

Response of Wistar Rats to Low Levels of Dietary Sudanese *Trichodesma africanum* L

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Abstract: *Trichodesma africanum* is not recognized in Sudanese folk medicine and villagers of Northern Kordofan State have observed casualties among grazing livestock as a result of consuming the plant when other pasture plants are scarce. The present study was conducted to investigate the effects on Wistar rats of various levels of dietary *T. africanum* aerial parts through clinical, biochemical, hematological and pathological parameters. *T. africanum* aerial parts were fed to rats at 2, 5, 10 and 20% of the standard diet for 12 weeks. Incorporation of *T. africanum* aerial parts in diet at 20% was lethal to Wistar rats 6-7 weeks after treatment and caused severe hepatonephrotoxicity and depression in growth and soft feces prior to death. These findings were accompanied by macrocytic normochromic type, leukocytosis due to lymphocytosis and alterations in hematology, serum enzyme activities and concentrations of total protein, albumin, cholesterol and urea and other serum constituents. The results of the present study indicated that *T. africanum* aerial parts were toxic to rats when fed at 2, 5 and 10% of the diet for 12 weeks and lethal to rats when fed at 20% of the diet for 6-7 weeks of treatment. Our conclusion from this study is that *T. africanum* is toxic to livestock and can result in dysfunction of various organs, alteration in serbiochemical and hematological parameters, in addition to that; it may cause death if consumed in elevated doses.

Key words: Enterohepatonephro toxicity, hematology, pathology, serum biochemistry, *Trichodesma africanum*

INTRODUCTION

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. From time to time additional species of plants are discovered to be toxic to animals. *Trichodesma africanum* L, a member of the family Boraginaceae, is locally known as *Hiraisha* and is common in Western Sudan. The villagers of Northern Kordofan State have noticed casualties among grazing livestock as a result of consuming this plant when other pasture plants are scarce (personal observation).

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 (European Food Safety Authority, 2007).

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

In Australia and Central Asia, *Trichodesma* poisoning of horses and cattle, known as *Suiljuk* disease, was found to affect the liver (Bull *et al.*, 1968).

It was the subject of experimental investigations which showed conclusively that the disease was due to the consumption of the plant *T. africanum* another plant of the family Boraginaceae (Bull *et al.*, 1968).

These authors found that *T. africanum* contains two pyrrolizidine alkaloids, trichodesmine and incanine. The present study was planned to examine the effects of various levels of dietary *T. africanum* on the growth, organ pathology, biochemical, and hematological characteristics of rats.

MATERIALS AND METHODS

Plant material: *Trichodesma africanum* aerial parts (Boraginaceae); vernacular name as ELhiraisha were collected from the vicinity of ELObeid, Northern Kordofan State, in November, 2006, shade-dried, ground and mixed in a standard rat diet.

Experimental design: Thirty clinically healthy male Wistar rats were housed within the premises of the Medicinal and Aromatic Plants Research Institute, National Research Center, Khartoum, under light/dark cycle and were allowed free access to feed and drinking water.

Table 1: Feed intake and growth changes in rats fed *T. africanum* for 12 weeks

Groups	Feed intake (g)	Body weight gain (g)	Feed efficiency (Body weight gain/feed intake)
Six weeks			
Control (Normal diet)	321±4.0 ^c	56.0±1.7 ^b	0.17±0.01 ^a
<i>T. africanum</i> (2%)	513±6.4 ^{ab}	60.0±1.7 ^{ab}	0.12±0.01 ^c
<i>T. africanum</i> (5%)	322±3.5 ^c	57.0±1.7 ^b	0.18±0.01 ^a
<i>T. africanum</i> (10%)	470±2.9 ^b	73.0±2.9 ^a	0.16±0.01 ^b
<i>T. africanum</i> (20%)	583±5.9 ^a	65.0±1.7 ^a	0.11±0.004 ^c
Twelve weeks			
Control (Normal diet)	102±2.3 ^c	33.0±1.2 ^a	0.32±0.02 ^a
<i>T. africanum</i> (2%)	295±2.9 ^a	4.5±0.2 ^b	0.02±0.02 ^c
<i>T. africanum</i> (5%)	198±2.9 ^b	3.0±0.2 ^b	0.02±0.01 ^c
<i>T. africanum</i> (10%)	295±5.8 ^a	3.7±2.3 ^b	0.03±0.01 ^c
<i>T. africanum</i> (20%)	ND	ND	ND

