Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 3 (2): 134-140, 2008
ISSN 1816-496X

Evaluation of Toxicity of *Rhanterium epapposum* in Wistar Rats

Shama I. Younis and S.E.I. Adam
Department of Biochemistry, School of Biotechnology,
Faculty of Science and Technology, El Neelain University,
P.O. Box 12702, Khartoum, Sudan

Abstract: We present the first reported study of the effects of feeding *Rhanterium epapposum* aerial parts at 20, 50, 100 and 200 g kg^{-1} of standard diet to male Wistar rats for 12 weeks. The criteria of assessment of the plant toxicity were the effects on growth, organs of the body, hematological and serobiochemical parameters of rats. Depression in growth and hepatonephropathy were severe in rats fed diets containing 100 and 200 g kg^{-1} of *R. epapposum* aerial parts. These findings were accompanied by macrocytic hypochromic anemia, leukocytosis due to lymphocytosis and alterations of serum concentrations of urea, total protein, globulin and other serum constituents. Toxicity may be frequent in animals that ingest this plant in a dry year. While this plant has traditionally been used in Sudan and other Afro-Asian countries it may show toxic effects in human that result from over-dosage because, in general, there is no standardized dosage system in traditional medical practice.

Key words: Hematology, pathology, *Rhanterium epapposum*, serum biochemistry, toxicity

INTRODUCTION

Plants and plant extracts have been used since the dawn of civilization by mankind. The use of ethnobotanical preparations is still continued all around the world, they offer a unique reasonable resource for the discovery of potential new drugs and modern medicine has developed a rational strategy for drug discovery which involves the study of plants and plant materials based on their ethnobotanical usage (Leung and Foster, 1996).

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

From time to time additional species of plants are discovered to be toxic for both human and animal. *Rhanterium epapposum* (Asteraceae), locally known as Al-Arfaj is prevalent in various parts of Sudan and other Afro-Asian countries and used in folk medicine by people in rural areas as a remedy for skin infections and gastrointestinal disturbances and as an insecticide (Ageel *et al*., 1987; El-Shanawany, 1997). 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). A description of the morphological properties of this plant has been given by Migahid (1996).

In Sudan and probably other countries, medical practitioners are not aware of the usage and toxicity especially those remedies that are used by tribal cultures other than their own. This has probably led to absence of records of cases of the country.

The evaluation and utilization of medicinal plants form an important part of the proposed medium- term program (WHO, 1987).

Corresponding Author: Shama I. Younis, Department of Biochemistry, School of Biotechnology, Faculty of Science and Technology, El Neelain University, P.O. Box 12702, Khartoum, Sudan Fax: 00249 83771154

R. epapposum is commonly used in the treatment of various disorders, with lack of information on it's toxicity, the study, therefore, investigates the effects of various levels of dietary component of *R. epapposum* aerial parts on the growth, organs of the body, hematological and serobiochemical parameters of Wistar rats.

MATERIALS AND METHODS

Plant Material

Rhanterium epapposum aerial parts were collected from the vicinity of ELHassahisa City, Central Sudan, shade-dried, ground and mixed in a standard rat diet.

Experimental Design

Thirty healthy male Wistar rats were kept within the premises of the Medicinal and Aromatic Plants Research Institute, National Research Center, Khartoum, under light/dark cycle with feed and water *ad libitum*.

The rats were randomly assigned to five groups of six rats each. Rats in group 1 were fed the untreated diet and served as controls. Groups 2, 3, 4 and 5 were fed diets containing 20, 50, 100 and 200 g kg^{-1}, respectively. All rats were fed their designated experimental diets for 12 weeks.

Average feed intake, body weight gain and feed efficiency (body weight gain/feed intake) were measured weekly for each group. After 6 weeks of treatment, 3 randomly selected rats from each group were killed by decapitation. The remaining 3 rats/group were similarly killed after 12 weeks. Blood samples were collected from each of the killed rats for hematology and serum analysis.

Pathological Examinations

Post-mortem findings were recorded for all rats and specimens of intestines, liver, spleen, kidneys and heart were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned 6 μm and stained with hematoxylin and eosin for histopathologic examinations.

