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Toxicopathological Effects of *Ambrosia maritima* L. (*Compositae*) Extracts in Rats

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Abstract: The toxic effect of the methanolic and water extracts of *Ambrosia maritima (A.maritima)* on rats treated for three weeks with different doses was determined. The methaolic extract was injected intramuscularly and the water extract was given orally. The results revealed that a single intramuscular dose of 2000 mg kg^{-1} Body weight (Bwt) of the methanolic extract was fatal, but a daily doses of 500 and 250 mg kg^{-1} (Bwt) caused inappetence, deceased activity, lameness and even paralysis, although body weights were not affected. However diarrhoea was the most prominent sign in rats receiving the water extract at the doses 1000 and 500 mg kg^{-1} (Bwt) while at the dose 250 mg kg^{-1} (Bwt) no clinical signs were observed. Lesions in both extracts consisted of generalized congestion, fatty change of the liver, degeneration of renal tubules and pancreatic hyperplasia.

Key words: *Ambrosia maritima*, toxicology, pathology, propylene glycol rats

INTRODUCTION

Ambrosia maritima which is locally in Sudan known as Damsissa is widely used in the folk medicine as antidiabetic (El-Gazali *et al*., 1987). It has also been reported to have molluscicidal effects (El-Magdoub *et al*., 1977; Vassiliades and Diaw, 1980; El-Sawy *et al*., 1984; Abou-Basha *et al*., 1994).

Oral administration of 5 - 5.6 mg kg^{-1} of dried powdered leaves or methanolic extract of *A. maritima* as well as incorporation of 50000 ppm of the powdered leaves in feed for four weeks revealed no toxic effect in rats (Alard *et al*., 1991). Depression of average body weight and inefficiency of feed utilization were reported in chicks fed a diet containing 10% of A. maritima (Bakhiet and Adam, 1996).

The present study was planned to investigate the toxic effect of both methanolic and water extract of the plant in Wister albino rats.

MATERIALS AND METHODS

A. maritima was collected from the river bank of the Blue Nile, Khartoum, Sudan and authenticated in Medicinal and aromatic Plants Research Institute (MAPRI) Khartoum,Sudan. The plant was dried in shade and ground coarsely. The methanolic extract of the plant was prepared according to Harborne and the water extract was prepared by maceration of the coarsely ground plant with distilled water.

Animals: Forty male Wister albino rats were used in this study. They were kept in the premises of MAPRI. And were given food and water *ad libitum*. They were acclimatized for one week.

Experimental design: Twenty male albino rats were divided randomly into four groups, each of 5 rats. The mean body weights of the groups were similar. One group served as control and injected intramuscularly with the propylene glycol where as the other received the methanolic extract of the plant at the doses of 250, 500 and 2000 mg kg^{-1} (Bwt). Similarly in water extract study 20 rats divided into four equal groups were used. The control group received distilled water and the other three groups were drenched with the water extract of the plant at the does of 250, 500 and 1000 mg kg^{-1} (Bwt) using stomach tube. The rats received the extracts for three weeks. Clinical signs and mortality rates were recorded. Average body weights were determined at the end of experiment.

Blood collection: Blood samples were collected weekly from the orbital plexus by means of heperinized capillary glass tubes according Schalm (1965). Blood was collected either on EDTA for hematological studies or glass centrifuge tubes to separate the serum by centrifugation of blood at 5000 rpm for 5 min. Sera were immediately separated and stored at -20°C for biochemical analysis.

Haematological and biochemical methods: Red and White Blood Cells (RBC and WBC), Haemoglobin concentration

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(Hb) and Packed Cells Volume (PCV) were determined according to Schalm (1965). Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemologbin Concentration (MCHC) were calculated from RBC, PCV and Hb values.

Sera were analyzed for the activity of Alkaline Phosphates (ALP) and cholesterol concentration using commercial kits Chemie (1972) and Richmond (1973), respectively. Total protein, calcium and phosphorus were determined according to Weichselbaum (1946), Trinder (1960) and Varley (1963), respectively.

At the end of the experimental period rats were dissected and liver and kidney were excised immediately and weighed. The relative organ weights were calculated. Specimens from different organs were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5 ìm and stained with haematoxyline and eosin according to Drury and Willington (1980).

Data were analyzed for significance using student's T-test according to Mendenhall (1971).