Values are means ± SE. Means within columns not sharing common letter (s) are significantly different (p<0.05); ND: not determined as rats in group 5 died between weeks 6 and 7 of treatment

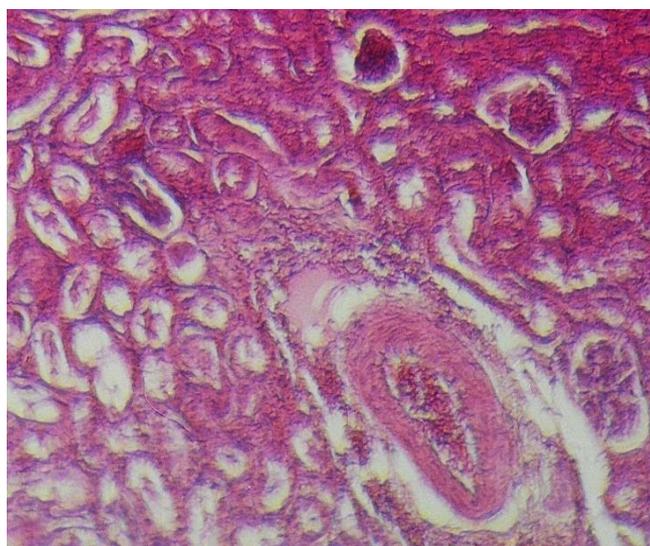


Fig. 1: Degeneration and necrosis of renal tubular cells, packing of glomerular tufts and acidophilic homogeneous material in affected tubules of a rat fed 10% *T. africanum* for 6 weeks, H&E X100

The rats were randomly allotted to five groups, each of six rats. Group1 was fed the normal rat diet and served as control. Groups 2-5 were fed diets containing 2%(w/w), 5%(w/w), 10%(w/w) and 20%(w/w) of *T. africanum* aerial parts, respectively. All rats were fed their designated experimental diets for 12 weeks.

Average feed intake, body weight, body weight gain and feed efficiency for each group were recorded weekly. Lots of 3 rats from each group were anaesthetized with diethyl ether and killed at 6 and 12 weeks. Blood samples and specimens of various organs were collected at slaughter, processed for histopathologic examinations.

Blood analysis: For hematological parameter blood samples were collected in dry test tubes containing EDTA for determination of Hemoglobin (Hb) concentration, Packed Cell Volume (PCV), Red Blood Cell (Rbc), White Blood Cell (WBC), differential WBC counts, Mean Corpuscular Volume (MCV), Mean Corpuscular

Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were estimated by standard methods (Schalm *et al.*, 1975).

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

Statistical analysis: The significance of differences between means was compared at each time point using Duncan's multiple range test after ANOVA for one-way classified data (Snedecor and Cochran, 1989).

RESULTS

Effects on growth: The effects of dietary *T. africanum* on body weight gain, feed intake and feed efficiency of the

