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EVALUATION OF 2-WEEKS TOXICITY OF AQUEOUS AND METHANOLIC EXTRACTS OF *Artemisia herba alba* (SHEEH) AERIAL PARTS ON WISTAR RATS

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ABSTRACT
The genus *Artemisia* has been used extensively in folk medicine by many cultures since ancient time (European medicine, North African and Arabic traditional medicine. *Artemisia herba alba* (locally known as Sheeh) is a herb wildly distributed in rural northern Sudan. It is considered a multipurpose medicinal plant and widely used in folk medicine for the treatment of many body ailments the most important of which are diabetes and malaria and as antimicrobial agent. The toxicity of two oral doses, 75 and 300mg/kg/day, of each of aqueous and methanolic extracts of *A. herba alba* aerial parts in Wistar rats for two weeks was evaluated. The results indicated that the extract was toxic exhibiting its effects by lowering body weight gain, and significant hematological and serobiochemical changes. These toxicity changes were highly correlated with dysfunction of vital organs namely, liver, kidney and intestines, no lesion were seen in other heart or spleen.

KEYWORDS: *Artemisia herba alba*, aqueous and methanolic extracts, Wistar rats, toxicity.

INTRODUCTION

For several years, in folk medicine, macerated plant extracts were used as treatments for several diseases. Some of plant treats disease but what their real effects don't take the required interest.[1] Medicinal plants constituents such as flavonoids, triterpenes, alkaloids, tannins, sterols and anthraquinones have been shown to be associated with antimicrobial, anthelmintic, antimalarial and antispasmodic activities.[2]

Artemisia herba alba Also, known also as desert wormwood (known in Arabic as sheeh), belongs to the plant family Asteraceae, has been used in folk medicine by many cultures since ancient times.[3] Different secondary metabolites have been isolated from *A. herba-alba*, the most important of which being the sesquiterpene lactones that occur with great structural diversity within the genus Artemisia. Additional studies have focused on flavonoids and essential oils [4] with an intraspecific variation in these constituents depending on the geographical area.[5] In folk medicine it is used to treat arterial hypertension and/or diabetes[6, 7] and the herbal tea has been used as analgesic and hemostatic agents.[8] *A. herba-alba* based teas were used in Iraqi folk medicine for the treatment of diabetes mellitus.[9] An aqueous extract of aerial parts of the plant has shown a hypoglycemic effect in alloxan-induced diabetic rabbits and mice.[10, 11, 12]

Artemisia herba-alba was found to possess moderate antioxidant activity[13], anti-venom [14], Antimicrobial [15; 16], nematicidal [17], antispasmodic [15], anthelmintic [18, 19], [20] reported this herb as a traditional remedy of enteritis, and various intestinal disturbances, among the Bedouins in the Negev desert.

Although, many plant families such as Apocynacea, Asteraceae, Cucurbitaceae and others, are considered important as folk herbal remedies and able to cure various ailments, many cases of poisoning by plants occur due to over dosage which is attributed to the absence of standardized dosage system in traditional herbal medicine.

Because of the common use of *Artemisia herba alba* in the treatment of various disorders as well as the lack of information in rodents and livestock, The objective of this study is to investigate the toxicity of two different doses of methanolic and aqueous extract of *Artemisia herba alba* aerial parts on growth, organ pathology, hematology and serobiochemical parameters.

MATERIALS AND METHODS

Experimental animals

Thirty male Wistar rats with average body weight ranged from 80 to 85g were used in this study. The rats were apparently clinically healthy and housed within the premises of El-Neelain University Animal House under standard husbandry conditions (30°C ± 2°, 60–70% relative humidity and 12h day-night cycle) and fed on the rat diet (flour 55.3%, meat 35%, edible oil 7.5%, sodium chloride 1.5% and vitamins and minerals 0.7) and water provided *ad. Libitum.* Animal experiments were designed and conducted in accordance with the guidelines of Institutional Animal Ethical Committee.