RESULTS AND DISCUSSION

Rats received 2000mg kg^{-1} Bwt methanolic extract died within 2days while the other treated groups (250 and 500 mg kg^{-1} (Bw)) showed depression, inappetence and paralysis. On the other hand, diarhoea was evident in those treated with water extract at the dose rate of 500 and 1000 mg kg^{-1} (Bwt).

There were no significant differences between the body weights of the control group and rats treated with either extract. However, a significant increase in the relative liver weights occurred in rats receiving 500 mg kg^{-1} (Bwt) water extract ($p < 0.05$) (Table 1).

The main gross lesions observed were dark gangreneous myositis in rats injected with 500 mg kg^{-1} (Bwt) methanlic extract (Fig. 1).

The haematological and biological data of rats receiving methanolic or water extract are shown in Table (2a and b). Generally RBC significantly decreased in rats treated with 250 and 500 mg kg^{-1} (Bwt) methanolic extract while WBC significantly increased ($p<0.001$) and in rats treated with 1000 mg kg^{-1} (Bwt) water extract WBC significantly increased ($p<0.001$). On the other hand, no significant changes were recorded in Hb, PCV, MCV and MCHC.

There was a significant increase in (ALP) activity and cholesterol concentration ($p<0.001$) and total protein ($p<0.05$) at the dose 500 mg kg^{-1} (Bwt) methanolic extract.

The inorganic phosphorus concentration increased significantly at the doses 250 and 500 mg kg^{-1} (Bwt) methanolic extract ($p<0.05$ and $p<0.001$), respectively.

At the dose 500 mg kg^{-1} (Bwt) water extract there was significant increase in cholesterol and Ca concentration ($p<0.01$ and $p<0.05$), respectively and no changes in other parameters

Histopathological changes in both methanolic and water extracts were similar with variation in the intensity.

Table 1: Body weights and relative organ weight of male albino rats treated with methanlic and water extracts of *Ambrosia maritima* for 3 weeks

	Dose (mg kg^{-1})	Body weight (g)	Liver (%)	Kidney (%)
Methanlic extract	0	96.5±12.6^{NS}	4.2±0.22^{NS}	0.95±0.06^{NS}
(Intramuscularly)	250	88.0±4.7^{NS}	4.6±0.18^{NS}	0.98±0.09^{NS}
	500	84.0±15.0^{NS}	4.8±0.60^{NS}	0.99±0.11^{NS}
Water extract	0	130.0±20.4^{NS}	3.4±0.40^{NS}	0.78±0.10^{NS}
(orally)	250	150.0±32.2^{NS}	3.5±0.20^{NS}	0.70±0.10^{NS}
	500	134.0±37.6^{NS}	4.1±0.30*	0.70±0.10^{NS}
	1000	132.5±29.0^{NS}	3.6±0.30^{NS}	0.70±0.14^{NS}

Values are means±SD, NS: Not Significant, *($p<0.05$)

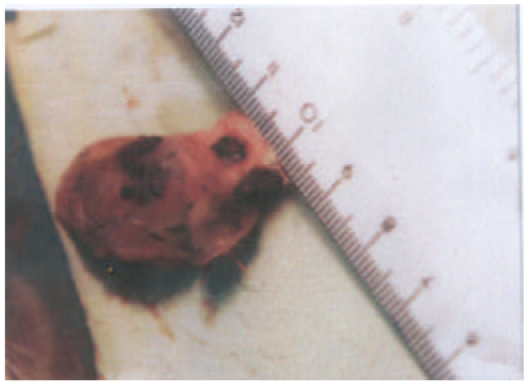

Fig. 1: Thigh muscle from rats receiving 500mg kg^{-1} Bwt *A. maritima* methanolic extract given I/M. Note: Darkness areas of dry gangrene

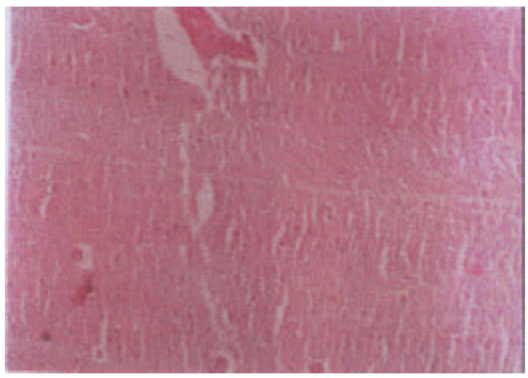

Fig. 2: Brain from rat receiving 500 mg kg^{-1} Bwt *A. maritimea* methanolic extract given I/M. Note: Congestion. (H and E×100)

