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Toxicity of *Abutilon glacum* Seeds' Extracts (Water and Methanol) on Wistar Rats

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Abstract: Current study was carried out to evaluate the toxicity of *Abutilon glacum* seeds extracts on experimental rats. The rats were allotted at random to five groups, each of six rats. One group served as control. Two groups were given aqueous extract of the seeds part of the plant and other two groups were given methanolic extract at 75 and 300 mg/kg/day orally for the tow extracts. All rats were dosed their designated experimental doses for 2 weeks. The mortality and weight gain, serobiochemical and hematological parameters were recorded in addition to pathological changes. The study showed that, the administration of aqueous and methanol extracts of *A. glacum* seeds has a toxic effects that resulted in alterations in Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP) activities, changes in the concentration of urea, cholesterol and other serobichemical parameters, also pathological changes in fatal organs demonstrated as lesions in liver, kidney and intestine, fatty cytoplasmic vaculation and necrosis of the hepatocytes and necrosis of the centrilobular hepatocytes, glomerular alteration and degeneration of the epithelial cells of renal tubules with acidophilic homogeneous substance in affected renal tubules, catarrhal enteritis and lymphocytic infiltration in intestinal lamina. We concluded that toxicity from oral administration of 300 mg/kg/day of *A. glacum* seeds extracts for 2 weeks was sever as evidenced by consistent extensive damage to liver and kidney. The damage to these organs caused by daily oral doses of plant extract at 75 mg/kg/day for 2 weeks was less marked.

Keywords: *Abutilon glacum*, alkaloids, flavonoide, gargadan, herbal medicine, pathological changes

INTRODUCTION

Use of medicinal plants still play a vital role covers the basic health needs in the developing countries. Plant materials remain an important resource to combat serious diseases in the world.

Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value (Nostro *et al*., 2000). According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (Pierangeli and Windell, 2009). The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, falvonoid and phenolic compounds (Edeogal *et al*., 2005). Within the recent years, infections have increased to a great extent and antibiotics resistance effects become an ever-increasing therapeutic problem (Mahesh and Satish, 2008).

Abutilon glacum of Family malvaceae is locally known as Gargadan. It is found in many and various areas in Sudan as well as other Afro-Asian countries (Rafik and Dina, 2000); in Sudan it is widely spread in Northern and central Sudan (Gamal *et al*., 1997). The plant is used locally as traditional medicine in rural areas' people as diuretic and purgative (Al-yahya *et al*., 1990). The leaf of the plant is prescribed for Diarrhea, gonorroea and bronchitis (Brooks *et al*., 1998), Also the Seeds used as demulcent, diuretic (Yogeshkumar and Sumitra, 2007).

Abutilon species were reported to possess activities such as hepatoprotective, antiplasmodial, hypoglycemic and Antimicrobial activity (Kang-Jin *et al*., 2007).

There are several compounds that have been identified in the leaves and seeds. The plant contains many compounds including flavonoids (Matlawska and Silkorska, 2002). As well as steroids, saponins, carbohydrates, coumarins and flavonoids (Sammia *et al*., 2008).

However, many cases of poisoning by medicinal plants resulted from over-dosage because, in general, there is no standardized dosage system in traditional medical practice. Some plants used in folk medicine of different countries have such narrow therapeutic indices that their use is dangerous and should be carefully researched.

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Fig. 1: Seeds of *Abutilon glacum*

Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine.

This study was carried out to investigate the effects of aqueous and methanolic extracts of the plant on Wistar rats in two different doses 75 and 300 mg/kg/day orally for two weeks.

MATERIALS AND METHODS

Plant material: *A. glaucum* seeds (Fig. 1) were purchased from a local market in Khartoum Bahari, Sudan (April, 2011). The plant tissues were cleaned, shade-dried and ground by a mechanical grinder.

