Biochemical Effects of Methanolic Extract of *Ximenia americana* Leaves in Rats

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ABSTRACT

This study was carried out to investigate the toxic effects of methanolic extract of *Ximenia americana* leaves on albino rats. Twenty rats were evenly divided into four groups A, B, C and D. Group A served as control. Groups B, C and D were orally administered with methanolic extract of *X. americana* leaves at doses of 12.5, 25 and 50 mg/kg body weight (bwt), respectively, for 21 days. Clinical signs and mortality were recorded. The rats were weighed at the beginning and end of the experiment. Haematological examination and serum activity, including total protein, albumin, alinine transferase and alkaline phosphatase, were investigated at the end of experiment. Specimens from liver, kidney, heart and brain were taken for histopathology. The rats receiving 50 mg/kg bwt showed depression and paresis. Mortality occurred in the groups receiving 25 and 50 mg/kg (bwt). Significant reduction in the body weight occurred in the group given 50 mg/kg bwt. Haematological examination did not show significant changes in all the treated groups. The rats receiving 12.5 mg/kg bwt of the extract showed no obvious changes. There was a significant decrease in total protein and albumin in the group dosed with 50 mg/ kg bwt in the third week. However, a significant increase occurred in ALT in the groups receiving 25 and 50 m/ kg bwt and ALP in the group given 50 mg/kg bwt. Haematology examination did not show any significant change. It is concluded that this study generated a base line for the safe use of *Ximenia americana* leaves in folk medicine

Key words: *Ximenia americana*; leaves; toxicity
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INTRODUCTION

Recognition and development of medicinal and economic benefits of traditional medicinal plants is increasing in both developing and industrialized countries (WHO, 1998). *Ximenia americana* is a plant which grows widely in the tropical and temperate regions of the world. It is extensively used as herbal remedies in treating malaria, leproutic ulcers and skin infections of mixed origin. *X. americana* is consider to be one of most commonly used herb in Sudanese folk medicine. Many studies were carried out on *X. americana* leaves, bark, seed and fruit with reference to its chemical constitute and

biological activity. Although it is widely used in folk medicine the toxicity in rats is not studied. Hence the main objective of this study was to study toxic effects on rats.

MATERIALS AND METHODS

Ximenia Americana leaves were collected from plant nursery in Khartoum. The leaves were air-dried and reduced to coarse powder, then, extracted with chloroform and methanol according to the Harborne method (1984). Twenty healthy Wistar albino rats of both sexes weighting 110-224 gm were supplied by Medicinal and Aromatic Plant Research Institute (MAPRI), Sudan. They were kept in animal housing under standard conditions at the Department of Pathology, Veterinary Medicine, University of Khartoum. They had free access to water and standard diet and were left for a week for acclimatization. At the end of the adaptation period the rats were evenly divided randomly into four groups. Group A was left as control, while groups B, C and D were administrated orally with methanolic extract of *X. americana* leaves, daily using nasogastric tube at concentrations of 12.5, 25, 50 mg/kg Bwt, respectively for 21 days.

Clinical signs, mortality and body weights at the start and the end of the experiment were recorded. Serum samples were collected to investigate total serum protein concentration, albumin concentration, alanine transferase (ALT) and alkaline phosphate (ALP) activity. Samples from the liver, kidney, heart and brain were fixed in 10% formalin for histopathology. Statistical analysis of Variance (ANOVA) was conducted according to Gomez and Gomex (1984) with an aid of (SAS, 1998)

RESULTS

The results indicated that rats received 12.5 mg/kg (bwt) of methanolic extract of *X. Americana* leaves showed no clinical signs, whereas depression and difficulty in breathing were observed in rats received 25 mg and 50 mg/kg Bwt. Signs of paresis in the group dosed with 50 mg/kg Bwt was evident. However, mortality was 40% in rats received 50 mg and 20% in rats received 25 mg after two weeks of dosing.