Table 2a: Haematological changes in male albino rats treated with methanolic and water extracts of *Ambrosia maritima* for 3 weeks

	Dose (mg kg^{-1} Bwt)	TP (g 100 mL^{-1})	ALP (U L^{-1})	Cholesterol (mg d^{-1}l)	Ca (mg 100 mL^{-1})	P (mg 100 mL^{-1})
Methanolic	0	6.20.±7^{NS}	325.0±16.7^{NS}	81.4±1.3^{NS}	8.2±2.3^{NS}	2.7±0.7^{NS}
extract(I M^{-1})	25	6.5±0.5^{NS}	350.0±15.3^{NS}	79.7±1.9^{NS}	8.6±1.4^{NS}	5.1±0.3*
	500	8.1±1.4*	774.0±9.1***	115.5±4.9***	9.9±0.9^{NS}	9.4±0.6***
Water extract	0	6.6±0.4^{NS}	343.0±15.8^{NS}	73.4±5.3^{NS}	7.6±0.3^{NS}	4.9±0.2^{NS}
(orally)	250	6.1±0.1^{NS}	342.0±1.6^{NS}	71.5±15.^{NS}	7.7±0.2^{NS}	4.9±0.1^{NS}
	500	6.7±0.1^{NS}	343.3±16.8^{NS}	93.4±3.6**	8.1±0.2*	4.9±0.2^{NS}
	1000	6.8±0.1^{NS}	342.5±9.1^{NS}	73.9±4.2^{NS}	8.1±0.4^{N}	4.9±0.1^{NS}

Values are mean±SD, *p<0.05, **p<0.01, ***p<0.001, NS: Not Significant

Table 2b: Serum changese in male albino rats treated with methanolic and water extracts of *Ambrosia maritima* for 3 weeks

	Dose (mg kg^{-1})	PCV (%)	Hb (g d^{-1})	RBC (10^{6})	WBC (10^{3})	MCV (%)	MCHC (%)
Methanolic	0	27.7±0.6^{NS}	11.9±0.8^{NS}	5.04±0.07^{NS}	2.0±0.13^{NS}	55.0±0.8^{NS}	42.5±5.0^{NS}
extract (I/M)	25	27.4±3.3^{NS}	10.3±1.2^{NS}	4.3±0.19***	4.7±0.27***	63.5±8.1^{NS}	38.0±6.2^{NS}
	500	27.8±5.6^{NS}	10.6±0.74^{NS}	4.3±0.21***	6.5±0.4***	64.9±12.3^{NS}	39.1±2.2^{NS}
	0	32.8±3.2^{NS}	11.6±0.6^{NS}	5.1±0.2^{NS}	1.6±0.2^{NS}	63.8±3.8^{NS}	35.5±3.3^{NS}
Water	250	34.6±1.4^{NS}	11.4±0.8^{NS}	5.2±0.2^{NS}	1.7±0.2^{NS}	66.6±3.8^{NS}	34.6±1.3^{NS}
extract (orally)	500	34.0±2.7^{NS}	11.4±0.8^{NS}	5.3±0.3^{NS}	1.7±0.2^{NS}	64.6±6.3^{NS}	33.4±3.1^{NS}
	1000	34.5±1.0^{NS}	12.1±0.2^{NS}	5.1±0.1^{NS}	2.6±0.2***	87.5±2.6^{NS}	34.8±1.5^{NS}

Values are mean±SD, *p<0.05, **p<0.01, ***p<0.001, NS: Not Significant

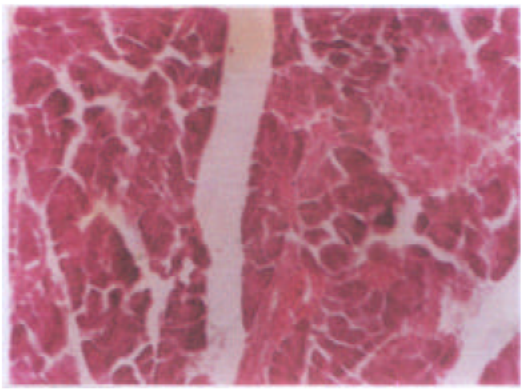

Fig. 3: Pancreas from rat receiving 1000 mg kg^{-1} Bwt *A. maritima* water extract given orally. Note: Congestion and hyperplasia of the pancreatic cells (H and E×400)