Plant materials

The aerial part of *Artemisia herba alba* (Fig. 1) was purchased from Khartoum herbal market and was botanically authenticated by the scientists at the Medicinal and Aromatic Plant Institute. The plant was cleaned and coarsely ground through mechanical grinder and the methanol and aqueous extracts were prepared and used in this study.

Fig. 1. *Artemisia herba alba* aerial part

Preparation of plant extract

Extraction was carried out using the Soxhlet extraction technique. A quantity of 100g of the coarsely powdered aerial parts were precisely weighed and extracted with 250ml methanol (99.8%), for 2 hr through soxhlet apparatus, then, the extract was separated from solvent using rotary evaporator and the air dried powder was used for the treatment (methanol extract). The plant residues were further extracted with distilled water over night at room temperature (25-30°C) filtered through NO.1 Whatman paper and freeze dried.

Experimental design

The rats were divided randomly to five groups each of six animals. The rats in group 1 were untreated controls and were given only distilled water daily for 14 days. The aqueous extract of *A. herba alba* aerial parts was given orally to rats orally at 75mg/kg/day for group 2 and 300mg/kg/day for group 3. The rats of groups 4 and 5 were given the methanol extract at 75 and 300mg/kg/day respectively. The treatment doses were administered to rats using stomach tubes.

For each group, body weight of rats was recorded weekly. Clinical signs and mortality rate were also recorded all through the experimental period. Blood samples for hematology and serum analyses and tissue samples for histopathology were taken at day 7, when half the animals in each group was sacrificed and at the end of the experimental period (day 14) after scarifying the remaining animals under mild chloroform anesthesia.

Hematological and serobiochemical analyses

The blood for measurement of complete blood count (CBC) was collected in EDTA blood containers and immediately analyzed through automated Haematology analyzer (Sysmex KX-21, Japan, 1999). while blood samples for serum chemistry were collected in heparinized sample containers and centrifuged immediately at 3500 *rpm* for 5min, the plasma was separated in a new plain sample container properly labeled according to the study group, rat number, time and date of collection and stored in refrigerator at -4 $^{\circ}$C until the used within one week of collection thought Roche diagnostic Hitachi 902 analyser (Germany).

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 5 µm and stained with hematoxylin and eosin stains [21], for histopathologic examinations.

Statistical analysis

Statistical Package for Social Science (SPSS) was used for the analysis of the data, the values of body weight and biochemical estimations were expressed as mean ± standard error of mean (S.E.M.) and analyzed through hoc Dunnet's simple *t*-test. Differences between groups were considered significant at $P < 0.05$ levels.[22]

RESULTS

Growth changes

The effects of plant extracts on body weight of treated rats were presented in Table 1. There was no significant increase on the body weight of all treated groups in the first week. A significant decrease (P<0.05) was observed in second weeks when compared to normal control (G1). No death was recorded among the rats along the treatment periods.

Table 1. Body weight and body weight gain in rats given oral doses of aqueous and methanolic extracts of *Artemisia herba alba* extract for 2 weeks

Groups	Body weight(g) Day 0	Body weight gain(g) One week	Body weight gain (g) Two weeks
1	85.0 ± 2.2	9.0 ± 2.7	11 ± 5
2	81.6 ± 2.7	11.2 ± 2.7 ^{NS}	4.3 ± 4.9*
3	83.6 ± 2.7	10.7 ± 2.8^{NS}	2.7 ± 5.1*
4	82.6 ± 2.7	10.9 ± 2.6^{NS}	3.5 ± 0.00*
5	81.6 ± 2.7	11.9 ± 2.6 ^{NS}	4.9 ± 5.0*

Values are expressed as mean ± S.E; NS not significant; * Significant = (P<0.05).