Blood Analysis

Erythrocytes (RBC), leukocytes (WBC), differential WBC counts, hemoglobin (Hb) concentration, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were evaluated (Schalm *et al*., 1975).

Serum samples were analysed for the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and concentrations of total protein, albumin, bilirubin, cholesterol and urea by commercial kits (Linear Chemicals, Barcelona, Spain). Serum globulin was determined by subtracting albumin from total protein concentration.

Statistical Analysis

Statistical significance was assessed by the unpaired Student's t-test (Snedecor and Cochran, 1989).

RESULTS

Effect on Growth

The effects of treatment with diets containing 20, 50, 100 or 200 g kg^{-1} of aerial parts of *R. epapposum* on feed intake, body weight gain and feed efficiency of the rats are shown in Table 1. The rats fed diet consisting of 200 g kg^{-1} *R. epapposum* aerial parts (group 5) for 6 and 12 weeks had

Table 1: Feed intake, body weight gain and feed efficiency of rats fed *R. epapposum* for 12 weeks

Treatment groups	Feed intake (g)	Body weight gain (g)	Feed efficiency (body weight gain/feed intake)
Six weeks			
Control (normal diet)	321.3±1.3	56.2±1.2	0.18±0.01
R. epapposum **(g kg^{-1})**			
20	468.8±3.9***	64.2±0.6***	0.14±0.01***
50	264.2±2.3**	42.7±1.6***	0.16±0.01**
100	386.2±3.5**	65.8±1.7***	0.17±0.1*
200	497.9±1.7***	55.0±1.3^{NS}	0.11±0.01***
Twelve weeks			
Control (normal diet)	101.7±1.6	32.5±0.9	0.32±1.7
R. epapposum **(g kg^{-1})**			
20	201.7±2.3**	50.0±1.8***	0.25±1*
50	159.5±2**	43.0±1.2**	0.27±1.7*
100	240.8±2.3***	48.3±1.3***	0.20±0.01**
200	326.7±2.9***	30.2±0.7^{NS}	0.09±0.01***

Values are means±SE; NS = Not Significant; * = p<0.05; ** = p<0.01; *** = p<0.001

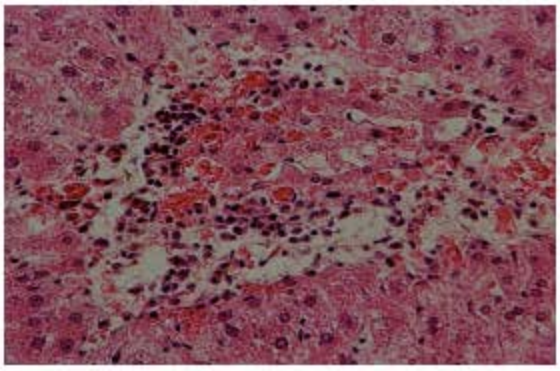

Fig. 1: Liver of a rat fed 200 g kg^{-1} *R. epapposum* diet for 12 weeks, showing cytoplasmic fatty vacuolation and necrosis of the hepatocytes. H and E X200

the lowest (p<0.001) feed efficiency but none of the animals died during the course of the experiment. The feed efficiency of the rats in groups 2, 3 and 4 was lower (p<0.05-0.001) than control (group 1) at weeks 6 and 12.

Pathologic Changes

After 6 weeks of treatment, no significant lesions were observed in the tissues of the rats on 20g kg^{-1} (group 2) or 50 g kg^{-1} *R. epapposum* aerial parts (group 3). Reduction in cytoplasmic basophilia with small fatty vacuoles in the centrilobular hepatocytes and in the cells of the renal proximal convoluted tubules as well as congestion of the blood vessels of intestinal lamina propria and heart were observed in rats on 100 g kg^{-1} (group 4) and 200 g kg^{-1} *R. epapposum* diets (group 5). In these groups, lesions were consistent and included cytoplasmic fatty vacuolation or necrosis of the centrilobular hepatocytes (Fig. 1), lymphocytic infiltration, degeneration or necrosis of the epithelial cells of the renal tubules, acidophilic homogeneous material in affected renal tubules, foci of lymphocytic accumulation in the cortex, segmentation, packing or necrosis of the glomerular tufts after 12 weeks of treatment. No significant lesions were observed in the spleen of the test rats but the heart blood vessels of the rats in groups 4 and 5 were congested with focal fatty vacuolation of some of the cardiac muscle fibers and lymphocytic accumulation. The liver (Fig. 2) and other tissues of the control (group 1) showed no lesions throughout the 12-week feeding period.

