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Comparative Toxicity of *Trichodesma africanum* and *Rhanterium epapposum* Aerial Parts Aqueous and Methanolic Extracts on Wistar Rats

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ABSTRACT

The effects on rats of the aqueous and methanol extracts prepared from *Rhanterium epapposum* and *Trichodesma africanum* aerial parts, given at different dose levels (300, 75 mg/kg/day) and by different routes of administration (orally or intra muscularly (i.m.) were investigated. The results indicated that the plant extracts are toxic and lethal to rats by whatever route (oral or im) it was given. The characteristic features of toxicity from aerial parts of *R. epapposum* and *T. africanum* were hepatonephrotoxicity, leukocytosis due to lymphocytosis or leukopenia due to neutropenia and anaemia. The anaemia was macrocytic normochromic, as indicated by the high Mean Corpuscular Volume (MCV) and normal Mean Corpuscular Hemoglobin Concentration (MCHC) values. These changes were evidenced by alterations in Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) activity and in total protein, albumin, globulin, cholesterol and urea concentrations. The occurrence of myositis brought about by the plant methanol extract given via intra muscular route probably contributed to the increase in AST activity. As conclude that the two extracts of both plant were toxic in both routs of administration and that, their toxicity result from the fact they contain chemical compound capable of damaging tissues and cells.

Key words: *Trichodesma africanum*, *Rhanterium epapposum*, toxicity, myositis, hepatonephrotoxicity

INTRODUCTION

Plants provide substitution for drugs in some rural areas. There is a rich abundance of plants reputed in traditional medicine to possess protective and therapeutic properties. The herbal medicine products are dietary supplements that people take to improve their health. There are many herbs have been used for a long time for claimed health benefits (Kayode and Kayode, 2011). However, some are not effective and some may cause health problems due to the increase and repeated use of them (Vaghasiya *et al*., 2011), this occurs as result of the fact that many medicinal plant contain toxic compounds like flavonoid, anthraquinones, tannins, triterpenes, sterols, alkaloids and volatile oil (Amna *et al*., 2011).

In tropical and subtropical countries, drought and the acute shortage of grass on pastures are frequent and these conditions may force animals to consume varying amounts of poisonous plants, which can cause damage to vital organs.

Abrus precatorius (Leguminosae) and *Jatropha curcas* (Euphorbiaceae) contain the phytotoxin, toxalbumins, abrin and curcin, respectively (Lin *et al*., 2003; Botha and Penrith, 2008). Ingestion of both plants by sheep, calves, goats and chicks causes catarrhal enteritis and hepatocellular necrosis (Joubert *et al*., 1984; Omer *et al*., 1992; Abdel Gadir *et al*., 2003), also it was reported that an ingestion of *J. tanjorensis* leaves lead to toxicological effects expressed as changes in weight, biochemical and ultrasonographic parameters of rabbits (Akhigbe *et al*., 2009) while plant parts of *A. precatorius* reported to be purgative, emetic and tonic (Arora *et al*., 2011).

Trichodesma africanum L., a member of the family Boraginaceae, is locally known as Hiraisha and is common in Western Sudan. Livestock grazing on *Trichodesma africanum* when other pasture plants are scarce. *Rhanterium epapposum* Oliv., a member of the family Asteraceae and locally known as Al-Arfaj, its widely distributed in various regions of Sudan and other Afro-Asian countries and used in local traditional medicine by people in rural areas as a remedy for skin infections and gastrointestinal disturbances and as an insecticide, Phytochemical analysis of the aerial parts of *R. epapposum* showed the presence of flavonoids, tannins, sterols, triterpenes and volatile oils (Al-Yahya *et al*., 1990).

Phytochemical analysis of the aerial parts of *T. africanum* demonstrated the presence of alkaloids, sterols, triterpenes, tannins and anthraquinones (El-Moaty, 2009) and pyrrolizidine alkaloid and other compounds (EFSA, 2007).

Hence, it appears those pyrrolizidine alkaloids are among the most widely distributed natural toxins affecting wildlife and livestock (Roeder, 2000).

The present study was planned to investigate the effects of various levels of aqueous and methanol extracts from the multipurpose medicinal plant, *R. epapposum* and the pasture plant, *Trichodesma africanum,* in Northern Kordofan State, on male Wistar rats for safety evaluation and nature of their effects.

MATERIALS AND METHODS

Plant material: *R. epapposum* and *T. africanum* aerial parts were collected from El-Hassahisa areas, Jazeera State and El Obeid areas, Northern Kordofan State, air-dried in the shade, ground separately by a mechanical grinder.

Animals and administration of plants material: Sixty, 2-month-old clinically healthy, male Wistar rats, were kept and fed with the premises of the Medicinal and Aromatic Plants Research Institute, National Research Center, Khartoum. The Rats were allotted at random to 9 groups, each of 6 rats.

Experimental design 1: Rats in group 1 served as untreated control. Rats in groups 2 and 3 were given aqueous extract of the aerial parts of the *R. epapposum* at 75 and 300 mg/kg/day via the oral route, respectively. The rats in groups 4 and 5 were given aqueous extract at 75 and 300 mg/kg/day via i.m. route.

The methanol extract dissolved in normal saline was administered to rats via oral route at 75 mg/kg/day (group 6) or 300 mg/kg/day (group 7) and intramuscularly at 75 mg/kg/day

(group 8) or 300 mg/kg/day (group 9). Dosing was continued until the rats died or were killed under diethyl ether anesthesia after 2 weeks of treatment.

Experimental design 2: The rats in group 1 were untreated controls. The aqueous extract from *T. africanum* aerial parts was given to rats orally at 75 mg/kg/day (group 2) and 300 mg/kg/day (group 3) and via i.m. route at 75 mg/kg/day (group 4) or 300 mg/kg/day (group 5).