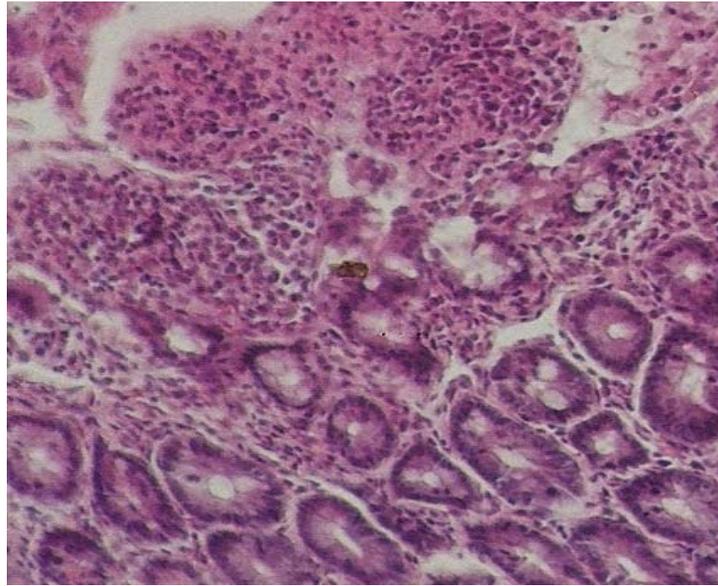


Fig. 2: Catarrhal enteritis and lymphocytic infiltration in intestinal lamina propria of a rat fed 10% *T. africanum* for 12 weeks, H&E X100

Table 2: Hematologic changes in *T. africanum* -fed rats for 12 weeks

Parameters	Treatment groups				
	1. Control (Normal diet)	2. <i>T. africanum</i> (2%)	3. <i>T. africanum</i> (5%)	4. <i>T. africanum</i> (10%)	5. <i>T. africanum</i> (20%)
Six weeks					
Hb (g/dL)	17.3±1.3 ^a	15.1±0.6 ^{ab}	13.8±1.0 ^b	17.1±1.3 ^a	13.0±1.2 ^b
RBC (×10 ⁶ mm ³)	3.7±0.3 ^a	2.8±0.2 ^{ab}	3.9±0.5 ^a	1.9±0.1 ^b	0.9±0.01 ^c
PCV (%)	36.0±1.7 ^{ab}	29.5±1.4 ^b	40.0±2.8 ^a	42.0±1.7 ^a	26.5±2.0 ^b
MCV (m ³)	98.6±3.2 ^c	104.6±2.6 ^b	103.4±3.6 ^b	221.1±6.0 ^{ab}	294.4±8.3 ^a
MCH (pg)	47.4±2.5 ^b	53.5±2.0 ^b	35.7±1.1 ^c	90.0±4.0 ^{ab}	142.9±2.5 ^a
MCHC (%)	48.1±1.8 ^a	51.2±3.0 ^a	34.5±2.5 ^b	40.7±2.1 ^{ab}	49.0±2.3 ^a
WBC (×10 ³ mm ³)	3.4±0.2 ^{ab}	2.8±0.2 ^b	5.0±0.2 ²	2±0.1 ^b	1.5±0.01 ^c
Neutrophils (%)	10.5±1.2 ^c	61.5±3.4 ^a	54.0±3.4 ^{ab}	29.0±1.7 ^b	60.0±1.7 ^a
Lymphocytes (%)	89.5±2.3 ^a	38.5±1.7 ^b	46.0±2.3 ^b	71.0±3.4 ^{ab}	40.0±1.2 ^b
Twelve weeks					
Hb (g/dL)	12.3±0.6 ^b	12.5±0.7 ^b	13.7±0.4 ^b	15.0±0.5 ^a	ND
RBC (×10 ⁶ mm ³)	5.1±0.3 ^a	3.8±0.4 ^b	5.6±0.3 ^a	4.2±0.2 ^b	ND
PCV (%)	24.0±1.7 ^b	26.5±1.4 ^{ab}	40.0±1.7 ^a	36.0±2.9 ^a	ND
MCV (m ³)	47.0±1.7 ^b	69.7±3.2 ^{ab}	71.4±3.1 ^{ab}	85.7±1.6 ^a	ND
MCH (pg)	24.0±2.3 ^b	32.9±1.7 ^a	34.3±1.9 ^a	35.7±2.1 ^a	ND
MCHC (%)	51.3±1.9 ^a	47.2±2.4 ^{ab}	34.5±1.4 ^b	41.7±3.3 ^{ab}	ND
WBC (×10 ³ mm ³)	3.1±0.2 ^b	4.2±0.2 ^{ab}	5.7±0.4 ^a	5.9±0.3 ^a	ND
Neutrophils (%)	60.5±3.1 ^{ab}	35.5±2.0 ^c	38.6±1.3 ^b	65.5±2.3 ^a	ND
Lymphocytes (%)	39.5±1.7 ^{ab}	64.5±1.7 ^a	61.4±2.9 ^a	34.5±1.7 ^b	ND