The effects of various doses of *X. americana* methanolic extract on body weights of rats are shown in table (1). There were no significant difference between body weights of the control and those received 12.5 and 25mg/kg Bwt of *X. americana* leaves methanolic extract. However, the control groups showed a significant increase (9.3%) in body weight after three weeks while no significant changes in body weight were observed in groups received 12.5 mg (2%) and 25 mg/kg Bwt (0.8%). There was significant ($P < 0.05$) decrease in body weight in the group received 50 mg/kg bwt (3.7%). There were no significant differences in the total protein, albumin, globulin concentration, ALP and ALT activities in groups of rats which received 12.5 mg and 25

mg compared to the control. However, a significant (P< 0.05) decrease was recorded in total protein and albumin, and significant increase in ALP and ALT activity in the group of rats received 50 mg/kgBwt methanolic extract of *X. americana* leaves (Table, 2). Haematological values were not significantly different from the control. The liver in the groups of rats dosed with 25 mg and 50 mg/kg Bwt of *X. Americana* leaves methanolic extract showed congestion, sinusoidal dilatation vaculation, degeneration and necrosis of hepatocytes. The kidney in the group of rats dosed with 25mg/kg showed congestion, necrosis of tubules and slight haemorrhages but the group dosed with 50 mg/kg showed interstitial and glomerular edema, congestion, haemorrhage and necrosis of tubules.

DISCUSSION

Herbal medicine became a topic of augmented global importance, having impact on both world health and international trade (Akerele, 1988). The current study investigated toxicity of *X. americana* leaves methanolic extract which is considered one of most commonly used herb in Sudanese folk medicine. This study has shown that methanolic extract of *Ximenia americana* leaves causing 20% mortality in rats at dose with 25mg/kgBwt at third week and 40% mortality in rats dosed with 50mg/kgBwt after two weeks. This indicated the effects were dose dependant. The chemical constituent of plant extracts may be the direct cause of the mortality.

When methanolic extract given to rats at dose of 12.5 mg/kg there were no signs of toxicity as indicated by non significant alteration in hematology and serum constituents. However, doses of 25 mg/kg and 50 mg/kg Bwt exhibited signs of poisoning which was illustrated by depression, nervous signs and difficulty in breathing. Since there is no pathological changes in the brain the nervous sign may be related to liver damage. Similar results were reported by Abd Elwahab (2000) who pointed out that *X. americana* leaves methanolic extract produce paralysis when injected intravenously into young chicks. In contrast, Maikai *et al*. (2008) pointed out that *X. americana* is not toxic to mice even at dose of 1000 mg/kg bwt.

The body weights showed significant increase in the control group and minor increase in groups dosed with 12.5 mg/kg Bwt and 25 mg/kg Bwt, while significant reduction was observed in the group treated with 50 mg/kg Bwt after 21 days. This indicated that methanolic extract of the plant which contains tannins had an inhibitory effect on the growth rate. Kumar and Singh (1984) pointed out that tannins interfere with absorption of nutrients. The methanolic extract of plant showed no significant effect on haematological values at all doses although haemorrhage was evident. This indicated that the plant is free of active constituent that could have an inhibitory effect on synthesis of haemopoietic system.

There was a significant reduction in serum total protein concentration in the groups given 50mg/kg Bwt. This is due to a sequence of reduction of albumin fraction

that may be attributed to liver damage which affected synthesis of albumin fraction. However, there was no change in globulin, but albumin/globulin ratio decreased due to reduction in albumin. The activities of enzymes ALT in serum used routinely to assess functional activity of the liver in both clinical and experimental settings (Yanpellawar *et al*., 2002). The activity of ALT increased in the groups received 25 mg and 50 mg/kg Bwt. This is an indication of hepatocellular damage which was evident in histopathological examination. ALP activity increased in the group received 50 mg/kg Bwt only. This may be due to the high dose that sensitized the hepatocytes causing loss of the functional integrity to cell membrane.