The methanol extracts from plant aerial parts were administered in normal saline to rats via the oral route at 75 mg/kg/day (group 6) and 300 mg/kg/day (group 7) and via i.m. route at 75 mg/kg/day (group 8) and 300 mg/kg/day (group 9). Dosing was continued until the rats died or were killed under diethyl ether anesthesia after 2 weeks of treatment.

Average feed intake, body weight gain and feed efficiency were measured weekly for each group. Clinical signs and mortality rates were recorded. After one weeks of treatment, 3 randomly selected rats from each group were killed by decapitation. The remaining 3 rats/group were similarly killed after 2 weeks. Blood samples were collected from each of the killed rats for hematology and clinical chemistry. All rats were examined to identify gross lesions and specimens of the liver, kidneys, heart, spleen, intestines and muscles at site of injection, were fixed in 10% neutral buffered formalin and processed for histopathology.

Blood analysis: Hemoglobin (Hb) concentration, Packed Cell Volume (PCV), Red Blood Cells (RBCs), White Blood Cells (WBCs), differential WBC count, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were estimated by standard methods (Schalm *et al*., 1975).

Sera were analyzed for the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and concentrations of total protein, *albumin*, globulin, bilirubin, cholesterol and urea by using commercial kits (Linear Chemicals, Barcelona, Spain).

Pathological examinations: At necropsy, all rats were examined to identify gross lesions and specimens of liver, kidneys, heart, spleen and intestines were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 6 μm and stained with hematoxylin and eosin (H and E) for histopathologic examinations.

Statistical analysis: All measured data were subjected to the analysis using SPSS version 11.5, the significance of differences between means were compared at each for all groups, $p<0.05$ was considered statistically significant.

RESULTS

Effects on Wistar rats of *R. epapposum* extracts given by different routes of administration

Clinical findings: Depression, huddling together, reluctance to move, locomotor disturbances, paresis of the limbs and recumbency were first observed in rats receiving daily doses of water extract at 300 mg/kg/day orally (group 3, 7) and 300 mg/kg/day i.m. (group 5, 9) 6 days post-dosing. One rat in group 3 died 10 days post-dosing. In rats receiving 75 mg/kg/day i.m. (group 4) and

75 mg/kg/day orally (group 2), the symptoms were mild and no death occurred among the rats. Control (group 1) had no clinical signs and was killed with survivors in other groups on day 15.

Pathological changes: At necropsy, there was congestion or haemorrhage in the liver, kidneys and heart and varying degrees of muscular haemorrhage at the site of injection in groups 4, 5, 9 and 10. On microscopy, there was diffuse cytoplasmic vacuolation and necrosis of the hepatocytes, vacuolar degeneration, segmentation or necrosis of the glomerular tubules, acidophilic homogeneous material in affected renal tubules (Fig. 1) mild enteritis, myositis (Fig. 2) and accumulation of lymphocytes in vital organs.

Haematological changes: After treatment with water extract of the plant, the values of RBCs and PCV in groups 2 and 3 were lower ($p<0.05$) and MCV values were higher ($p<0.05$) in groups 3 and 4 than control and other groups. MCHC were significantly changed in all groups having higher values ($p<0.05$) compared to control. In two weeks, RBCs count were lower in groups 3, 4 and 5 compared to control and MCV was significantly higher in all groups shows significant increase in MCH groups 3 and 4 have lower p-value compared to control (Table 1).After treatment

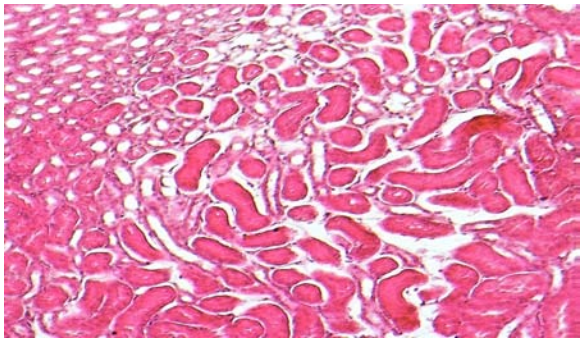

Fig. 1: Vacuolar degeneration and necrosis of renal tubules with acidophilic homogeneous substance in affected tubules of a rat receiving 300 mg/kg/day of methanol extract of *R. epapposum* via i.m. route for 6 days, H and E x200

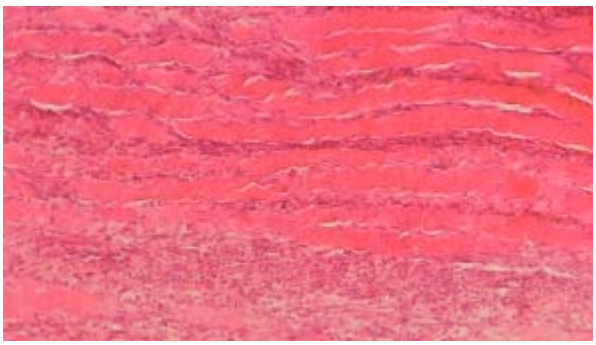

Fig. 2: Myositis at site of injection in a rat receiving 300 mg/kg/day of methanol extract of *R. epapposum* via i.m. route for 6 days, H and E x100

Table 1: Haematological changes in rats given *R. epapposum* aqueous and methanol extracts by different routes of administration for 2 weeks