1: control group
2: 75mg/kg/day aqueous extract;
3: 300mg/kg/day aqueous extract
4: 75.mg/kg/day methanolic extract
5: 300mg/kg /day methanolic extract

Hematological changes

Hematological changes produced by the administered doses of the methanolic and aqueous extracts of *A. herba alba* was shown in Table 2. The effect of the plant extract administration after the first week on the hematological parameters was mild. After the end of the experimental period, there were significant increases (P<0.05−P<0.001) in the values of Hb, RBCs and PCV in all the treated groups when compared to the control rat group 1. Significant decrease (P<0.05− P<0.01) in the values of MCV and MCH in the test groups and the MCHC value in G32, G3 and G4 was also observed. The two doses of each extract (methanolic and aqueous) produced a significant increase (P<0.05−P<0.01) in the counts of WBCs and lymphocytes, whereas the granulocytes counts was significantly reduced (P<0.05) when compared to the controls.

Serobiochemical changes

The effect of extracts doses on serum enzymes and metabolites are given in Table 3. At the end of the two weeks, the activities of the enzymes ALT and ALP were highly (P<0.05 − P<0.01) reduced. AST activity and cholesterol concentration of the treated groups, the total protein of groups 3, 4, and 5, albumin and bilirubin concentrations of groups 3 and 4 were significantly increased (P<0.05 − P<0.01) when compared to the normal control group (G1).

Pathological changes

Neither gross lesion nor microscopic changes were seen in the vital organs of the control rats of group 1. In the rats given 300 mg/kg/day aqueous extract and 75 mg/kg/day methanolic extracts, group 3 and 4, there were necrosis of centrilobular hepatocytes, lymphcytic infiltration and hemorrhage (Fig.2), and disquamination of the intestinal epithelium with lymphocytic infiltration (Fig. 3). Group 3 and 4 showed severe glomerular and renal tubular degeneration with fatty cytoplasmic vaculation (Fig. 4). The rats given 75 mg/kg/day aqueous extract and 300 mg/kg/day (group 2 and 5) methanolic extract showed dilatation of the renal convoluted tubules glomerular necrosis with lymphocytic infiltration Fig 5. No lesions were observed in spleen or heart of the test rats.

Table 2. Haematological changes in rats given rats given oral doses of aqueous and methanolic extracts of *Artemisia herba alba* extract for 2 weeks.

Parameters	Control	Aqueous		Methanol	
	1. Normal diet	2. *A. herba-alba* (75mg/kg/day)	3. *A . herba-alba* (300 mg/kg/day)	4. *A . herba-alba* (75mg/kg/day)	5. *A . herba-alba* (300 mg/kg/day)
One week					
Hb (g/dl)	14.10 ± 0.9	17.90 ± 1.0*	15.50 ± 1.0^{NS}	15.30 ± 0.8^{NS}	13.30 ± 0.8^{NS}
RBC (X10^{6}mm^3)	9.70 ± 0.7	10.40 ± 2.6^{NS}	11.00 ± 0.8 *	9.90 ± 2.1^{NS}	8.50 ± 0.3^{NS}
PCV (%)	61.30 ± 3.1	66.20 ± 3.0 *	71.50 ± 3.1 *	63.00 ± 2.9^{NS}	55.10 ± 3.0*
MCV (m3)	63.20 ± 1.2	63.65 ± 1.1^{NS}	65.00 ± 1.2^{NS}	63.64 ± 1.3^{NS}	64.82 ± 1.2^{NS}
MCH (pg)	14.20 ± 0.2	17.40 ± 0.4*	14.90 ± 2.4^{NS}	15.50 ± 0.2^{NS}	15.60 ± 0.4^{NS}
MCHC (%)	23.00 ± 0.1	27.04 ± 0.2*	21.68 ± 0.2*	24.04 ± 0.1^{NS}	24.14 ± 0.1^{NS}
WBC (X10^{3}mm^3)	5.80 ± 0.8	4.20 ± 0.9*	4.10± 0.9*	5.20 ± 0.8 ^{NS}	4.90 ± 0.7*
Lymphocytes (%)	67.40 ± 3.4	67.50 ± 6.6^{NS}	70.10 ± 3.4 ^{NS}	68.40 ± 3.3 ^{NS}	62.70 ± 3.5*
Granulocyte (%)	32.60 ± 3.4	32.50 ± 6.6^{NS}	29.90 ± 3.4 ^{NS}	31.60 ± 3.5^{NS}	37.30 ± 3.3*
Two weeks					
Hb (g/dl)	14.20 ± 0.2	16.50 ± 0.2*	17.10 ± 0.3*	17.70 ± 0.1*	19.80 ± 0.2*
RBC (X10^{6}mm^3)	8.80 ± 0.2	11.80 ± 0.3*	16.60 ± 0.2*	20.70 ± 0.3**	11.50 ± 0.2*
PCV (%)	58.10 ± 0.9	75.60 ± 2.8*	85.50 ± 0.9***	91.00 ± 0.8***	63.20 ±0.8*
MCV (m^3)	66.02 ± 1.0	64.07 ± 1.0 ^{NS}	51.51 ± 0.9 **	43.91 ± 0.5 **	37.57 ±0.4* *
MCH (pg)	16.10 ± 0.1	13.90 ± 0.5*	12.00 ± 0.1*	9.20 ± 0.1*	11.30 ±0.1*
MCHC (%)	24.44± 0.6	21.82 ± 0.4 *	20.00 ± 1.0*	19.45 ± 0.9*	31.33 ± 1.0**
WBC (X10^{3}mm^3)	4.55 ± 0.5	6.20 ± 0.6*	7.30 ± 0.4*	7.70 ± 0.5*	7.30 ± 0.5*
Lymphocyte (%)	54.80 ± 1.0	68.30 ± 0.9*	65.80 ± 0.1**	72.30 ± 0.3**	73.00 ± 0.9**
Granulocyte (%)	45.20 ± 0.2	31.80 ± 0.1*	34.20 ± 0.2*	27.70 ± 0.1*	27.00 ± 0.3*