Values are means±SE. Means within rows not sharing common letter (s) are significantly different (p<0.05); ND = not determined as rats in group 5 died between weeks 6 and 7 of treatment

rats are presented in Table 1. After 6 weeks of treatment; the rats fed diets consisting of 2% (Group 2) and 20% *T. africanum* (Group 5) had the lowest feed efficiency compared to the control. After 12 weeks of treatment, according to control, the rats fed 2% (Group 2), 5% (Group 3) and 10% *T. africanum* (Group 4) had the lowest growth rate but none of the rats died during the course of the experiment. However, the rats of group 5 fed on 20% *T. africanum* diet died between weeks 6 and 7 and showed soft feces and frequently huddled together prior to death.

Pathologic changes: Six weeks after beginning the test feeding, significant changes were observed in the tissues of the rats fed on diets containing 2% (Group 2), 5% (Group 3), 10% (Group 4) and 20% of *T. africanum* (Group 5) and compared with rats on the control diet (Group 1). The lesions in the test rats included congestion of the renal blood vessels, tubular cell degeneration or necrosis, packing of glomerular tufts and acidophilic homogeneous material in affected tubules (Fig. 1) with lymphocytic infiltration in the cortex, intestinal lamina

Table 3: Changes in serum constituents of *T. africanum* - fed rats for 12 weeks

Parameters	Treatment groups				
	1. Control (Normal diet)	2. <i>T. africanum</i> (2%)	3. <i>T. africanum</i> (5%)	4. <i>T. africanum</i> (10%)	5. <i>T. africanum</i> (20%)
Six weeks					
AST (iu)	45.0±1.7 ^{ab}	31.5±1.4 ^b	53.6±2.0 ^a	28.5±1.4 ^b	45.5±1.7 ^{ab}
ALT(iu)	17.0±1.1 ^{ab}	11.3±0.7 ^b	14.3±1.2 ^{ab}	14.8±1.6 ^{ab}	37.8±2.1 ^a
ALP (iu)	149.0±2.9 ^{ab}	121.0±2.3 ^b	39.9±2.2 ^a	336.0±9.2 ^a	79.7±3.0 ^{bc}
Total protein (g/dL)	7.6±0.5 ^a	7.2±0.2 ^a	7.4±0.3 ^a	7.6±0.5 ^a	6.7±0.4 ^a
Albumin (g/dL)	5.0±0.2 ^a	4.9±0.2 ^a	3.7±0.1 ^b	4.3±0.2 ^a	5.3±0.3 ^a
Globulin (g/dL)	2.6±0.1 ^{ab}	2.3±0.2 ^b	3.7±0.2 ^a	3.3±0.1 ^a	1.4±0.1 ^{ab}
Bilirubin (mg/dL)	0.8±0.01 ^a	0.7±0.01 ^b	0.5±0.01 ^b	0.5±0.003 ^b	0.6±0.1 ^{ab}
Cholesterol (mg/dL)	82.3±4.2 ^{ab}	63.5±3.7 ^b	52.6±2.0 ^{bc}	48.8±1.6 ^{bc}	95.2±3.0 ^a
Urea (mg/dL)	36.6±2.0 ^{ab}	42.3±1.3 ^{ab}	76.4±3.1 ^a	29.6±2.9 ^b	37.8±3.3 ^{ab}
Twelve weeks					
AST (iu)	41.8±2.4 ^a	22.0±1.7 ^b	25.0±2.3 ^b	20.8±1.6 ^b	ND
ALT (iu)	14.2±0.7 ^a	13.2±1.2 ^a	12.3±0.8 ^a	13.6±0.8 ^a	ND
ALP (iu)	317.9±7.4 ^a	198±4.6 ^b	320.0±6.9 ^a	204.0±2.8 ^b	ND
Total protein (g/dL)	8.9±0.4 ^{ab}	8.8±0.2 ^{ab}	5.5±0.2 ^b	12.4±0.5 ^a	ND
Albumin (g/dL)	2.2±0.1	3.4±0.22.	1 ±0.01	3.6±0.14	ND
Globulin (g/dL)	6.7±0.4 ^{ab}	5.4±0.2 ^b	3.4±0.2 ^c	8.8±0.3 ^a	ND
Bilirubin (mg/dL)	0.7±0.01 ^a	0.7±0.01 ^a	0.6±0.004 ^a	0.6±0.01 ^a	ND
Cholesterol (mg/dL)	81.6±1.7 ^{ab}	86.3±1.9 ^a	55.0±1.1 ^c	68.4±2.5 ^b	ND
Urea (mg/dL)	60.4±3.1 ^b	80.1±1.7 ^a	37.5±2.6 ^c	55.4±2.0 ^b	ND