Study design:

Experimental design: Thirty two-month-old Wistar rats were housed within the premises of the Medicinal and Aromatic Plants Research Institute, National Centre for Research, Khartoum, with feed and water provided *Ad libitum*. The rats were allotted at random to five groups, each of 6 rats. Group 1 continued to be fed the normal diet and served as control. Groups 2 and 3 were given aqueous extract of the seeds part of the plant at 75 and 300 mg/kg/day via the oral route, respectively. The rats in groups 4 and 5 were given methanolic extract at 75 and 300 mg/kg/day via the oral route, respectively. All rats were dosed their designated experimental oral doses for 2 weeks.

Lots of 3 rats from each group were anaesthetized with diethyl ether and killed at one and 2 weeks.

Average body weight and body weight gain for each group were recorded weekly. Blood samples were collected at slaughter. At necropsy, all rats were examined to identify gross lesions and specimens of the liver, kidneys, heart, spleen and intestines were immediately fixed in 10% neutral buffered formalin and processed for histopathology.

Hematological methods: These techniques were performed according to an Automated Haematology Analyzer (Human GambH, max-planck-Ring 21, D-65205 wiesbaden, Germany). The parameters measured were Hemoglobin concentration (Hb), Packed Cell Volume (PCV), Red Blood Cells (RBCs), platelets count, White Blood Cells (WBCs), differential WBCs counts and erythrocytes indices; Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC).

Serobiochemical methods: Blood samples were collected and allowed to clot and sera were separated by centrifugation at 3000 rpm for 5 min and stored at -20°C until analyzed. The following methods for enzyme activity of control and test rats were performed according to the instructions in the manual of the Roche Diagnostic Hitachi 902 Analyzer (Germany, 1996).

Pathological methods: Necropsy was conducted to identify gross lesion, after anesthetizing, the rats were dissected. Specimens of the liver, kidneys, heart, spleen and intestines were collected and immediately fixed in 10% neutral buffered formalin.

The organs were embedded in paraffin wax, sectioned at 5 µm diameter and stained routinely with Hematoxylin and Eosin (H & E) (Andrew *et al*., 2008).

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

Growth changes: The effect of body weight and body weight gain of rats given daily oral doses of *A. glacum* seeds extract is represented in Table 1. The control rats (Group 1) had the higher ($p<0.05$) body weight gains than groups 2, 3, 4 and 5 at both weeks.

Hematological changes: Hematological changes for rats given daily oral doses of *A. glacum* seeds aqueous extract at 75 mg/kg (Group 2) and 300 mg/kg (Group 3) for 2 weeks are presented in Table 2. One week after treatment, the values of Hb and RBCs in group 3 were higher ($p<0.05$) than control. The values of MCH, MCHC and WBCs were lower ($p<0.05$) in group 3 than control and other groups. The values of RBCs and PCV in group 4 and lymphocytes in group 5 were higher ($p<0.05$) than the control group. The values of Hb in group 5, WBCs in group 4, MCH in group 4 were lower ($p<0.05$) than the control. Two weeks after treatment the values of RBCs, PCV, in group 3, MCV and MCH in group 2 and WBCs in group 3 were higher ($p<0.05$) than the control group, the value of MCH in group 3, MCHC and lymphocytes in group 2 and group 3 was lower ($p<0.05$) than control. The values of PCV and WBCs and lymphocytes in group 4 were lower ($p<0.05$) than the control.

Serobiochemical changes: Serobiochemical changes for rats given daily oral doses of *A. glacum* seeds,

Table 1: Body weight and body weight gain in rats orally given *A. glacum* extracts for 2 weeks

	Parameters		
Treatment groups	Body weight (g) 0 week	Body weight gain (g) 1 week	Body weight gain (g) 2 weeks
1.Control (normal diet)	87.5±3.4	23.0±1.9	12.0±0.9
2. 75 mg/kg/day aqueous extract	85.8±3.1	16.0±2.4*	6.0±3.20*
3. 300 mg/kg/day aqueous extract	85.8±3.1	15.7±2.0*	1.4±3.00*
4. 75 mg/kg/day methanolic extract	85.8±3.1	14.3±1.7*	6.0±2.20*
5. 300 mg/kg/day methanolic extract	85.8±3.1	17.7±2.2*	5.0±25*