Values are expressed as means ± S.E; NS = not significant; *Significant = (P<0.05).

Table 3. Serobiochemical analysis of rats given oral doses of aqueous and methanolic extracts of *Artemisia herba alba* extract for 2 weeks

Parameter	Control	Aqueous		Methanolic	
	(Normal diet)	2. *A. herba-alba* (75mg/kg/day)	3. *A. herba-alba* (300 mg/kg/day)	4.*A. herba-alba* (75mg/kg/day)	5. *A. herba-alba* (300 mg/kg/day)
One week					
ALT (iu)	48.00 ± 5.0	36.30 ± 4.9*	44.00 ± 4.8^{NS}	54.00 ± 5.0*	39.70 ± 5.0*
AST (iu)	31.70 ± 3.9	24.40 ± 4.0*	31.40 ± 2.7^{NS}	29.20 ± 3.8*	38.80 ± 3.9*
ALP (iu)	264.00 ± 8.4	157.70 ± 8.3*	184.00 ± 8.5*	255.00 ± 8.4*	272.60 ± 8.5*
Total protein (g/dl)	8.30 ± 0.1	8.30 ± 0.1^{NS}	7.90 ± 0.1^{NS}	7.20 ± 0.1^{NS}	7.90 ± 0.2^{NS}
Albumin (g/dl)	4.40 ± 0.1	4.50 ± 0.0^{NS}	4.40 ± 1.0^{NS}	3.80 ± 0.2^{NS}	4.2 ± 0.2^{NS}
Globulin (g/dl)	3.10 ± 0.1	3.70 ± 0.1^{NS}	3.40 ± 0.0^{NS}	3.30 ± 0.2^{NS}	3.70± 0.2^{NS}
Bilirubin (mg/dl)	0.20 ± 0.03	0.10 ± 0.0*	0.200 ± 0.0^{NS}	0.20 ± 0.0^{NS}	0.10 ± 0.0*
Cholesterol (mg/dl)	82.30 ± 0.3	105.00 ± 0.3*	96.0 ± 0.3^{NS}	129.00 ± 0.3*	101.60 ± 0.3*
Two weeks					
ALT(iu)	64.70 ± 11.8	56.00 ± 11.9*	58.70 ± 11.7*	53.00 ± 11.8*	53.00 ± 11.8*
AST (iu)	35.00 ± 2.5	41.60 ± 2.5*	48.00 ± 2.6**	46.00 ± 0.4**	41.60 ± 0.9*
ALP (iu)	443.30 ± 9.9	259.30 ± 9.7*	208.00 ± 9.6**	205.50 ± 2.4**	181.30± 2.6**
Total protein (g/dl)	8.40 ± 0.2	8.70 ± 0.1^{NS}	9.80 ± 0.1*	9.70 ± 0.1*	9.50 ± 0.2*
Albumin (g/dl)	4.60± 0.2	4.80 ± 0.2^{NS}	5.10 ± 0.2*	5.90 ± 0.3*	4.90 ± 0.10^{NS}
Globulin (g/dl)	3.80 ± 0.5	3.90 ± 0.2^{NS}	4.70 ± 0.1*	3.30 ± 0.2 ^{NS}	4.60 ± 0.6 ^{NS}
Bilirubin (mg/dl)	0.20± 0.0	0.20 ± 0.1^{NS}	0.70 ± 0.1*	0.53 ± 0.2*	0.20 ± 0.0^{NS}
Cholesterol (mg/dl)	87.70 ± 6.0	109.70 ± 5.0*	98.30 ± 7.0*	98.00 ± 6.0*	105.30 ± 6.0*

