluated in mice to assess its safety or otherwise, since the findings are important considering the usage of the plants by human beings.

*Corresponding author. E-mail: ambrosev2003@yahoo.com.

MATERIALS AND METHODS

Collection of plant material

Fresh stem bark of *X. americana* was collected from Afaka village, 35 km to Kaduna and taken to Department of Biological Sciences, Ahmadu Bello University Zaria for identification. The voucher number is 1612. The bark was dried at room temperature before crushing into powder then stored in air-tight container and kept at 4°C until needed.

Table 1. Phytochemical screening of *Ximenia Americana*.

Extract	Alkaloids	Anthraquinones	Cardiac glycosides	Flavonoids	Pylobatannins	Saponnins	Tannins	Terpenoids
Water extract (WE)	++	++	+++	++	+++	++	+++	+++

+++ = Highly present, ++ = moderately present.

Table 2. Post mortem gross pathology result of acute toxicity of mice administered aqueous extract of *Ximenia Americana*.

Organ	Dose (mg/ kg body weight)		
	10	100	1000
Heart	None	None	None
Kidney	None	None	None
Liver	None	None	None
Lungs	Congestion	Congestion	Congestion
Spleen	None	None	None

Plant extraction

Two hundred (200) grams of the powdered bark was soaked in 1000 ml of distilled water for 24 h. It was then filtered through a cheese cloth before further filtration using a Whatman No. 1 filter paper. The filterate was concentrated in hot air oven at 50°C for two days and subsequently air-dried. The brownish black extract was pounded using pestle and mortar into powder and stored in air-tight container and kept at 4°C till needed.

Animals

Swiss albino mice (20-32 g) aged 8- 12 weeks, bred in the College of Agriculture and Animal Science, Ahmadu Bello University, Mando Road Kaduna were used for the study. They were kept in clean plastic cages in a 12 h light/dark cycle with litter changed every week. They were fed with mice cubes specially prepared by ECWA feed, Jos Plateau State. They were watered *ad libitum.* A standard protocol was observed in accordance with the Good Laboratory Practice (GLP) Regulations of the WHO (1998). The animal Laboratory care of CCAC (1993) was strictly followed.

Phytochemical screening

The aqueous stem bark extract was screened as described by Sofowora (1993), Trease and Evans (1989) and Harborne (1973).

Acute toxicity studies

Thirty male Swiss albino mice of average weight 25.3 g were acclimatized for a week in cleaned cages and randomly divided into 3 groups of 3 animals each. Groups 1, 2 and 3 were intraperitoneally/orally administered 10, 100 and 1000 mg/kg body weight aqueous bark extract reconstituted in water following the method of Lorke (1983). The fourth group which is the control was administered water orally/intraperitoneally. The animals were observed frequently from the day of treatment. The nature and time of any adverse effect was noted, Observation was carried out for 14 days and the experiment terminated. All animals were weighed and euthanized in a chloroform chamber and gross pathologic examination conducted. Sections of tissues such as lung, kidney, spleen, liver, and heart were obtained for histopathological studies.

Determination of median lethal dose (LD_{50})

Based on the result of the acute toxicity test, white Swiss albino mice of average weight 25.3 g divided into 3 groups of one animal per group were intraperitoneally/orally administered 1600, 2900 and 5000 mg/kg body weight, aqueous bark extract in water. Death was monitored over a period of 24 h. LD_{50} was then determined using the method of Lorke (1983). The acute toxicity LD_{50} was calculated as the geometric mean of the dose that resulted in 100% mortality and that which caused no mortality at all. The animals were observed and the studies terminated after two weeks. Recovery and body weight gain after each investigation was taken as a sign of surviving the test.

Haematology

The packed cell volume (PCV), haemoglobin and white blood cell were measured using the standard microhematocrit method (Schalm et al., 1975) and plasma protein was determined by method of Coles (1986).

Statistics

The mean and standard deviation and the level of significance for the differences between means were computed by student's t test.