Table (1). The Mean body weights of rats given methanolic extract of *X. americana* leaves

Treated groups (mg/Kg Bwt)	**Body weights(g)**	
	Day 0	**Day 21**
0	150.22±20	164.2±16.46
12.5	149.5±9.99	152.0±10.52
25	149.3±63	150.5±11.93
50	149.4±10.52	143.8±2.44*

Values are expressed as mean ± SD P< 0.05

Table 2. Hematological changes in albino rats given methanolic extract of *Ximenia americana* (leaves)

Duration	Treated groups (mg/kg BWT)	RBC (mm^3)	WBC (10/ml)	Hb (%)	PCV (%)	MCV (fi)
7	0	5.99±0.62	3.40±0.22	74.0±3.07	44.0±2.28	75.84±6.09
	12.5	6.48±0.42^{NS}	3.29±0.52^{NS}	72.6±3.01^{NS}	44.0±1.61^{NS}	69.41±6.05^{NS}
	25	5.85±0.41^{NS}	3.26±0.32^{NS}	80. ±3.44^{NS}	47.0±2.40^{NS}	81.57±4.83^{NS}
	50	6.25±0.63^{NS}	2.71±0.19^{NS}	75.5±3.82^{NS}	41.6±1.44^{NS}	70.72±9.71^{NS}
21	0	5.70±0.48	3.54±0.15	76.15±3.76	48.0±0.95	86.98±8.49
	12.5	5.22±0.62^{NS}	3.16±0.43^{NS}	72.59±3.01^{NS}	49.20±1.62^{NS}	94.25±19.01^{NS}
	25	5.39±0.26^{NS}	3.28±0.23^{NS}	77.53±3.2^{NS}	50.80±1.39^{NS}	95.40±6.00^{NS}
	50	5.56±0.86^{NS}	2.46±0.46^{NS}	80.5±4.5^{NS}	47.0±2.68^{NS}.	103.07±25.00^{NS}

Values are expressed as mean ± SD

NS= Not significant

MCHC (g/dl)
23.38±1.12
24.75±0.49^{NS}
25.49±0.54^{NS}
27.34±1.55^{NS}
23.88±1.49
22.22±1.15^{NS}
22.89±2.26^{NS}
26.08±2.28^{NS}

Table 3. Changes in serum constituents of albino rats dose with methanolic extract of *X. americama* leaves

Duration day	Treated groups (mg/kg BWT)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Albumin/ globulin ratio	ALT U/L	ALP U/L
7	0	6.10±0.20	4.00±0.13	2.10±0.19	1.99±0.16	49.60±9.92	32.90±5.52
	12.5	6.10±0.52^{NS}	4.30±0.15^{NS}	1.80±0.49^{NS}	2.30±0.27^{NS}	49.50±5.84^{NS}	33.00±5.50^{NS}
	25	6.10±0.32^{NS}	4.40±0.15^{NS}	1.70±0.30^{NS}	2.50±0.75^{NS}	44.60±4.90^{NS}	49.50±6.74*
	50	6.20±0.38^{NS}	4.20±0.28^{NS}	2.00±0.23^{NS}	2.15±0.30^{NS}	51.80±6.14^{NS}	38.50±6.74^{NS}
15	0	6.58±0.16	4.71±0.09	1.87±0.44	2.51±0.42	60.20±8.83	38.42±6.77
	12.5	6.74±0.20^{NS}	5.24±0.09^{NS}	1.50±0.49^{NS}	3.48±0.67^{NS}	60.40±5.36^{NS}	49.40±10.21^{NS}
	25	6.70±0.37^{NS}	5.00±0.09^{NS}	1.70±0.30^{NS}	2.94±0.48^{NS}	61.50±3.92^{NS}	61.63±10.10^{NS}
	50	6.54±0.37^{NS}	4.54±0.27^{NS}	2.00±0.23^{NS}		64.40±7.28^{NS}	71.40±10.98*
21	0	6.62±0.24	4.83±0.09	1.79±0.24	2.94±0.44	65.00±3.54	44.00±6.74
	12.5	6.56±0.23^{NS}	4.78±0.31^{NS}	1.78±0.40^{NS}	3.50±0.93^{NS}	66.40±7.31^{NS}	47.40±8.70^{NS}
	25	6.08±0.34^{NS}	4.59±0.26^{NS}	1.49±0.21^{NS}	3.36±0.52^{NS}	78.00±9.65*	61.63±10.10^{NS}
	50	5.00±0.01*	3.10±0.09*	1.90±0.07^{NS}	1.63±0.61*	80.00±5.38*	72.10±5.01*

Values expressed as mean ± SD NS ≡ Not significant * (P<0.05

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