TOXICITY OF *ARGEMONE MEXICANA* SEED, SEED OIL AND THEIR EXTRACTS ON ALBINO RATS

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ABSTRACT

Albino rats received *A. mexicana* seed, seed oil and ethanolic seed extracts in different dosages and routes of administration suffered hyperae-sthesia, inappetence, intermittent diarrhea, emaciation and decrease in body weight. Hepatorenal lesions accompanied with increase in serum GOT activity and urea concentration were the pathological findings in rats.

INTRODUCTION

Argemone mexicana L. a member of the family Papaveraceae is commonly found in Sudan.Bui and Muraveva (1973), and (Muraveva and Bui, 1974) studied *A. mexicana* of Sudanese origin and reported the presence of alkaloids protopine, allocryptopine sanguinarine and chelerthrine and Khalid, (1985), found no trace of morphine or codeine. (Oliver-Bever, 1986) reported that protopine stimulates the heart, blood pressure and respiration, as well as the striated and smooth muscles and allocryptopine slows down the heart and prolongs systole in rats, frogs, cats and rabbits. Sanguinarine inhibits neutral protease activity (Sakamoto, 1986) and prolongs the ventricular refractory period (Whittle *et al.,* 1980). The potential role of sanguinarine found in *Argemone mexicana L.* seed oil, as aetiology of epidemic dropsy glaucoma was suggested by (Sarker, 1948). Forensic scientists claim that numerous fatalities have occurred in Sudan due to addition of *A. mexicana L.* seeds to native alcoholic drinks (Aragi or Merrisa) probably arising from increase the narcotic potency. Therefore, information on toxicity is needed by law enforcement bodies. An experiment with rats was conducted to elucidate the clinicopathological effect of *A. mexicana* seed and seed extracts.

MATERIALS AND METHODS

Plant Material: *A. mexicana* seeds were collected from Hillet Kuku, Khartoum North, and ground with a mortar and pestle and then mixed in the diet.

Animals: One hundred and twenty albino rats of both sexes, 45days of age and of 200gm average body weight were used. The rats were clinically healthy and housed within the premises of the Faculty of Pharmacy, University of Khartoum, and fed on rat diet (flour 75.3%, meat 15%, edible oil 7.5%, sodium chloride 1.5% and vitamins + amino acids 0.7%) and water provided *ad. Libitum.*

Administration of Plant-Seed, Seed Oil and Extract: 120albino rats of both sexes, 45days of age and 200gm average body weight were used. The rats were clinically healthy and housed within the premises of the Faculty of Pharmacy, University of Khartoum, and fed on rat diet consisted of (flour 75.3, meat 15, edible oil 7.5, sodium chloride 1.5 and vitamins + amino acid 0.7%). The rats were divided randomly into 6 groups of 20 each. Group1 rats were fed on ration containing 10% ground seeds while group 2 rats were fed on ration containing 10% seed oil (n-hexane extract). 50mg/kg of direct

ethanolic seed extract was dissolved in water and then administered intraperitoneally (i/p) to each rat in group 70% Ethanolic extract (obtained from the seeds remaining after extraction with n-hexane) at 250 and 500mg/kg were dissolved in drinking water of group 4 and 5 respectively. Group 6 rats were the undosed controls. Body weight of each rat was recorded weekly. Doses continued daily until the rats died or were slaughtered. Survivors groups 1, 2, 3, 4, 5 and 6 were slaughtered at days 31, 7, 25, 22, 12 and 31 respectively.

Serobiochemical Methods: Blood samples were collected immediately after slaughter by severing the cervical blood vessels were allowed to clot, centrifuged at 3000rpm and the separated sera were stored at $-20°C$ until analyzed, for the activities of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP), and for the concentrations of uric acid, urea and total cholesterol by commercial kits (Cromatest Laboratories Knickerbocker, S.A.E. Barcelona, Spain). Serum total protein concentration was measured by a refractometer (No. 43098, Atago, Japan).

Pathological Methods: Necropsy was made immediately after death or slaughter to identify gross lesions and specimens of heart, lungs, stomach, liver, kidneys, intestines, spleen, muscles, spinal cord and sciatic nerve were fixed in 10% neutral buffered formalin and processed for histopathology and stained with haematoxylin and eosin (H&E).

Statistical Methods: Statistical Package for Social Science (SPSS) was used for the analysis of the data. Duncan's multiple range test in the one-way ANOVA was used.