RESULTS

The results revealed that the aqueous extract of stem bark of *X. americana* had high contents of cardiac glycosides, phlobatannins, tannins and terpenoids. Alkaloids, anthraquinones, flavonoids and saponins were moderately present (Table 1). Clinical signs of toxicity observed in all the cases include initial excitement, restlessness, difficulty in breathing, loss of appetite, gene-ral weakness and depression which was seen as the doses increased. No death was observed in the first 24 h and throughout the period of experiment. There were variable changes in the body weight of the animals in all the treated groups (Table 2). The control group gained weight during the 14 days period of observation, while the treated groups showed variable weight loss. These changes however, were significantly ($p<0.05$) different from the control. The relative weights of the

Table 3. Effect of administering different doses of *Ximenia Americana* aqueous extract on body weight of mice over a period of two weeks.

Dose (mg/kg b.wt)	Day 0	Day 7	Day 14
Control	28.50±0.90	28.70±0.78	29.00±1.20
10	20.20±0.35	20.40±0.67	20.60±0.10
100	30.30±0.27	30.00±1.00	30.20±0.70
1000	30.00±1.12	29.10±0.50	28.90±1.10*
1600	19.00	19.20	17.80*
2900	24.50	24.30	23.00*
5000	29.00	28.60	26.40*

*Significantly different from the control ($p < 0.05$).

Table 4. Effect of administering different doses of *Ximenia Americana* aqueous extract on the organ weight of mice (g/100 g).

Dose (mg/kg b.wt)	Heart	Liver	Lungs	Kidney	Spleen
10	0.40±0.12	1.70±0.11	0.40±0.10	0.80±0.06	0.30±0.80
100	0.20±0.07	1.70±0.20	0.20±0.14	0.80±0.10	0.30±0.12
1000	0.40±0.14	2.30±0.06	0.50±0.03	0.90±0.07	0.30±0.13
Control	0.10±0.08	1.00±0.40	0.10±0.10	0.60±0.30	0.40±0.12
1600	0.50	1.70	0.80	0.70	0.50
2900	0.40	1.70	0.30	0.80	1.10
5000	0.40	1.30	0.40	0.70	0.60

*Significantly different from the control ($p < 0.05$).

Table 5. Effect of different doses of *Ximenia Americana* aqueous extract on the haematology profile of the mice.

Dose (mg/kg b.wt)	PCV (%)	Hb (g/dl)	WBC (x10^9/L)	Total plasma protein (g/dl)
Control	52.00±2.00	17.40±0.10	7.20±1.30	5.50±2.30
10	51.00±1.19	16.50±0.06	7.10±1.00	5.00±0.98
100	50.00±0.20	17.00±0.48	6.50±0.03	5.10±0.72
1000	49.00±2.48	16.80±0.30	6.90±0.45	6.00±1.00
1600	49.00	16.50	6.20	5.20
2900	48.00	16.80	5.90	5.00
5000	48.00	16.40	6.00	4.80

*Significantly different from the control ($p < 0.05$).

organs (heart, liver, spleen, kidney and lungs) were not significantly ($p<0.05$) different from the control (Table 3). However, the lungs of the group treated at 2900 mg/kg body weight were significantly different ($p<0.05$). Gross pathological changes as a result of administering the doses of extract were not observed in any of the organs, except congestion of the lungs noticed (Table 4). Histopathological examination of the organs did not reveal any abnormalities (data not shown). The packed cell volume, hemoglobin, white blood cell and total plasma protein were not significantly ($p<0.05$) different from the control (Table 5). However, the white blood cell and total plasma protein of group treated with 5000 mg/kg body weight showed decreased values.