Values are expressed as means ± S.E; NS = not significant; *Significant = (P<0.05).

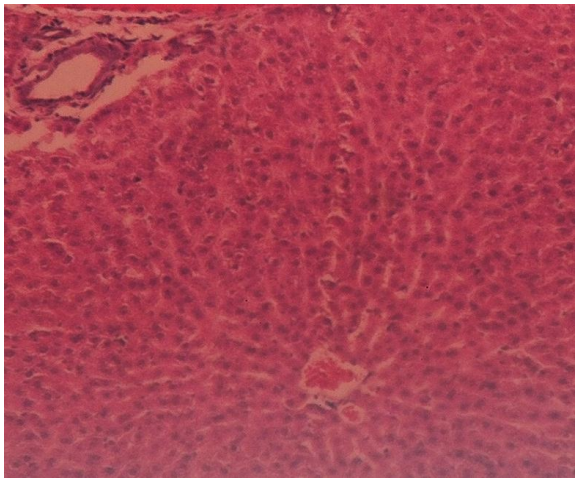

Fig 2. Necrosis of centrilobular hepatocytes, lymphcytic infiltration and hemorrhage in a rat given oral doses of *A. herba alba* methanolic extract 75 mg/kg for two weeks . H&E X 100.

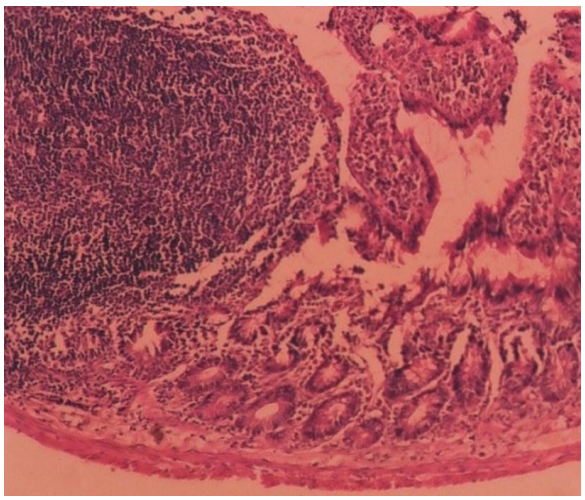

Fig.3. Disquamation of the intestinal epithelium and lymphocytic infiltration in rat receiving 300 mg/kg oral dose of *A. herba alba* aqueous extract for 2 weeks, H&E X 100.