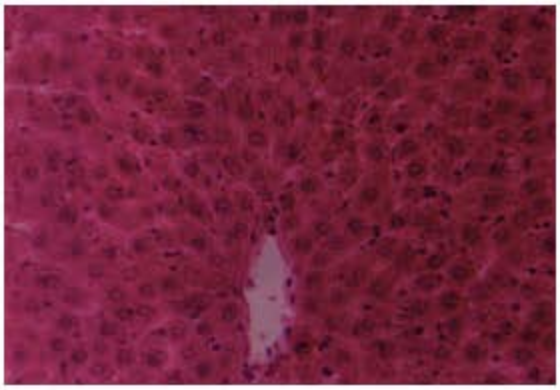

Fig. 2: Liver of a rat without any diet for 12 weeks, showing no lesions. H and E X200

Table 2: Hematologic changes in rats fed *R. epapposum* for 12 weeks

	Treatment groups				
Parameters	Control (normal diet)	*R. epapposum* (20 g kg^{-1})	*R. epapposum* (50 g kg^{-1})	*R. epapposum* (100 g kg^{-1})	*R. epapposum* (200 g kg^{-1})
Six weeks					
Hb (g dL^{-1})	17.3±0.8	14.0±0.6**	15.0±0.8*	15.6±1.2*	12.0±0.5***
RBC (×10^{6} mm^{3})	3.7±0.2	1.7±0.2***	3.3±0.3*	1.3±0.2***	3.3±0.1*
PCV (%)	36.0±2.3	31.0±1.7*	40.3±1.3^{NS}	31.5±1.5*	27.0±1.7**
MCV (m^{3})	98.6±2.7	187.9±4.6**	122.9±7.4*	248.0±7.5***	82.8±2.8*
MCH (pg)	47.4±2.0	84.9±3.5**	45.7±2.0^{NS}	122.8±5.8***	36.8±2.2*
MCHC (%)	48.1±1.2	42.2±1.3*	37.3±1.7*	49.5±1.5^{NS}	44.4±1.4*
WBC (×10^{3} mm^{3})	3.4±0.2	1.6±0.1***	5.2±0.2***	2.6±0.3**	3.3±0.2^{NS}
Neutrophils (%)	10.0±1.2	30.0±2.3**	51.6±3.5***	30.0±1.2**	65.0±2.9***
Lymphocytes (%)	90.0±2.3	70.0±1.7*	48.4±2.0**	70.0±2.3*	35.0±1.2***
Twelve weeks					
Hb (g dL^{-1})	12.3±0.6	14.3±0.5^{NS}	13.9±0.6^{NS}	14.3±0.5^{NS}	12.0±0.5^{NS}
RBC (×10^{6} mm^{3})	5.1±0.3	3.9±0.3**	5.8±0.5^{NS}	4.3±0.3*	3.7±0.2**
PCV (%)	24.0±2.3	29.3±2.4^{NS}	40.3±1.3***	36.0±1.7***	38.0±1.7***
MCV (m^{3})	47.1±2.0	75.1±1.7*	69.5±1.7*	83.7±2.1**	102.7±3.2***
MCH (pg)	24.1±1.8	36.7±1.6***	24.0±2.3^{NS}	33.3±1.3**	32.4±1.4**
MCHC (%)	51.3±3.0	48.8±1.2^{NS}	34.5±2.0**	39.7±2.2*	31.6±2.1**
WBC (×10^{3} mm^{3})	3.1±0.2	4.0±0.2*	5.6±0.3***	3.6±0.2*	4.7±0.3**
Neutrophils (%)	59.7±2.6	36.5±2.0**	56.01.7^{NS}	31.0±2.3***	50.5±2.0*
Lymphocytes (%)	40.3±3.5	63.5±2.0***	44.0±1.7^{NS}	69.0±2.9***	49.5±2.0*