	Groups (aqueous extract)					Groups (methanol extract)			
Parameters	1	2	3	4	5	6	7	8	9
One week									
Hb (g dL^{-1})	12.7±0.4^{ab}	11.2±0.44^{b}	10.3±0.6^{b}	14.0±0.29^{a}	11.3±0.17^{b}	14.8±0.44^{a}	15.5±0.29^{a}	14.0±0.28^{a}	14.3±0.17^{a}
RBC ($\times10^{6}$ mm^{3})	7.1±0.15^{a}	5.4±0.76^{b}	5.0±0.25^{b}	6.5±0.52^{a}	7.0±0.34^{a}	6.0±0.27^{b}	6.9±0.35^{a}	5.4±0.42^{c}	6.2±0.19^{ab}
PCV (%)	42.5±1.8^{a}	26.7±4.8^{b}	31.3±1.8^{ab}	41.3±2.7^{a}	34.7±1.8^{ab}	41.7±2.3^{a}	42.0±1.8^{a}	37.7±1.8^{b}	39.0±1.7^{b}
MCV (m^{3})	59.9±1.6^{ab}	49.4±6.2^{b}	62.1±5.10^{a}	63.5±5.6^{a}	49.3±6.2^{b}	69.9±0.67^{a}	60.6±1.3^{b}	69.8±0.59^{a}	62.6±0.7^{ab}
MCH (pg)	17.9±1.0^{b}	20.7±2.2^{a}	20.5±1.30^{a}	21.5±1.9^{a}	16.1±0.6^{b}	24.8±0.76^{a}	22.4±1.2^{a}	25.9±1.0^{a}	23.0±1.2^{a}
MCHC (%)	29.9±0.49^{b}	42.0±2.5^{a}	32.9±0.67^{ab}	34.0±0.76^{ab}	32.6±0.94^{ab}	35.5±0.88^{a}	36.9±0.49^{a}	37.1±0.95^{a}	36.7±1.4^{a}
WBC ($\times10^{3}$ mm^{3})	4.7±0.12^{a}	3.7±0.60^{b}	4.5±0.1^{ab}	3.5±0.2^{b}	5.4±0.9^{a}	5.3±0.63^{ab}	4.9±2.7^{a}	4.9±0.06^{b}	6.5±0.27^{a}
Neutrophils (%)	61.0±0.29^{a}	65.7±1.2^{a}	68.5±2.9^{a}	53.5±0.9^{b}	66.5±2.5^{a}	54.4±7.2^{b}	65.3±0.52^{a}	52.5±6.6^{b}	62.2±5.3^{a}
Lymphocytes (%)	39.0±0.87^{ab}	34.3±0.58^{b}	31.5±3.2^{b}	46.5±1.0^{a}	33.5±2.0^{b}	45.6±1.8^{a}	34.7±0.52^{b}	47.5±1.6^{a}	37.8±1^{b}
Two weeks									
Hb (g dL^{-1})	12.7±0.35^{a}	12.7±0.35^{a}	11.5±0.8^{a}	10.1±0.25^{b}	11.1±0.69^{a}	12.4±0.95^{a}	11.8±0.13^{a}	12.7±0.92^{a}	12.1±0.6^{a}
RBC ($\times10^{6}$ mm^{3})	7.1±0.15^{a}	6.3±0.35^{ab}	5.7±0.27^{b}	5.8±0.2^{b}	4.5±0.23^{c}	6.5±0.26^{ab}	5.3±0.11^{b}	6.0±0.89^{b}	5.6±0.67^{b}
PCV (%)	42.5±1.8^{a}	40.0±10^{a}	37.0±1.7^{ab}	36.0±1.5^{ab}	34.5±0.29^{b}	40.0±1.7^{a}	37.0±1.5^{b}	36.0±3.0^{b}	34.5±2.6^{b}
MCV (m^{3})	59.9±1.6^{b}	63.5±1.9^{ab}	64.9±6.3^{ab}	62.1±0.5^{ab}	76.7±3.0^{a}	60.0±2.0^{b}	86.8±0.98^{a}	65.5±0.29^{ab}	66.1±1.5^{ab}
MCH (pg)	17.9±1^{b}	20.2±1.3^{ab}	20.2±0.9^{ab}	22.9±0.95^{a}	24.7±0.9^{a}	19.0±0.76^{b}	22.3±0.9^{a}	21.2±0.88^{a}	21.6±1^{b}
MCHC (%)	29.9±0.49^{b}	31.8±0.75^{a}	31.1±0.95^{a}	28.1±0.5^{b}	32.2±0.9^{a}	31.8±1.2^{ab}	76.7±0.35^{a}	28.1±0.79^{b}	32.2±0.42^{ab}
WBC ($\times10^{3}$ mm^{3})	4.7±0.12^{a}	3.5±0.36^{ab}	4.3±0.12^{a}	4.4±0.21^{a}	3.0±0.26^{b}	3.6±0.3^{b}	2.7±0.11^{c}	5.2±0.9^{a}	5.2±0.42^{a}
Neutrophils (%)	61.0± 0.29^{a}	59.0±3.7^{a}	52.0±0.50^{b}	53.0±0.29^{b}	59.8±0.87^{a}	54.3±3.8^{ab}	59.8±1.2^{ab}	50.3±7.6^{b}	54.0±0.91^{ab}
Lymphocytes (%)	39.0±0.87^{b}	41.0±0.87^{b}	48.0±5.10^{a}	47.0±0.78^{a}	40.2±1.2^{b}	45.7±0.74^{a}	40.2±0.18^{b}	49.7±1.5^{a}	46.0±0.47^{a}

Values are Mean±SE, Means within rows with no common letter(s) are significantly different ($p<0.05$), Hb: Hemoglobin, RBC: Red blood cells, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, WBC: White blood cells

with water extract of the plant, the values of RBCs and PCV in groups 2 and 3 were lower ($p<0.05$) and MCV values were higher ($p<0.05$) in groups 3 and 4 than control and other groups. MCH and MCHC were higher value in all groups compared to control. lymphocytes in groups 7 and 9 were slightly lower than other groups. In two weeks PCV value in groups 7, 8 and 9 has lower p value than other groups, group 7 shows the most significant increase in MCV and the lowest p value for WBCs count. After one week of treatment, by methanolic extracts the values of RBCs in groups 8 and 9 and PCV in group 8 were lower ($p<0.05$) than control and other groups. MCV values were higher.