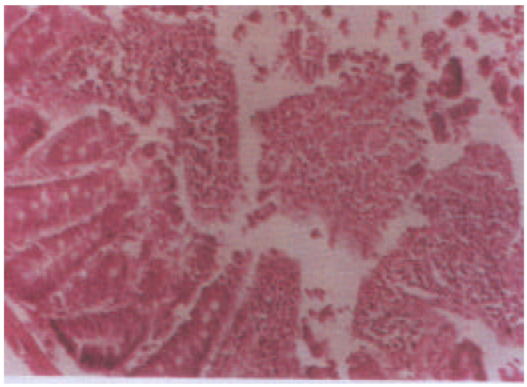

Fig. 4: Intestine from rat receiving 250 mg kg^{-1} Bwt *A. maritim*a water extract given orally. Note: Sloughing of the intestinal mucosal epithelia (H and E×100)

These included congestion and haemorrhage of the internal organs and the brain (Fig. 2).

Fatty changes in the liver, tubular necrosis, degeneration and mononuclear cellular infiltration in the kidney and hyperplasia of the pancreatic cells were observed (Fig. 3). Increased goblet cells in the intestinal mucosa (Fig. 4) were found in rats treated orally.

The present study demonstrated that 2000 mg kg^{-1} (Bwt) methanolic extract of *Ambrosia maritima* intramuscularly was lethal. This may be due to its endotheliotoxic effect of blood vessels.

The daily doses 250 and 500 mg kg^{-1} (Bwt) of the methanolic extract manifested its toxicity by clinical signs which are more pronounced at the dose 500 mg kg^{-1} (Bwt) as demonstrated by inappetence and lamenees. These signs may be attributed to the changes observed in the liver and muscle. On the other hand, the daily oral administration of water extract of *A. maritima* at a rate of 250, 500 and 1000 mg kg^{-1} (Bwt) were not lethal and less toxic as manifested by watery feaces. These suggest that *A. maritima* has the potential of causing toxicosis. In contrary Vassiliades and Diaw (1980) found that *A. maritima* caused no adverse effects in guppies and mice. The muscle lesions in rats injected with methanolic extract and intestinal erosion in the orally treated rats may be attributed to the bitterness of the plant which causes the irritation.

The mean body weights of the rats were not affected. This may be due to the nutritive value of the plant. This is in harmony with Bakhiet and Adam (1996) who reported improvement in body weights of chickens fed diet mixed with 2% of *A. maritima* but when *A. maritima* was increased to 10% to the feed the body weight was decreased.

Generalized congestion and haemorrhage were found in all organs including the brain in rats treated with both extracts. This may be due to influence of the plant constituents on the endothelium leading to injury and hence affect the blood vessels permeability. Similar lesions were reported in animals fed garlic or ginger, which were attributed to suppression in the synthesis of thromboxane and prostacycin (Ali and Mohammed, 1986).

The liver lesions were associated with significant increase in total protein and alkaline phosphatase activity in the intramuscularly treated rats at dose of 500 mg kg^{-1} (Bwt) but not in the orally treated groups, could mean that this concentration has a sensitizing effect on the liver cells leading to increase metabolic activity specially the amino acid synthesis (Ford *et al*., 1968).

The elevated activity of ALP in the group that received 500 mg kg^{-1} (Bwt) of the methanolic extract suggests hepatic dysfunction (Cornelius and Kaneko, 1963). Liver damage seen as fatty change is also manifested by the raised cholesterol level in rats treated by both extracts at the dose 500 mg kg^{-1} (Bwt) which was clearer in the methanolic extract.

Renal injury was demonstrated by degeneration of the tubuler epithelium. The decreased values of RBCs in the intramuscularly treated rats may be attributed to renal damage which affects erythropoitien production.

WBC count increased in the treated animals by both routes may ascribe to necrosis occurred specially at the site of contact of the plant with the tissue and it is possible that the plant constituent stimulate the immune system.