DISCUSSION

Acute toxicity test gives clues on the range of doses that could be toxic to the animal; it could also be used to estimate the therapeutic index (LD_{50}/ED_{50}) of drugs and xenobiotics (Rang et al., 2001). Phytochemicals are thought to have a positive or negative effect on an animal. Tannins and anthraquinones are thought to have both proxidant and antioxidant effects on the body. While the antioxidant protects the tissues and organs, the proxidant damages the tissues and organs. The weight changes of the animals during the period of observation which was more visible at higher doses, suggest the presence of tannins and other phenolics which are

thought to interfere with absorption of nutrients making them unavailable and thereby reducing feed intake (Kumar and Singh, 1984). Even though the animals were fed with adequate diet, the aqueous extract at higher doses could have caused the interference since phyto-chemical studies showed the presence of tannins and other compounds which interferes with absorption of nutrient such as proteins and minerals resulting in weight loss. The organ weights were not significantly different form the control. This suggests that the aqueous extracts did not interfere with the organs. The congestion seen in the lungs could be as a result of the inhalation of the chloroform used to euthanize the animals. It appears that the different dose of the aqueous extracts did not affect the hematological parameters of the animals. This is very surprising because the extracts contained the presence of saponnins which has been reported (Sofowora, 1993) to have deleterious hemolysing effect on circulating erythrocytes.

The presence of anthraquinones in the extracts, which has been reported (Huang et al., 1992) to produce free radicals and hydrogen peroxide during its oxidation to semiquinone in the body, is thought to damage the cells of the body. The absence of gross and histopathological lesions in the organs could suggest the level of safety of the aqueous extract on the animals.

In conclusion, to our knowledge, this is the first investigation on the toxicity studies of *X. Americana*. This study has shown that acute administration of the aqueous bark extract of *X. americana* may be safe as the LD_{50} could not be determined at the doses given. Though the phytochemical screening revealed many chemical constituents, which could affect the animal positively or negatively as a result of prolong usage. It is recommended that a long-term study be conducted.

ACKNOWLEDGEMENTS

This work was sponsored in part by Carnegie Foundation and Ahmadu Bello University, Zaria, Nigeria. We are indeed grateful to them.

REFERENCES

Arbonnier M (2004). Trees, Shrubs and lianas of West African dry zones. Margraf Publishers CIRAD GMBH,MNHN.

Ake AI, Guinko S (1991). In: Plants used in traditional medicine in West Africa. F. Hoffman, La Roche Ltd. Basel Switzerland .pp. 100

CCAC (1993). Canadian Council on Animal care Guide Vol. 1 (2nd Ed).

Harborne, JB (1973). Phytochemical methods. London Chapman and Hall Ltd. pp.49-188.

Huang KC, Chang JH, Tung SF, Wu RT, Foegh ML (1992). Immuno-supressive effect of emodin a free radical generator. Eur. J. Pharm. 211: 359-364

Kumar R, Singh M (1984). Tannins: their adverse role in ruminant Nutrition. J. Agric. Food Chem. 32: 447-453.

Lorke D (1983). A new approach to practical acute toxicity testing. Arch. Toxicol. 54: 275-287

Ogunleye DS, Ibitoye SF (2003). Studies of antimicrobial activity and chemical constituents of *Ximenia americana*. Trop. J. Pharm. Res. 2(2): 239- 241.

Onyekwelu NA, Igweh AC, Halid I (2000). Antitrypanosomal and antimicrobial effects of *Ximenia americana* root extract. J. Pharm. Res. Drug Dev., (In Press).

Rang HP, Dale M, Ritter J (2001). Pharmacology. 4th ed. (USA ed.) New York, Churchill Livingstone.

Sofowora A (1993). Medicinal plants and Traditional Medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria, p. 289.

Trease GE, Evans WC (1989). Pharmacognosy. 11th Edition Brailliar Trindel Can. Macmillan Publishers.

Maikai VA, Nok AJ, Alawa CBI, Adaudi AO (2007). The effect of *Ximenia americana* in mice experimentally infected with *Trypanosoma congolense*. Int. J. Biosci. 2(1): 48-52.