Changes in serum constituents: These data are presented in Table 2. After one week of treatment with aqueous extract, the activities of AST and ALP in groups 2, 3, 4 and 5 and ALT in group 3 were higher ($p<0.05$) than control and other groups. The concentration of albumin in group 4 was lower ($p<0.05$) and that of globulin was higher ($p<0.05$) in groups 2,3,4 and 5 than other groups. After 2 weeks of treatment, the activities of AST were higher ($p<0.05$) in groups 2-5. The activity of ALP was lower in groups 2, 4 and 5 and total protein concentration was higher ($p<0.05$) in group 2 than other groups. Globulin concentration was higher ($p<0.05$) in groups 3 and 4 than other groups. Urea was higher ($p<0.05$) in groups 2, 3 and 5 than other groups.

After one week of treatment with methanol extract, the activities of AST and ALP in groups 6-9 and of ALT in groups 8 and 9 were higher ($p<0.05$) than control and other groups. The concentrations of globulin were higher ($p<0.05$) in groups 6, 8 and 9 than control. Cholesterol concentration in group 6 was the lowest ($p<0.05$) among all groups. After 2 weeks of treatment, the

Table 2: Changes in serum constituents of rats given *R. epapposum* aqueous and methanol extracts by different routes of administration for 2 weeks

	Groups (aqueous extract)					Groups (methanol extract)			
Parameters	1	2	3	4	5	6	7	8	9
One week									
AST (IU)	26.8±1.3^{b}	49.4±2.2^{a}	48.3±2.4^{a}	35.7±1.9^{ab}	37.3±1.8^{ab}	50.6±2.1^{a}	54.7±1.9^{a}	38.4±1.1^{ab}	49.7±2.2^{a}
ALT (IU)	14.6±1.3a^{b}	15.7±1.5^{ab}	20.9±1.2^{a}	7.0±0.9^{c}	12.1±1.3^{b}	17.7±1.4^{ab}	18.0±1.9^{ab}	32.0±2.3^{a}	15.7±1.1^{b}
ALP (IU)	339.4±4.7^{ab}	137.1±3.6^{c}	427.2±4.2^{a}	244.3±4.6^{b}	187.7±3.3^{bc}	200.0±0.9	183.0±1.9^{b}	241.0±3.1^{ab}	112±3.2^{c}
Total protein (g dL^{-1})	5.3±1.2^{b}	7.2±0.9^{b}	8.55±1.1^{a}	6.83±1.2^{ab}	8.1±1.3^{a}	6.1±1.7^{a}	5.8±1.3^{a}	6.4±1.9^{a}	5.9±1.6^{a}
Albumin (g dL^{-1})	2.4±0.9^{b}	2.5±0.9^{b}	4.15±1.1^{a}	2.0±0.4^{c}	2.9±0.5^{ab}	1.3±1.7^{a}	2.4±0.02^{a}	2.3±0.05^{ab}	1.32±0.05^{b}
Globulin (g dL^{-1})	2.9±0.9^{c}	4.7±1.2^{a}	4.4±1.1^{a}	4.83±1.2^{a}	5.2±1.3^{ab}	4.8±1.1^{ab}	3.4±1.1^{b}	4.1±1.3^{a}	4.58±1.2^{ab}
Bilirubin (mg dL^{-1})	0.93±0.03^{ab}	0.5±0.05^{b}	0.7±0.06^{ab}	0.88±0.4^{ab}	1.5±0.03^{a}	0.8±0.04^{ab}	0.5±0.01^{b}	1.2±0.09^{a}	0.76±0.04^{ab}
Cholesterol (mg dL^{-1})	73.4±2.9^{ab}	85.9±3.3^{a}	69.7±2.1^{ab}	65.2±2.2^{b}	74.1±2.1^{ab}	51.8±1.9^{b}	65.2±1.9^{ab}	69.6±2.1^{ab}	75.6±2.5^{a}
Urea (mg dL^{-1})	51.4±1.9^{a}	54.8±2.0^{a}	56.2±1.7^{a}	53.6±1.5^{a}	56.7±1.5^{a}	58.1±1.7^{a}	42.0±1.4^{b}	57.2±1.9^{a}	55.0±1.6^{ab}
Two weeks									
AST (IU)	26. ±1.3^{c}	38.4±1.9^{b}	54.7±2.2^{a}	50.1±2.1^{a}	46.3±2.4^{ab}	54.5±1.9^{a}	37.2±1.1^{ab}	40.5±1.4^{ab}	57.4±2.1^{a}
ALT (IU)	14.6±1.9^{b}	12.0±1.5^{ab}	8.7±0.8^{c}	27.9±1.9^{a}	12.2±1.3^{b}	8.7±1.0^{c}	23.9±1.1^{ab}	36.7±1.3^{a}	31.4±1.4^{a}
ALP (IU)	339±4.7^{a}	211.6±4.3^{b}	339.0±5.1^{a}	155.0±3.3^{c}	264.0±4.1^{ab}	281.0±1.9^{b}	395.0±4.5^{a}	222.0±2.7^{c}	288.0±3.6^{b}
Total protein (g dL^{-1})	5.3±1.2^{ab}	3.9±1.1^{b}	6.7±1.4^{a}	6.5±1.3^{a}	5.7±0.9^{ab}	7.0±0.8^{ab}	7.2±0.6^{ab}	9.0±1.2^{a}	6.6±0.7^{b}
Albumin (g dL^{-1})	2.4±0.8^{b}	2.8±0.7^{a}	2.5±0.6^{b}	2.5±0.8^{ab}	2.7±0.9^{a}	2.6±0.2^{a}	2.4±0.1^{ab}	2.1±0.05^{b}	2.8±0.06^{a}
Globulin (g dL^{-1})	2.9±0.7^{ab}	1.1±0.06^{b}	4.2±1.1^{b}	4.0±0.9^{a}	3.0±0.9^{ab}	4.4±0.8^{b}	4.8±0.9^{ab}	6.9±0.7^{a}	3.8±0.5^{bc}
Bilirubin (mg dL^{-1})	0.9±0.04^{ab}	1.2±0.04^{a}	0.9±0.05^{ab}	0.8±0.02^{ab}	0.6±0.01^{b}	1.3±0.07^{ab}	1.4±0.07^{ab}	1.8±0.06^{a}	1.2±0.04^{ab}
Cholesterol (mg dL^{-1})	73.4±2.5^{ab}	84.4±3.3^{a}	77.1±2.1^{ab}	73.4±2.2^{ab}	72.6±1.5^{b}	80.0±3.1^{a}	63.7±1.9^{b}	64.5±1.8^{b}	71.1±2.0^{ab}
Urea (mg dL^{-1})	51.4±1.9^{a}	83.0±3.1^{a}	71.0±2.2^{ab}	54.3±2.4^{b}	68.0±1.8^{ab}	33.8±1.5^{b}	53.8±1.9^{ab}	48.6±1.6^{ab}	57.1±0.7^{a}