Values are means±SE; NS = Not Significant; * = p<0.05; ** = p<0.01; *** = p<0.001

Hematologic Changes

In rats on 200 g kg^{-1} *R. epapposum* diet (group 5) for 6 weeks, the values of Hb, RBC, PCV, MCH and MCHC were lower (p<0.05-0.001) and those of lymphocytes were lower (p<0.05-0.001) than control (Group 1). In rats on 100 g kg^{-1} *R. epapposum* diet (Group 4), the RBC, PCV, WBC and lymphocytes were lower (p<0.05-0.001) and MCV and MCH were higher (p<0.001) than control and other groups. The Hb, RBC, PCV, MCHC and WBC values in group 2 and MCHC and lymphocytes in group 3 were lower (p<0.05-0.001) than other groups. After 12 weeks of treatment, the values of RBC in groups 2, 4 and 5 and MCHC in groups 3, 4 and 5 were lower (p<0.05-0.001) and MCV, WBC and lymphocytes were higher (p<0.05-0.001) than control and other groups (Table 2).

Serobiochemical Changes

After 6 weeks of treatment, the activities of serum ALT did not significantly change among the groups and that of AST decreased (p<0.05) in groups 2, 3 and 4. The activity of ALP in groups 2-5

Table 3: Changes in serum constituents of the *R. epapposum*-fed rats for 12 weeks

Parameters	Treatment groups				
	Control (normal diet)	*R. epapposum* (20 g kg^{-1})	*R. epapposum* (50 g kg^{-1})	*R. epapposum* (100 g kg^{-1})	*R. epapposum* (200 g kg^{-1})
Six weeks					
AST (iu)	41.8±1.6	29.0±1.7*	27.3±0.8*	31.8±2.2*	37.8±1.6^{NS}
ALT (iu)	14.2±0.6	13.3±0.8^{NS}	13.7±0.8^{NS}	13.5±0.9^{NS}	12.0±0.5^{NS}
ALP (iu)	317.9±4.5	141.7±3.8**	274.2±5.3*	57.1±2.9***	106.7±3.8**
Total protein (g dL^{-1})	8.9±0.3	5.8±0.4**	8.5±0.3^{NS}	7.0±0.2*	5.6±0.2**
Albumin (g dL^{-1})	2.2±0.1	4.6±0.3***	3.1±0.2*	5.2±0.3***	4.8±0.4***
Globulin (g dL^{-1})	6.7±0.4	1.2±0.1**	5.4±0.4*	1.9±0.2**	0.8±0.01***
Bilirubin (mg dL^{-1})	0.7±0.01	0.7±0.01^{NS}	0.7±0.01^{NS}	0.5±0.003*	0.5±0.003*
Cholesterol (mg dL^{-1})	81.6±3.5	74.1±2.4*	63.2±1.8**	89.3±4.7^{NS}	61.5±2.6**
Urea (mg dL^{-1})	60.4±3.1	35.8±1.6**	67.0±3.4*	34.3±1.9**	29.6±2.0**
Twelve weeks					
AST (iu)	31.8±1.0	22.7±1.0**	20.4±1.4**	25.9±1.7^{NS}	30.1±1.2^{NS}
ALT (iu)	14.5±0.7	14.9±0.9^{NS}	14.2±0.7^{NS}	12.0±0.6^{NS}	14.5±0.3^{NS}
ALP (iu)	410.9±9.1	301.3±6.5**	203.3±4.7***	350.1±4.7*	152.9±3.9***
Total protein (g dL^{-1})	7.0±0.5	12.7±0.9**	6.9±0.4^{NS}	12.0±0.5**	9.5±0.6*
Albumin (g dL^{-1})	2.4±0.2	4.2±0.3**	3.45±0.2**	3.7±0.3**	3.2±0.2*
Globulin (g dL^{-1})	4.6±0.2	8.5±0.3***	2.55±0.2**	8.3±0.4***	6.3±0.2**
Bilirubin (mg dL^{-1})	0.7±0.003	0.6±0.01^{NS}	0.58±0.002^{NS}	0.5±0.003*	0.5±0.01*
Cholesterol (mg dL^{-1})	77.0±2.9	63.2±3.0*	65.7±1.6^{NS}	67.4±3.7^{NS}	34.4±2.5***
Urea (mg dL^{-1})	36.6±1.5	89.0±2.3***	52.9±2.8**	80.0±1.7***	74.5±2.6***