The pancreatic cells were hyperplastic in rats treated by either route. This may explain the folk use of the plant as hypoglycemic. Ahmed (1987) pointed out that 2% *A. maritima* leaves extract produced a notable reduction in blood glucose level in mice and rabbits.

Contrary to Hassieb *et al*. (1993) who stated that *A. maritima* was not toxic even at concentration of 1:500 in drinking water and Vassiliades and Diaw (1980) who stated that up to 1000 ppm had no toxic affects in mice we found that the plant has enterohepatic toxicity.

CONCLUSION

Although the extract of *A. maritima* results in signs of toxicity more careful investigation with lower doses is needed to test its safety. The investigation should also concentrate on isolation of ingredients rather than whole extract in order to identify the active non-harmful ingredients without side effects.

REFERENCES

Abu-Basha, L.M., M.H. El-Sayed, A.F. Allam and M.M. Osman, 1994. The effect of *Ambrosia maritime* (Damisissa) on the variability of *Lymnacea calliaudi*, an experimental study. J. Egyptian Soc. Parasitol., 24: 513.

Ahmed, H.E., 1987. Sesquiterpene lactones of *Ambrosia maritime L.* (*Compositae*). M.Sc. Thesis, University of Khartoum, Sudan.

Alard, F., C. Stievenant, P. Vanprays, L. Thilemans and S. Geerts, 1991. Toxicity and mutagenicity of the molluscicidal plant *Ambrosia martitima* L. Drug Chem. Toxicol., 14: 353.

Ali, M. and S.Y. Mohammed, 1986. Selective suppression of prostacycline by aqueous extract of garlic in rabbits prostaglandin. Lukat Med., 25: 139.

Bakhiet, A.O. and S.E.I. Adam, 1996. Effect of *Ambrosia maritime L.* on Bovan type Chicks. J. Herbs Spices and Med. Plants, 4: 51.

Chemie, D.G.K., 1972. J. Clin. Chem. Clin. Biochem., 10: 182-192.

Cornelius, C.E. and J.J. Kaneko, 1963. Clinical Biochemistry of Domestic Animals, Academic Press, New York and London.

Drury, R.N.B. and E.A. Wallington, 1980. Corleton's histo-pathological techniques. (4th Edn.), Oxford University Press, New York, Toronto.

El-Gazali, G.E.B., E.A. Bari, A.K. Bashir and A.M. Salih, 1987. Medicinal plants of Sudan Part II. Medicinal plants of Nuba Mountains, Medicinal and Aromatic Plant Research Institute, Khartoum, Sudan.

El Magdoub, A.A.I., M.F. Elsawy, H.K. Bassiouny, I.A. El Sayed, R.A. Galil and E.M. Hassan, 1977. An evaluation of the plant *Ambrosia maritima* as a molluscicidal. Irish Vet. J., 34: 157.

El Sawy, M.F., T.F.C. Duncan, M.R. Marshall and H.K. Bassiouny, 1984. The molluscidal properties of *Ambriosa martima* L. (Compisite) Results from a field trial using plant material. Trop. Med. Parasit., 35: 100.

Ford, E.J.H., H.E. Ritchic and E. Thorpe, 1968. Serum changes following the feeding of ragwort (*Senecio jacopoea*) to calves. J. Compar. Pathol., 78: 207.

Hassieb, M.M., A.A. El-Swak, E.M. El-Manakhly and D.A. Abdel-Aal, 1993. Patholgoical effects of the Egyptian wild herb.

Mendenhall, W., 1971. Introduction to probability and statistics (35th Edn.), Wadsworth Publishing Company Inc. Belmont California, USA.

Richmond, W., 1973. Clin. Chem., 19: 1350.

Schalm, O.W., 1965. Veterinary haematology. (4th Edn.) Lea and Febiyer Philadelphia, USA.

Trinder, P., 1960. Colorimetric micro determination of calcium in serum. Analyst, 85: 889.

Varley, 1963. J. Clin. Med., 27: 955.

Vassiliades, G. and O.T. Diaw, 1980. Molluscidal action of Senegalese strains of *Ambriosa martima* L. Laboraory trials. Revue, d Elevage et, de medicine vetrinaire, des. Pays, Tropic aus, 33: 401.

Weichselbaum, T.E., 1946. An accurate and rapid method for the determination of proteins in small amounts in blood serum and plasma. Am. J. Clin. Path., pp: 16.