Values are Mean±SE, Means within rows with no common letter(s) are significantly different ($p<0.05$), AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase

activities of AST in groups 6-9 and those of ALT in groups 7-9 were higher ($p<0.05$) than control and other groups. The concentration of total protein and globulin were higher ($p<0.05$) in all groups compared to the control. Group 6 shows the most significant decrease in urea concentration.

Effects on Wistar rats of *T. africanum* extracts given by different routes of administration

Clinical findings: Huddling together, depression, inability to move, locomotors disturbances, paresis of the limbs and recumbency were first seen in the rats receiving daily doses of aqueous extract at 300 mg kg^{-1} orally (group 3) and 300 mg kg^{-1} i.m. (group 5) 5 days post-dosing. Two rats from group 3 died on day 10. The clinical signs were mild in rats of groups 2 and 4 receiving the aqueous extract of the plant at 75 mg kg^{-1} *per os* or 75 mg/kg/day i.m. Survivors were killed 15 days post-dosing.

One rat receiving 300 mg/kg/day orally of methanol extract (group 7) died on day 10 but none of the rats receiving 300 mg/kg/day i.m. of methanol extract (group 9) died during this period and manifested obvious clinical signs of locomotor disturbances, paresis of the limbs and depression. The rats given 75 mg/kg/day orally (group 6) or 75 mg/kg/day i.m. of methanol extract (group 8) for 15 days showed less marked clinical disturbances. Survivors were killed 15 days post- dosing.

Pathologic changes: Post-mortem findings in rats receiving *T. africanum* water or methanol extract by different routes of administration included congestion or haemorrhage in the liver,

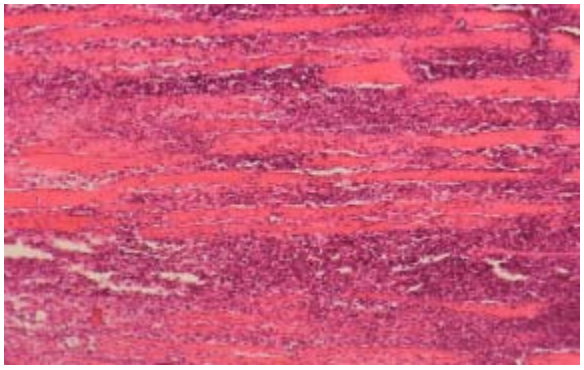

Fig. 3: Severe lymphocytic infiltration in a rat injected i.m. with 300 mg/kg/day of *T. africanum* methanol extract for 2 weeks, H and E x100

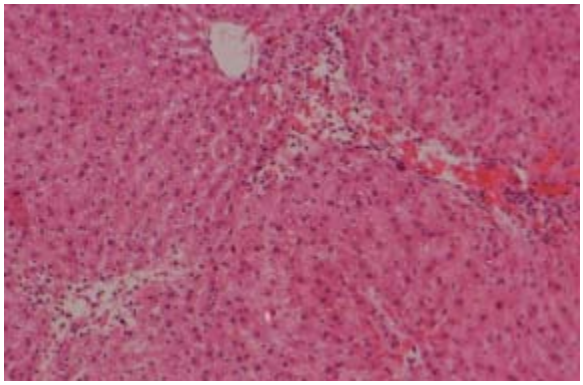

Fig. 4: Liver of rats receiving *T. africanum* oral doses of aqueous extract 300 mg kg^{-1} for 2 weeks, showing, fatty cytoplasmic vacuolation of the centrilobular hepatocytes, necrosis and hemorrhage, H and E x200

kidneys, heart and intestines and muscular haemorrhage at the sites of injection and atrophy in aqueous extract-dosed rats of groups 3, 4, 5 and methanol-dosed rats in groups 7-9. On microscopy, there was haemorrhagic myositis with lymphocytic accumulation (Fig. 3) between the muscle fibres that showed necrosis particularly in groups 4, 5, 7 and 9. Diffuse cytoplasmic vacuolation and necrosis of the hepatocytes (Fig. 4), vacuolar degeneration and necrosis of the renal tubules, splenic haemosiderosis, catarrhal enteritis and accumulation of lymphocytes in vital organs were observed. These changes were less marked in groups 2 and 6. Control rats (group 1) showed no significant lesions.

Haematological changes: After one week of treatment with aqueous extract,, the values of RBCs and PCV were lower ($p<0.05$) in groups 2, 4 and 5 than control and other groups. The values of MCHC in groups 2, 4 and 5 were higher ($p<0.05$) than control. The values of WBCs in groups 4 and 5 were lower ($p<0.05$) than other groups. After 2 weeks of treatment, values of RBCs were lower ($p<0.05$) in groups 2, 4 and 5 and PCV values were lower ($p<0.05$) in groups 2-5 than other groups. The values of MCV were higher ($p<0.05$) in groups 2 and 4 (Table 3).