Values are expressed as mean±S.E; *: Significant = ($p<0.05$)

Table 2: Hematological analysis of rats given *A. glacum* methanol and aqueous extract orally for 2 weeks

		Methanolic extract		Aqueous extract	
Parameter	1. Control (normal diet)	2. *A. glacum* (75 mg/kg/day)	3. *A. glacum* (300 mg/kg/day)	4. *A. glacum* (75 mg/kg/day)	5. *A. glacum* (300 mg/kg/day)
One week					
Hb (g/dL)	16.3±1.4	16.9±1.8^{NS}	18.9±1.5*	16.4±1.1^{NS}	13.8±1.9*
RBCs (X10^{6}mm^3)	10.1±0.8	11.3±1.2^{NS}	13.9±0.6*	12.1±0.9*	09.6±0.7^{NS}
PCV (%)	66.4±4.8	72.8±4.9*	90.9±4.4*	81.5±5.0*	62.0±4.5^{NS}
MCV (m^3)	66.3±0.9	64.3±1.0^{NS}	65.6±2.4^{NS}	67.3±0.9^{NS}	66.0±0.0^{NS}
MCH (pg)	16.1±0.2	15.0±0.1^{NS}	13.6±0.3*	13.5±0.3*	14.7±0.4^{NS}
MCHC (%)	24.4±3.4	23.2±3.7^{NS}	20.7±3.2*	20.6±3.8^{NS}	22.3±3.1^{NS}
WBCs (X10^{3}mm^3)	08.1±0.8	07.3±1.5^{NS}	04.4±0.6*	05.6±1.0*	04.9±0.6*
Lymphocytes (%)	54.0±5.9	60.0±5.8^{NS}	59.5±6.0^{NS}	59.1±5.8^{NS}	66.5±5.5*
Granulocytes (%)	46.6±5.9	40.0±5.7*	40.5±6.0*	40.9±5.8*	33.5±5.5*
Two weeks					
Hb (g/dL)	14.1±0.9	15.2±0.8^{NS}	14.5±1.2^{NS}	13.0±1.3^{NS}	17.5±0.6*
RBCs (X10^{6}mm^3)	09.9±0.6	09.1±0.6^{NS}	16.0±0.8*	08.5±0.6^{NS}	11.0±0.5*
PCV (%)	61.3±3.9	60.8±3.6^{NS}	98.9±3.4*	55.9±3.9*	71.2±3.2*
MCV (m^3)	61.7±1.2	66.7±1.5*	64.6±1.1^{NS}	66.0±1.0^{NS}	65.0±1.1^{NS}
MCH (pg)	14.2±0.2	17.0±0.4*	11.7±0.6*	15.3±0.6^{NS}	15.4±0.2^{NS}
MCHC (%)	23.3±0.9	16.6±0.6*	17.9±0.7*	23.2±8.1^{NS}	24.4±1.0^{NS}
WBCs (X10^{3}mm^3)	08.5±0.8	07.8±0.3^{NS}	10.1±0.9*	06.0±0.6*	07.2±0.2^{NS}
Lymphocytes (%)	67.4±3.4	61.6±3.9*	58.8±3.1*	60.5±3.0*	77.7±3.3*
Granulocytes (%)	32.6±3.4	38.4±3.9*	41.2±3.0*	39.5±3.8*	21.4±3.6*

Values are expressed as mean±S.E.; NS: Not significant; *: Significant = ($p<0.05$)

Table 3: Serobiochemical analysis of rats given *A. glacum* methanol and aqueous extract orally for 2 weeks