Values are means±SE. Means within rows not sharing common letter (s) are significantly different (p<0.05); ND: not determined as rats in group 5 died between weeks 6 and 7 of treatment

propria, and hepatic parenchyma and between the cardiac muscle fibers and focal catarrhal enteritis. After 12 weeks of treatment, the lesions were consistent and severe in groups 3 and 4 and the hepatic portal tracts and intestinal lamina propria had lymphocytic aggregates (Fig. 2). The damage to vital organs was more marked in rats of group 5 that died between weeks 6 and 7. No significant changes were observed in the spleen of the test rats or in the tissues of the control rats fed untreated diet (Group1).

Hematologic changes: The data are summarized in Table 2. After 6 weeks of treatment, the rats on 2% (Group2) and 20% *T. africanum* diet (Group 5) had lower (p<0.05) RBC, PCV, WBC and lymphocytes and higher (p<0.05) MCV, MCH and neutrophil values than control (Group 1). After 12 weeks of treatment, the values of PCV and MCV were higher (p<0.05) and those of RBC were lower (p<0.05) in groups 2 and 4 than control and other groups. MCHC values were lower (p<0.05) and WBC values were higher (p<0.05) in groups 2-4 than control. Hematologic changes in rats of group 5 were not described because these animals died between weeks 6 and 7.

Serobiochemical changes: The data are presented in Table 3. After 6 weeks of treatment, cholesterol level in group 5 and urea concentration in group 3 were higher (p<0.05) than control and other groups. In the test groups, the concentration of total protein did not change. Albumin concentration decreased in group 3 and globulin level increased in groups 3 and 4. The activity of ALT increased in group 5 and that of AST increased in group

3 compared with control and other groups. Bilirubin concentration changed within the normal established range. After 12 weeks of treatment, the activity of AST decreased in groups 2-4 and that of ALP decreased in groups 2 and 4 and was not determined in rats in group 5 as these animals died between weeks 6 and 7. The concentrations of total protein and globulin increased in group 4 and decreased in group 3. The concentrations of urea and cholesterol in group 2 were higher (p<0.05) than other groups.

DISCUSSION

The incorporation *T. africanum* aerial parts in the diets at 2, 5, 10 and 20% were chosen for several reasons. In chickens and rats, dietary levels of 2 or 10% represent non-toxic concentrations of some plants exemplified by *Nigella sativa* (Al-Homidan *et al.*, 2002) and *Thymus vulgaris* (Haroun *et al.*, 2002).

On the other hand, 2 or 5% dietary *Azadirachta indica* and *Rhazya stricta* are toxic to chickens (Ibrahim *et al.*, 1992) and rats (Adam, 1999). However, the inclusion of 20% in the diet of *Commiphora myrrha* oleogum resin is fatal to rats (Omer *et al.*, 1999).

No research has been done to investigate the toxicity of *T. africanum* aerial part. The present study suggests the seriousness of feeding *T. africanum* at 20% may be related to the concentration and characteristics of the compounds in the plant. Phytochemical investigations had been conducted on *T. africanum* aerial parts and demonstrated the presence of pyrrolizidine alkaloid and other compounds just as had been proven by (Omar *et al.*, 1983).