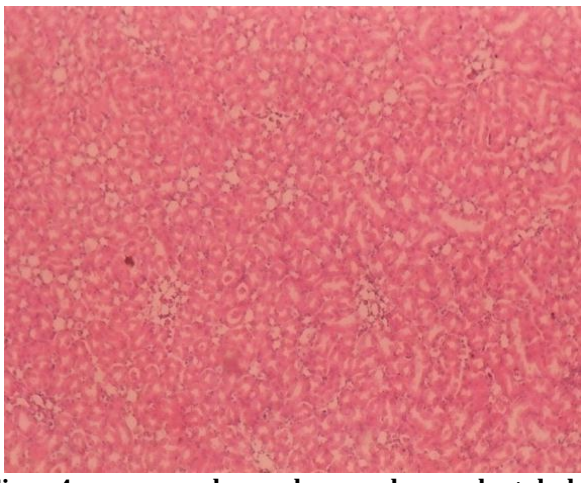

Fig. 4 severe glomerular and renal tubular degeneration with fatty cytoplasmic vaculation in a rat orally given 300 mg/kg/day aqueous extract of *A. herba alha* (H&EX100).

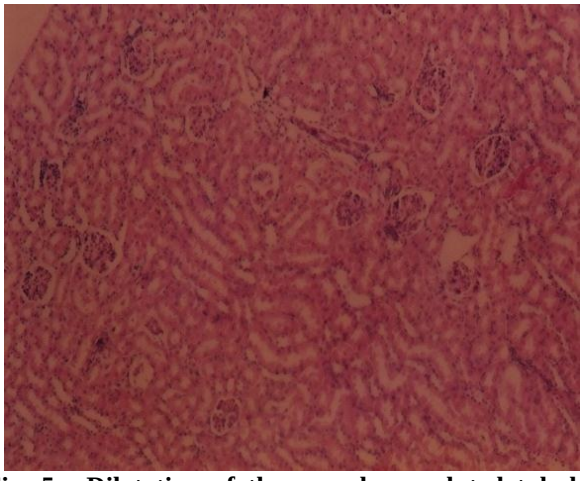

Fig. 5. Dilatation of the renal convoluted tubules glomerular necrosis with lymphocytic infiltration in a rat orally given 300 mg/kg/day methanolic extract of *A. herba alba* (H&EX100).

DISCUSSION

Artemisia herba alba is extensively used in Sudanese traditional medicine for the treatment of diabetes but very meager toxicological information is available therein. The results of the present research indicated that methanolic and aqueous extracts *A. herba alba* aerial parts at 75 and 300 mg/kg for two weeks are toxic as evidenced by growth impairment, histological, hematological and serobiochemical alterations and that the liver and kidney are the sensitive organs to toxic action of *A. herba alba*.

The *A. herba alba* aerial part has many phytochemical components having considerable concentrations of trepenoids, alkaloids, glycosides and sterols.[23] Whether these components have yielded individual or synergistic toxicities was only a symptomatic and/or histopathologic judgment, though some individual component has known toxicity symptoms and lesions.[24]

In the rats given 75, and 300 mg/kg of each of the aqueous and methanolic extracts of *A herba alba* aerial parts, the damage to vital organs could explain the depression in growth. The mechanism whereby *A. herba alba* aerial part exerts this effect cannot be stated in the present study but the injury to these organs could contribute to the reduction of ALT and ALP activities and increase of AST activity and cholesterol concentration. The decrease in MCV with a significant decrease in MCHC indicates microcytic hypochromic anaemia [25] found that the administration of *A. herba alb*a at different doses for 30 days induces dose-dependent decrease in fetus size without any other noticeable toxicological effects. On the other hand, a slight decrease in the relative ovarian weights and a significant decrease in embryo weights were observed by [26] in female rats received *A. herba alba* extract for 4 weeks. However extending the exposure period to 12

weeks with the same dosing rates revealed a significant decrease in both parameters.

A renal toxicity study carried out by [27] revealed an exceptional case of acute renal failure, but the mechanism by which the plant injured the kidney remains unknown.

CONCLUSION

The aqueous and methanolic extracts of *Artemisia herba alba* aerial parts administrated orally to Wistar rats at 75-300 mg/kg/day for two weeks are toxic but not fetal. The toxicity after the first week was mild when compared to severity at the end of the experimental period (two weeks).The toxicity is characterized by lower body weight gain, extensive tissue lesions and significant hematological and serobiochemical changes.

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