Values are means±SE; NS = Not Significant; * = p<0.05; ** = p<0.01; *** = p<0.001

was lower (p<0.05-0.001) than control. The concentrations of total protein and globulin in groups 2, 4 and 5 were lower (p<0.05-0.001) than control and group 3.The concentrations of bilirubin changed within the normal range. Cholesterol concentration in groups 2, 3 and 5 was lower (p<0.05-0.001) and urea concentration in group 3 was higher (p<0.05) than control and other groups. After 12 weeks of treatment, the activities of AST and ALT did not change but the activity of ALP in groups 2, 3, 4 and 5 was lower (p<0.05-0.001) than control (group 1). The concentrations of total protein in groups 2, 4 and 5 were higher (p<0.05-0.01) than 3 and control (group 1). Globulin concentration in groups 2, 4 and 5 was higher (p<0.01-0.001) and that in group 3 was lower (p<0.01) than control. Bilirubin concentration changed within the normal range. The concentration of cholesterol in groups 2 and 5 was lower (p<0.05-0.001) and that of urea in groups 2-5 was higher (p<0.01-0.001) than control (Table 3).

DISCUSSION

In spite of the common use of *R. epapposum* aerial parts in Sudan and other parts of the world to treat various disorders toxicological information on rodents, birds or livestock is not available.

It is well known that the susceptibility of animals to feeding plant material is dependent on the type of the active constituents and concentrations in the amount added to the diet as well as on the rate of their metabolic conversion in the liver to metabolites and consequent excretion (Adam, 1999; Ibrahim *et al*., 2004).

The choice of dietary inclusions of 20, 50, 100 and 200 g kg^{-1} of *R. epapposum* aerial parts was based on other studies in Wistar rats (Adam, 1998, 1999).

The results of the present study indicate that feeding rats with *R. epapposum* at 100 and 200 g kg^{-1} of the normal diet for 12 weeks is toxic but not lethal as evidenced by the presence of growth impairment, of lesions in the vital organs, hematological and serobiochemical alterations. Our previous investigations have shown that leaves of *Francoeuria crispa* (Asteraceae) incorporated at 100 g kg^{-1}of the normal diet are toxic to rats (Adam, 1998).

The development of cytoplasmic fatty vacuolation or necrosis of the centrilobular hepatocytes, degeneration or necrosis of the epithelial cells of the renal tubules, segmentation, packing or necrosis of the glomerular tufts with lymphocytic infiltration could explain growth depression. However, the mechanism whereby the plant constituents damage body tissues can not be defined from the present study. The decrease in serum AST and ALT activities could have been due to enzyme excretion. Neither hypercholesterolemia nor hyperbilirubinemia is a feature of *R epapposum* toxicity in rats. It has been found that hyperbilirubinemia is associated with periportal liver injury previously described by Gopinath and Ford (1972) in sheep, Ali and Adam (1978) in goats and by Adam (1999) in rats and the increase in urea concentration indicates a renal malfunction.

The anemia was of a macrocytic hypochromic type as indicated by increases in MCV and decreases in MCHC. Previous investigations showed normocytic normochromic anemia in rats on 100 g kg^{-1} *F. crispa* leaves for 8 weeks or normocytic hypochromic anemia in rats fed a diet containing 100 g kg^{-1} of *Cuminum cyminum* fruits for 6 weeks (Adam, 1998; Haroun *et al*., 2002).