RESULTS

Clinical Findings: In rats of groups 1, 2, 3, 4 and 5 hyperaesthesia, inappetence, intermittent diarrhea, emaciation, decrease in body weight, erection of hair and weakness of the fore and hind limbs ended with complete paralysis are the prominent manifestations. These signs appear on days 4, 3, 12, 6 and 5 and are more severe in rats of groups 2, 5, 4, 3 and 1, respectively. Significant decrease ($P < 0.05$) in mean body weight/rat is observed in groups 1 (197.5–171.0), 2 (196.5-177.2), 3 (206.5–179), 4 (237–179) and 5 (216-190) gm, at the end of the experiment. The controls (group 6) showed an increase in mean body weight/rat from 171-197.5gm.

Post-mortem Findings: The heart, liver, kidneys and intestine of rats in groups (1–5) showed slight to moderate congestion and/or haemorrhage. Catarrhal enteritis and fatty change and necrosis are seen in the liver (Fig. 1) and kidneys of the test rats. Severe inflammation is observed in the abdominal muscles and subcutaneous tissues at the site of injection (Fig. 2), in rats of (group 3). There are no lesions in the control-undosed rats (group 6).

Histopathological Findings: Slight centrilobuar hepatocellular necrosis or fatty vaculation is detected in the liver of rats in groups (1 and 3). The hepatic necrosis or fatty vaculation extended to the midzone in groups (2, 4, and 5). Congestion of the sinusoids and accumulation of lymphocytes are also seen in the liver of treated groups.

Some rats of groups (2, 3 and 5) show degeneration and necrosis of the renal glomeruli and cortical tubular cells. These tubules contain desquamated cells or acidophilic homogeneous material. Some rats in groups (2 and 5) show dilation of renal convoluted tubules. Scattered haemorrhagic foci are seen in the renal interstitial tissue specially in rats of groups (2 and 3). Lymphocytic aggregates are seen in the cortex of rats in (group 4).

Haemosiderin deposits are seen in the red pulp of the spleen of dosed rats specially in groups (2, 3 and 5). Severe lymphocytic infiltration is observed in the

gastrointestinal mucosae and submucosae and the lumen of the intestine contained desquamated epithelial cells groups (1, 2, 4, and 5).

There are slight emphysema, congestion, oedema and peribronchiolar lymphocytic infiltration in the treated rats specially in groups (2 and 5). Smalls cattered foci of haemorrhage are seen between the cardiac muscle fibres of rats in groups (2, 3 and 5). Severe myositis with lymphocytic infiltration, necrosis or oedema of muscle bundles is seen in (group 3) at site of administration. None of the dosed rats showed changes in peripheral nerves or the spinal cord.

Serum Chemistry: The results of serum analysis in rats are summarized in (Table 1). The 6 groups gave significantly different mean values for each of measured parameters. The significance was determined at the level of $P < 0.05$, and was also calculated within the groups. Activities of GOT in groups 1, 2, 3 and 5 rats were significantly higher than the rest, group 4 was intermediate. The control group 6 showed the least GOT mean value. However, groups 2, 3, 4 and 5 were not different. The overall serum GOT activity gave probability at ($P < 0.0001$).
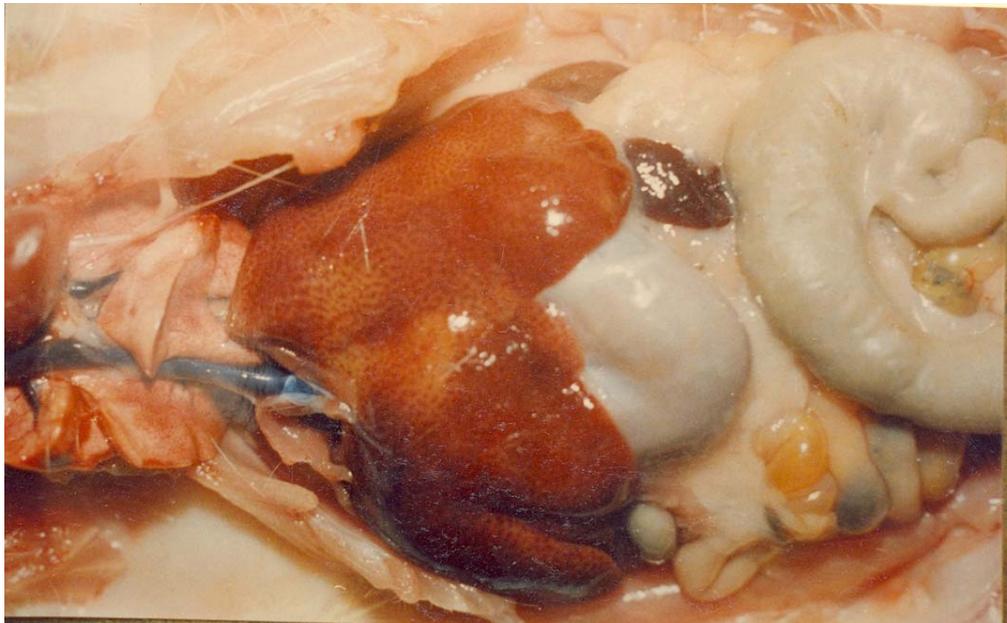

Fig. (1): Fatty change in liver of dosed rats

Fig. (2): Severe inflammation at the abdominal muscles and subcutaneous tissue at the site of injection in rats of group 3

Groups 2, 3, 4 and 5 had significantly higher GPT mean values, whereas the control group 6 rats showed the least mean value. This least value was not different from that exhibited by group 1 rats. The activity of serum GPT gave a probability at (P < 0.02).