		Methanolic extract		Aqueous extract	
Parameters	1. (Normal diet)	2. *A. glacum* (75 mg/kg/day)	3. *A. glacum* (300 mg/kg/day)	4. *A. glacum* (75 mg/kg/day)	5. *A. glacum* (300 mg/kg/day/)
One week					
AST (iu)	29.00±3.8	29.2±3.4^{NS}	24.00±3.5*	28.3±1.8^{NS}	29.9±2.1^{NS}
ALT (iu)	48.00±4.1	40.3±3.9*	24.00±3.5*	38.8±4.4*	41.3±4.3*
ALP (iu)	483.3±6.9	353.3±5.7*	261.3±6.8*	235.0±1.3*	369.6±1.5*
Total protein (g/dL)	07.30±0.2	07.3±0.3^{NS}	07.2±0.1^{NS}	08.3±0.5^{NS}	08.3±0.3^{NS}
Albumin (g/dL)	03.70±0.4	04.3±0.1^{NS}	04.0±0.1^{NS}	04.4±0.2^{NS}	04.4±0.1^{NS}
Globulin (g/dL)	03.60±0.3	03.3±0.4^{NS}	03.2±0.7^{NS}	03.8±0.3^{NS}	03.3±0.1^{NS}
Bilirubin (mg/dL)	00.20±0.0	00.1±0.0^{NS}	00.1±0.0^{NS}	00.1±0.0^{NS}	00.1±0.0^{NS}
Cholesterol (mg/dL)	108.7±5.4	111.3±6.4^{NS}	104.0±5.0^{NS}	124.0±6.5*	107.0±6.0^{NS}
Two weeks					
AST (iu)	31.20±3.9	37.80±3.8*	38.3±3.2*	48.50±3.5*	39.40±2.6*
ALT (iu)	47.70±5.4	60.70±5.1*	47.0±4.5^{NS}	75.50±5.5*	67.50±5.1*
ALP (iu)	260.0±4.2	425.7±4.1*	200.7±4.2*	442.5±4.3*	371.5±4.2*
Total protein (g/dL)	08.30±0.7	08.40±0.2^{NS}	08.4±0.1^{NS}	08.3±0.2^{NS}	07.7±0.7^{NS}
Albumin (g/dL)	04.40±0.7	04.70±0.1^{NS}	04.7±0.2^{NS}	04.5±0.1^{NS}	04.5±0.2^{NS}
Globulin (g/dL)	04.00±0.1	03.70±0.3^{NS}	04.0±0.2^{NS}	03.8±0.1^{NS}	03.2±0.5^{NS}
Bilirubin (mg/dL)	00.80±0.1	00.60±0.0^{NS}	00.7±0.0^{NS}	00.2±0.1^{NS}	0s0.2±0.1^{NS}
Cholesterol (mg/dL)	91.00±0.6	37.00±0.7*	108.0±0.4*	111.5±0.7*	99.00±0.5*

Values are expressed as mean±S.E.; NS: Not significant; *: Significant = ($p<0.05$)

aqueous extract at 75 mg/kg (Group 2) and 300 mg/kg (Group 3) for 2 weeks are presented in Table 3. One week after treatment the activities of ALT and ALP in group 2 and AST in group 3 were lower ($p<0.05$) than control, the activities of ALT and ALP in groups 4 and 5 were lower ($p<0.05$) than control group and the concentration of cholesterol in group 4 was higher ($p<0.05$) than control. Two weeks after treatment, The activities of AST in group 2 and 3, ALT and ALP in group 2 and the concentration of cholesterol in group 3 were higher ($p<0.05$) than the normal control group and the activity of ALP in group 3 and cholesterol in group

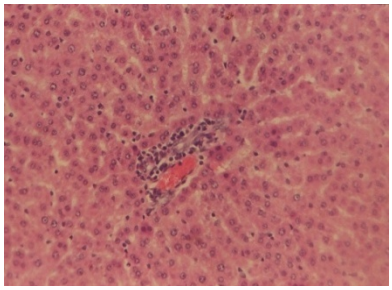

Fig. 2: Liver of the rats receiving daily oral doses of *A. glacum* seeds aqueous extract at 75 mg/kg for one week showing cytoplasmic fatty vaculation and necrosis of the hepatocyte H & E X100