Leukocytosis, notable in rats fed a diet containing 100 or 200 g kg^{-1} of *R. epapposum* aerial parts was a feature of plant toxicity in rats and was due to an increase in lymphocytes. These findings suggested that the plant aerial parts constituent(s) had a selective action on circulating WBC. Phytochemical studies on the aerial parts *R. epapposum* showed that flavonoids, tannins, sterols, triterpenes and volatile oils are their major active constituents (AL-Yahya *et al*., 1990).

We conclude that Wistar rats are susceptible to poisoning by *R. epapposum*. The toxicity from this plant was severe at concentration of 100 and 200 g kg^{-1} of the diet as evidenced by inefficient feed utilization, hepatonephropathy and hematologic and serobiochemical alterations.

We suggest that in spite of the need for phytotherapy in many developing countries, efforts must be exerted to identify and evaluate plants grazed by animals and/or used as remedies for the treatment of various ailments as their use maybe hazardous to health. We suggest non-use of R.epapposum aerial part as medicinal in man and animals ought to be prevented from grazing this plant. However, further studies are deemed necessary to investigate *R. epapposum* toxicity for livestock, female Wistar rats and other species of animals. Investigations into the isolation, characterization and quantification of the constituents in *R. epapposum* are necessary for determining their respective modes of action.

REFERENCES

Adam, S.E.I., 1998. Toxic effects of *Francoeuria crispa* in rats. Phytother. Res., 12: 476-479.

Adam, S.E.I., 1999. Experimental *Rhazya stricta* toxicosis in rats. Vet. Hum. Toxicol., 41: 5-8.

Ageel, A.M., M. Tariq, J.S. Mossa and M.A. AL-Yahya, 1987. Saudi Plants Used in Folk Medicine. King Abel Aziz City for Science and Technology, Riyadh.

Ali, B. and S.E.I. Adam, 1978. Effects of *Acanthospermum hispidum* on goats. J. Comp. Pathol., 88: 533-538.

AL-Yahya, M.A., I.A. Al-Meshal, J.S. Mossa, A.A. Al-Badr and M. Tariq, 1990. Saudi Plants: A Phytochemical and Biological Approach. King Saud University Press, Riyadh.

El-Shanawany, M.A., 1997. Plants Used in Saudi Folk Medicine. King Abdul Abdul Aziz City for Science and Technology, Riyadh.

Gopinath, C. and E.J.H. Ford, 1972. Location of liver injury and extent of bilirubinemia in experimental liver lesions. Vet. Pathol., 9: 99-108.

Haroun, E.M., O.M. Mahmoud and S.E.I. Adam, 2002. Effect of feeding *Cuminum cyminum* fruits, *Thymus vulgaris* leaves or their mixture to rats. Vet. Hum. Toxicol., 44: 67-69.

Ibrahim, I.A., S.M.A. ELBadwi, W.S. Abdel Gadir, A.O. Bakhiet, S.O. Yagoub and S.E.I. Adam, 2004. Susceptibility of Bovans chicks to low dietary levels of *Ammi visnaga* and *Artemisia herba-alba*. Vet. Hum. Toxicol., 46: 67-69.

Leung, A.Y. and S. Foster, 1996. Encyclopedia of Common Natural Ingredients Used in Foods, Drugs and Cosmetics. 2nd Edn., John Wiley and Sons, New York.

Migahid, A.M., 1996. Flora of Saudi Arabia. Vol. 1, 4th Edn., King Saud University Press, Riyadh.

Schalm, O.W., N.C. Jain and G.H. Carroll, 1975. Veterinary Hematology. 3rd Edn., Lea and Febiger, Philadelphia.

Snedecor, G.W. and W.C. Cochran, 1989. Statistical Methods. 8th Edn., Iowa State University Press, Ames, Iowa.

WHO, 1987. Global Medium-Term Program (Traditional Medicine) Covering a Specific Period 1990-1995 (WHO Document TRM/MTP/87.1).