The effects of *T. africanum* showed as changes on the body weight gain on first six weeks in which all groups has clear increasing in body weight gain but were decreased on the second six weeks. In rats fed a diet consisting of 20% *T. africanum* aerial parts damage to vital organs could explain the depression in feed intake and growth.

The mechanism whereby the plant constituents injured body tissues cannot be defined from the present study. In rats fed 10 or 20% *T. africanum*, damage to the vital organs probably contributed to the decrease in serum ALP activity, total protein, albumin and cholesterol concentrations as well as the increase in urea concentration. The hypoproteinaemia due to hypoalbuminaemia might have resulted from hepatocellular dysfunction as it was noticed that renal and intestinal damage can contribute to hypoproteinemia in rats fed *Artemisia abyssinica* (Adam *et al.*, 2000), chicks fed *Abrus precatorius* (Omer *et al.*, 1992), sheep fed *Aristolochia bracteata* (Barakat *et al.*, 1983) and goats fed *Croton macrostachys* or *Jatropha curcas* (Abdel *et al.*, 2003).

Occurrence of hypocholestromia is a further evidence of liver damage characterized by the development of cytoplasmic fatty vacuolation and necrosis of the centrilobular hepatocytes with lymphocytic infiltration, which we suggest its caused due to pyrrolizidine alkaloid (Huxtable and Cooper, 2000).

This result are confirmed by research which shows that The PAs, which have minimal toxicity in their original form, are metabolized in the liver and can become toxic metabolites (Huxtable and Cooper, 2000).

The absence of bilirubinaemia is not a puzzle, the damage to the centrilobular hepatocytes without significant bilirubinaemia or jaundice was observed in rats which had been fed *Francoeuria crispa* (Adam, 1998a) or *Artemisia abyssinica* (Adam *et al.*, 2000). It has been found that the rise in serum bilirubin is due to periportal liver injury or periportal proliferation of the bile ducts previously described by Gopinath and Ford (1972) in sheep, Ali and Adam (1978) in goats and in rats Adam (1999). The degeneration or necrosis of the renal tubular epithelium, packing of the glomerular tufts, acidophilic homogeneous material in the affected tubules and lymphocytic infiltration seen in the rats might have accounted for the rise in urea concentration and hypoproteinaemia.

The development of catarrhal enteritis with lymphocytic accumulation in the intestinal lamina propria might have probably contributed to hypoproteinaemia.

This finding is supported by other researches, in goats fed *Ruta graveolens* (ELAgraa *et al.*, 2002), *Croton macrostachys* or *Jatropha curcas* (Abdel *et al.*, 2003), also in sheep and rats fed *Cuminum cyminum* (Haroun

et al., 2002), all of them found that hepatic, renal and intestinal damage can contribute to hypoproteinaemia.

Hyperkalaemia was observed in rats fed 10% *T. africanum*. This might have affected the cardiac muscle function as evidenced by infiltration of lymphocytes, focal myocardial fatty change and congestion of the cardiac blood vessels. Hyperkalaemia might have probably contributed to death of rats fed 20% *T. africanum*.

As there were decreases in RBC, PCV and Hb and increases in MCV with no effect on MCHC, the anaemia was of a macrocytic normochromic type.

It has been shown that an increase in the size of the RBC may result from loading of the membrane with cholesterol at the periphery of the cell as opposed to its centre (Adam, 1998b).

This result was supported by researcher observe the same type of anaemia in sheep has been fed on *R. stricta* (Adam, 1998b).

The development of leukopenia due to neutropenia or leukocytosis due to lymphocytosis at stages of feeding *T. africanum* suggests that the plant constituents had an action on the circulating WBC or on their formation.

CONCLUSION

The study showed that *T. africanum* aerial parts were toxic to rats when fed at 2, 5 and 10% of the diet for 12 weeks and lethal to rats when fed at 20% of the diet for 6-7 weeks of treatment. Palmed for toxicity is pyrrolizidine alkaloids, *T. africanum* toxicity was demonstrated in dysfunction on various organs and alteration in serobiochemical and hematological parameters, death may occur as result of consumption of higher doses of *T. africanum*.

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

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