Table 3: Haematological changes in rats given *T. africanum* aqueous and methanol extracts by different routes of administration for 2 weeks

	Groups (aqueous extract)					Groups (methanol extract)			
Parameters	1	2	3	4	5	6	7	8	9
One week									
Hb (g dL^{-1})	12.7±0.4^{ab}	13.2±0.2^{a}	12.8±0.5^{a}	13.0±0.3^{a}	12.8±0.6^{a}	13.3±0.3^{a}	13.7±0.3^{a}	14.0±0.4^{a}	12.5±0.5^{a}
RBC ($\times10^{6}$ mm^{3})	7.1±0.15^{a}	5.2±0.3^{b}	6.2±0.4^{ab}	5.7±0.4^{ab}	5.8±0.3^{ab}	6.1±0.4^{b}	6.0±0.3^{b}	6.2±0.5^{b}	6.6±0.6^{ab}
PCV (%)	42.5±1.8^{a}	33.7±1.8^{b}	40.3±2.2^{a}	35.7±1.5^{b}	32.5±1.2^{b}	40.3±2.2^{a}	39.3±1.5^{a}	35.3±1.4^{b}	36.3±1.5^{b}
MCV (m^{3})	59.9±1.6^{ab}	64.8±1.3^{a}	64.7±1.2^{a}	62.6±1.5^{ab}	56.5±1.3^{b}	66.0±1.4^{a}	65.5±1.4^{a}	56.9±1.4^{b}	55.0±1.5^{b}
MCH (pg)	17.9±1^{b}	25.4±0.8^{a}	20.5±0.6^{ab}	22.8±0.5^{a}	22.3±1.3^{a}	21.8±1.4^{a}	22.8±1.3^{a}	22.6±1.5^{b}	18.9±1.2^{ab}
MCHC (%)	29.9±0.49^{b}	39.2±0.9^{a}	31.8±0.7^{b}	36.4±0.8^{ab}	39.4±0.7^{a}	33.0±0.4^{ab}	34.9±0.3ab	39.7±0.4^{a}	34.4±0.3^{a}
WBC ($\times10^{3}$ mm^{3})	4.7±0.12^{a}	3.0±0.8^{b}	3.0±0.8^{b}	6.3±0.6^{a}	6.0±0.3^{a}	3.0±0.2^{c}	3.7±0.3^{b}	4.3±0.4^{ab}	5.9±0.5^{a}
Neutrophils (%)	61.0±0.29^{a}	62.0±0.7^{a}	60.8±0.5^{b}	59.3±0.6^{b}	62.5±0.4^{a}	61.8±0.6^{a}	55.0±0.7^{b}	62.7±0.7^{a}	67.0±0.6^{a}
Lymphocytes (%)	39.0±0.87^{ab}	38.0±0.3^{b}	39.2±0.2^{b}	40.7±0.4^{b}	37.5±0.2^{b}	38.2± 0.3^{b}	45.0±0.6^{a}	37.3±0.4^{b}	33.0±0.8^{c}
Two weeks									
Hb (g dL^{-1})	12.7±0.35^{a}	10.4±0.3^{b}	12.3±0.3^{a}	11.3±0.4^{ab}	9.3±0.2^{b}	13.1±0.2^{a}	12.0±0.3^{a}	12.7±0.2^{a}	10.2±0.4^{b}
RBC ($\times10^{6}$ mm^{3})	7.1±0.15^{a}	5.4±0.2^{b}	6.6±0.3^{ab}	5.6±0.4^{b}	5.0±0.3^{b}	5.8±0.3^{ab}	7.7±0.5^{a}	5.3±0.3^{ab}	4.7±0.4^{b}
PCV (%)	42.5±1.8^{a}	36.3±1.6^{a}	35.0±1.4^{ab}	35.0±1.5^{ab}	33.3±1.2^{b}	43.3±2.2^{a}	40.0±1.6^{a}	39.3±1.5^{b}	36.3±1.5^{b}
MCV (m^{3})	59.9±1.6^{b}	67.2±1.4^{a}	53.0±1.3^{b}	62.5±1.5^{a}	65.7±1.4^{a}	74.7±1.5^{a}	52.0±1.2^{b}	69.0±1.6^{a}	75.6±1.8^{a}
MCH (pg)	17.9±1^{b}	19.3±0.9^{a}	18.6±0.7^{a}	20.2±0.6^{a}	18.3±0.8^{a}	22.6±1.4^{a}	15.6±1.6^{b}	24.0±1.3^{a}	21.7±1.4^{a}
MCHC (%)	29.9±0.49^{b}	28.7±0.7^{a}	32.2±0.6^{a}	32.3±0.2^{a}	27.9±0.3^{a}	30.3±0.3^{a}	30.0±2.0^{b}	32.3±0.4^{a}	28.0±0.2^{a}
WBC ($\times10^{3}$ mm^{3})	4.7±0.12^{a}	3.5±0.7^{b}	4.4±0.6^{ab}	3.4±0.7^{b}	4.5±0.5^{a}	3.4±0.3^{b}	2.8±0.1^{c}	5.7±0.4^{a}	4.8±0.1^{ab}
Neutrophils (%)	61.0± 0.29^{a}	59.0±0.3^{b}	60.5±0.2^{ab}	64.0±0.6^{a}	61.0±0.5^{ab}	52.5±0.6^{ab}	49.5±0.5^{b}	57.3±0.7^{ab}	64.0±0.8^{a}
Lymphocytes (%)	39.0±0.87^{b}	41.0±0.2^{a}	39.5±0.3^{a}	36.0±0.4^{b}	39.0±0.3^{a}	47.5±0.5^{ab}	50.5±0.4^{a}	42.7±0.3^{ab}	36.0±0.6^{b}

Values are Mean±SE, Means within rows with no common letter(s) are significantly different ($p<0.05$), Hb: Hemoglobin, RBC: Red blood cells, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, WBC: White blood cells

After one week of treatment with methanol extract, PCV values were lower ($p<0.05$) in groups 8 and 9 than other groups. MCHC values were higher ($p<0.05$) in all groups compared to the control.