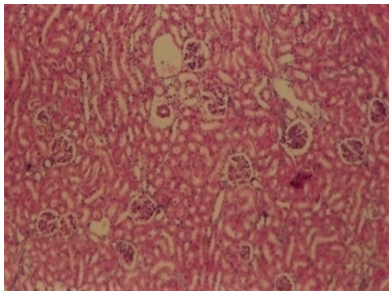

Fig. 3: Showing glomerular alteration, dilatation, vaculation and necrosis of scattered renal tubular cell of *A. glacum* seeds treated rat at 75 mg/kg methanol for one week H &E X100

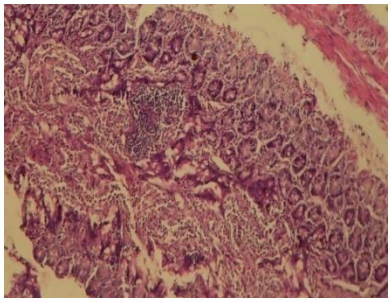

Fig. 4: Catarrhal enteritis and lymphocytic infiltration in intestinal lamina propria of a rat receiving daily oral doses of *A. glacum* seeds methanol Extract 300 mg/kg for 1 week H &E X100

2 were lower ($p<0.05$) than the control. The activities of ALT, AST and ALP in groups 4 and 5 and the concentration of cholesterol in groups 4 and 5 were higher ($p<0.05$) than the control.

Pathological changes: There were no lesions in the spleen, heart and other vital organs of control rats. There was a lesion in liver, kidney and intestine of all treated groups, there were fatty cytoplasmic vaculation and necrosis of the hepatocytes (Fig. 2), glomerular alteration, dilatation, vaculation and necrosis of scattered renal tubular (Fig. 3). Degeneration or necrosis of the epithelial cells of renal tubules with acidophilic homogeneous substance in affected renal tubules, catarrhal enteritis and lymphocytic infiltration in intestinal lamina (Fig. 4).

DISCUSSION

This study was conducted to show or investigate the toxic effect of the plant *A. glacum* on the experimental rats. The administrated doses were chosen at 75 and 300 mg/kg/day because these levels were toxic in other plants such as *Rh. epapposum* and *T. africanum* aerial parts' aqueous and methanol extracts given by different routes of administration which investigated by Shama and Adam (2008) and *M. esculenta* roots (Shama and Wasma, 2011) and The result of the present investigation indicated that *A. glacum* seeds were toxic but not fatal to rats in daily oral doses of (75 and 300 mg/kg) for two weeks, mimicking result in other study (Barlow *et al*., 2002).

The toxicity of plant material seems dependent of the types of active principles in the plant, the concentration added to the diet and the rate of their metabolic conversion in the liver to metabolites and their consequent excretion.

The phytochemical studies on *A. indicum* revealed the presence of alkaloids, glycosides, carbohydrates, steroids, saponins, carbohydrates, coumarins and flavonoids (Sammia *et al*., 2008; Khandelwal, 2002).

The results of present study showed that in rats given methanolic and aqueous extracts of *A. glacum* seeds at concentration 75 and 300 mg/kg there is damage and necrosis in the liver attributed to the decreased activity of ALT resulting from inability of hepatocytes to synthetize the enzyme. The Increases in the activity of AST is attributed to the damage in liver and heart.

There are no changes in albumin and total protein due to malabsorption in intestine resulting from desquamation or damage in other vital organs.

The toxicity from oral administration of 300 mg/kg/day of *A. glacum* seeds extracts for 2 weeks was sever as evidenced by consistent extensive damage to liver and kidney. The damage to these organs caused by daily oral doses of plant extract at 75 mg/kg/day for 2 weeks was less marked.

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