After 2 weeks of treatment with methanol extract of *T. africanum,* the values of RBCs were lower ($p<0.05$) in groups 6, 8 and 9. The values of PCV were lower ($p<0.05$) in group 9 and MCV values were higher ($p<0.05$) in all groups. Group 7 shows the lowest p-value in neutrophils and consequently in lymphocytes. The values of WBCs were lower ($p<0.05$) in groups 6 and 7 and those of lymphocytes were higher ($p<0.05$) in groups 6, 7 and 8 than other groups.

Changes in serum constituents: After one week of treatment with aqueous extract of the plant, the activities of AST in groups 2 and 3 and those of ALT in groups 4 and 5 were higher ($p<0.05$) than other groups. The activity of ALP was lower ($p<0.05$) in groups 2, 4 and 5 than control and other groups. The concentration of total protein was significantly increased in group 2 and that of globulin was higher ($p<0.05$) in all groups (Table 4).

After 2 weeks of treatment, the activities of AST were higher ($p<0.05$) in all groups. The activity of ALP was lower in groups 2, 3 and 5. Bilirubin concentration was lower in groups 3 and 4. Urea was lower ($p<0.05$) in groups 2-5 compared to the control.

After one week of treatment with methanol extract, the activities of AST and ALP were higher ($p<0.05$) in all groups but only group 7 and 8 shows significant change in regard to ALT. The

Table 4: Changes in serum constituents of rats given aqueous and methanol extracts of *T. africanum* by different routes of administration for 2 weeks

	Groups (aqueons extract)					Groups (methanol extract)			
Parameters	1	2	3	4	5	6	7	8	9
One week									
AST (IU)	26.8±1.3^{b}	40.2±0.98^{ab}	54.7±0.9^{a}	26.8±0.4^{b}	22.9±0.6^{c}	60.4±0.6^{a}	60.0±1.1^{a}	36.3±1.3^{ab}	55.3±1.2^{a}
ALT (IU)	14.6±1.3a^{b}	17.5±0.5^{ab}	18.3±0.4^{ab}	29.7±0.9^{a}	33.2±0.4^{a}	16.3±1.3^{a}	7.6±0.3^{b}	18.7±0.9^{a}	7.3±0.4^{b}
ALP (IU)	339.4±4.7^{ab}	268.0±1.5^{bc}	347±1.5b^{c}	535±4.1^{a}	280.0±2.7^{b}	411.0±2.0^{a}	198.1±1.4^{bc}	151.0±2.6^{c}	220.0±2.0^{b}
Total protein (g dL^{-1})	5.3±1.2^{b}	10.0±1.0^{a}	5.70±0.7^{b}	7.10±0.6^{ab}	7.0±0.6^{ab}	7.9±0.8^{ab}	6.9±0.26^{b}	8.1±0.5^{a}	7.8±0.4^{a}
Albumin (g dL^{-1})	2.4±0.9^{b}	2.6±0.21^{a}	1.75±0.05^{c}	2.15±0.15^{b}	2.1±015^{b}	2.4±0.23^{ab}	3.1±0.3^{a}	2.3±0.4^{ab}	2.1±1.5^{b}
Globulin (g dL^{-1})	2.9±0.9^{c}	7.4±0.4^{a}	3.95±0.05^{b}	4.95±0.5^{ab}	4.9±0.6^{ab}	5.5±0.05^{a}	3.8±0.05^{ab}	5.8±0.1^{a}	5.7±0.05^{a}
Bilirubin (mg dL^{-1})	0.93±0.03^{ab}	0.9±0.01^{a}	0.35±0.005^{b}	1.05±0.15^{a}	0.9±0.15^{a}	0.6±0.05^{c}	0.5±0.05^{c}	0.7±0.12^{b}	0.7±0.05^{ab}
Cholesterol (mg dL^{-1})	73.4±2.9^{ab}	74.1±0.49^{b}	74.1±0.9^{b}	84.5±0.8^{a}	78.5±0.8^{ab}	69.6±2^{ab}	50.4±2.1^{b}	71.1±1.4^{ab}	80.2±1.2^{a}
Urea (mg dL^{-1})	51.4±1.9^{a}	62.8±0.75^{ab}	34.3±0.4^{c}	43.8±0.4^{bc}	76.6±0.8^{a}	80.3±1.8^{a}	58.1±1.5^{b}	58.1±1.5^{b}	66.7±0.9^{ab}
Two weeks									
AST (IU)	26. ±1.3^{c}	60.3±0.9^{a}	44.8±0.99^{b}	68.7±0.3^{a}	44.8±0.9^{b}	33.4±1.1^{ab}	62.5±1.2^{a}	31.4±0.6^{ab}	68.6±1.1^{a}
ALT (IU)	14.6±1.9^{b}	19.3±9.5^{ab}	28.0±0.29^{a}	7.9±0.2^{c}	15.8±0.4^{b}	20.4±0.7^{ab}	12.8±0.6^{bc}	30.8±0.4^{a}	6.4±0.2^{c}
ALP (IU)	339±4.7^{a}	204.0±1.5^{ab}	295.0±1.7^{b}	328.7±1.7^{ab}	170.2±0.9^{c}	358.0±1.5^{a}	255.8±2.0^{bc}	234.0±2.0^{c}	269.0±2.0^{b}
Total protein (g dL^{-1})	5.3±1.2^{ab}	6.3±0.4^{a}	5.4±0.3^{b}	5.7±0.4^{ab}	6.7±0.15^{a}	6.0±0.32^{ab}	6.0±1.0^{ab}	6.8±0.15^{a}	6.8±0.2^{a}
Albumin (g dL^{-1})	2.4±0.8^{b}	2.6±0.4^{b}	2.2±0.15^{b}	2.7±0.1^{a}	2.1±0.12^{b}	2.8±0.15^{a}	2.8±0.15^{a}	2.7±0.17^{a}	2.7±0.3^{a}
Globulin (g dL^{-1})	2.9±0.7^{ab}	2.7±0.05^{a}	3.2±0.4a^{b}	3.0±0.5^{ab}	4.6±0.2^{a}	3.2±0.9^{ab}	3.2±0.25^{ab}	4.1±0.5^{a}	4.1±0.05^{a}
Bilirubin (mg dL^{-1})	0.9±0.04^{ab}	0.7±0.4^{b}	0.4±0.05^{b}	0.4±0.05^{b}	0.8±0.15^{a}	0.7±0.05^{b}	0.6±0.9^{b}	1.4±0.3^{ab}	2.0±0.29^{a}
Cholesterol (mg dL^{-1})	73.4±2.5^{ab}	72.6±0.05^{ab}	84.5±0.7^{a}	71.1±0.7^{ab}	65.2±1^{b}	81.5±1.3^{a}	71.1±0.95^{ab}	68.2±0.9^{b}	69.6±1.5^{b}
Urea (mg dL^{-1})	51.4±1.9^{a}	39.0±0.8^{ab}	40.7±0.4^{ab}	40.0±1.2^{ab}	36.7±0.9^{b}	50.5±0.8^{a}	41.4±0.7^{b}	51.0±0.8^{a}	41.4±0.8^{b}

Values are means±SE, Means within rows with no common letter(s) are significantly different ($p<0.05$), AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase

activity of ALP was higher ($p<0.05$) in group 6 and lower ($p<0.05$) in groups 7-9 than other groups. The concentrations of total protein and globulin in all groups were higher ($p<0.05$) than control. Urea concentration was higher ($p<0.05$) in group 6. After 2 weeks of treatment, the activities of AST and ALT were lower P value ($p<0.05$) in all groups. The activity of ALP was lower ($p<0.05$) in groups 7, 8 and 9 than other groups. The concentration globulin in all groups were higher ($p<0.05$) than control.

DISCUSSION

In the present study, the effects on rats of the aqueous and methanol extracts prepared from *R. epapposum* and *T. africanum* aerial parts and given at different dose levels by different routes of administration were investigated similar to other finding in regard to toxicity of *R. epapposum* to Wistar rats (Younis and Adam, 2008). The results indicated that the plant extracts are toxic and lethal to rats by whatever route (orally and i.m.) it was given. Previous phytochemical investigations of *T. africanum* have demonstrated the presence of pyrrolizidine alkaloid trichodesmine, β-amyrin, β-methyl oleanate, potassium nitrate, a nitrogen containing toxin, β-sitosterol and stigmasterol (Omar *et al*., 1983). *R. epapposum* contains flavonoids, tannins, sterols, triterpenes and volatile oils (Ageel *et al*., 1987; Al-Yahya *et al*., 1990).

The characteristic features of the extracts from aerial parts of *R. epapposum* and *T. africanum* were hepatonephrotoxicity, leukocytosis due to lymphocytosis or leukopenia due to neutropenia and anaemia. The anaemia was macrocytic normochromic, as indicated by the high MCV and normal

MCHC values. These changes were evidenced by alterations in AST, ALT and ALP activity and in total protein, albumin, globulin, cholesterol and urea concentrations. The occurrence of myositis brought about by the plant methanol extract given via i.m. route probably contributed to the increase in AST activity.

The presence of haemosiderin deposits in red pulp of the spleen might have resulted from slight destruction of RBCs by unknown toxic constituent (s) in aerial parts of *T. africanum*. It seems likely that splenic haemosiderosis might have been caused by saponin present in the extract prepared from the plant aerial parts. Iron was believed to be released from lysed RBCs to the circulation and passed to urine, perhaps bound to transferrin (Abu Damir *et al*., 1993).

Nakhla *et al*. (1991, 1992) conducted toxicologic studies on *Balanites aegyptiaca* and *Trigonella foenum-graecum*, popular medicinal plants in Sudan and other countries and found that injection by different routes of administration of the saponin in chicks caused hepatonephropathy and anaemia. Neither *Trigonella* saponin nor *Balanites* saponin caused bilirubinaemia probably due to the presence of lesions in the hepatic centrilobular zone of the test chicks. Tariq *et al*. (1985) injected rats via intraperitoneal route with ethanol and chloroform extracts from *T. anguina* and observed that both extracts produced sedation accompanied by rapid respiration.

Galal *et al*. (1991) were able to prepare ethanol extract as well as other fractions from the bark of *Albizia anthelmintica* to compare their toxicity in rats. These authors found that the butanolic fraction was more toxic and lethal to rats than ethanol extract and that both extracts caused varying degrees of hepatonephropathy.

Further studies are necessary to isolate and characterize the constituents in plants aerial parts and elucidate their exact modes of action and interaction.

CONCLUSION

The aerial parts of *R. epapposum* were toxic but not lethal, to Wistar rats at the concentrations in the test diets. The toxicity from *T. africanum* aerial parts was severe as evidenced by inefficiencies of food utilization, extensive tissue damage, significant serobiochemical and haematological changes and death among the rats 2 weeks after treatment.

The study also described the comparative toxicity to rats of *R. epapposum* and *T. africanum* aerial parts aqueous and methanol extracts and confirmed the development of vital organ lesions as well as myositis at site of injection being more marked in case of *T. africanum* extract.

RECOMMENDATIONS

Studies are, therefore, needed to elucidate the mechanism responsible for the development of vital organ lesions in rats and other animals.

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