The significant changes (P < 0.05) were seen in mean values of serum ALP activity when compared to control rats group 6 but this was within normal levels.

The highest serum total protein mean value was shown by group 2 rats. Groups 1, 5 and 6 gave significantly second higher total protein mean value. The control and group 4 were not significantly different, while groups 3 and 4 gave the least total protein contents. The probability within the group was found to be at (P < 0.0001).

Group 1 rats gave the highest serum cholesterol level. Groups 2 and 6 showed intermediate comparable results, while groups 3, 4 and 5 had significantly lower mean values. The probability was found at (P = 0.0001).

Serum urea gave a probability equal to 0.0001. Group 2 of rats gave the sole significantly higher mean urea value. The other groups of rats including the control were not significantly different from one another.

Groups 1,2 and 3 showed the highest mean uric acid values, Groups 4,5 and 6 gave the least mean values while Groups 1,3 and 6 gave intermediate results. Although there were significant differences in serum ALP, total protein, cholesterol and uric acid of rats, the data within the normal level.

Table (1): Changes in Serum Constituents of Rats Poisoned with *A. mexicana L.* Seed or Seed Extracts (M $\pm$ S.E)

Groups	Total Protein g/100ml	Cholesterol mg/100ml	Urea mg/100ml	Uric Acid mg/100ml
1 10% seed in (Ration) (*)	6.4 ± 0.31^{b} (5.6-7.1)	156.6 ± 5.0^{a} (145.35-167.91)	39.7 ± 4.7^{b} (29.1-50.3)	2.5 ± 0.2^{ab} (1.9-3.1)
2 10% 0il in (Ration) (*)	7.6 ± 0.2^{a} (7.1-8.0)	142.9 ± 4.3^{b} (132.75-153.00)	62.9 ± 11.2^{a} (63.6-89.3)	3.00 ± 0.39^{a} (2.07-3.9)
3 50mg/kg (I.P) (*)	5.2 ± 0.1^{d} (4.9-5.6)	133.3 ± 6.2^{bc} (118.2-148.3)	26.2 ± 2.7^{b} (19.7-32.8)	2.5 ± 0.3^{ab} (1.79-3.12)
4 250mg/kg (Orally) (*)	5.6 ± 0.1^{cd} (5.2-5.9)	125.52 ± 1.36^{c} (122.4-128.6)	27.96 ± 0.39^{b} (27.1-28.9)	1.7 ± 0.2^{bc} (1.0-2.0)
5 500mg/kg Orally (*)	6.3 ± 0.2^{b} (5.9-6.6)	137.2 ± 2.1^{bc} (132.1-142.5)	36.13 ± 0.8^{b} (34.3-38.0)	1.4 ± 0.2^{c} (0.94-1.83)
6 (Control) (*)	6.0 ± 0.1^{bc} (5.7-6.3)	140.28 ± 4.16^{b} (130.7-149.9)	26.23 ± 1.14^{b} (23.6-28.9)	1.7 ± 0.2^{bc} (1.3-2.2)

Groups	GOT i.u	GPT i.u	ALP i.u
1 **10% seed in (Ration)** (*)	**94.0 $\pm$ 6.7** ^{a} (78.8-109.2)	**33.7 $\pm$ 4.6** ^{bc} (23.4-44.0)	**127.8 $\pm$ 28.1** ^{b} (64.3-191.2)
2 **10% 0il in (Ration)** (*)	**81.9 $\pm$ 6.0** ^{ab} (67.8-96.0)	**41.4 $\pm$ 3.1** ^{ab} (34.4-48.9)	**91.4 $\pm$ 16.5** ^{b} (52.4-130.3)
3 **50mg/kg (I.P)** (*)	**82.9 $\pm$ 4.9** ^{ab} (71.0-94.8)	**45.0 $\pm$ 4.8** ^{a} (33.4-65.6)	**114.9 $\pm$ 31.2** ^{b} (38.5-191.3)
4 **250mg/kg (Orally)** (*)	**76.5 $\pm$ 5.2** ^{b} (64.7-88.3)	**43.5 $\pm$ 3.3** ^{ab} (36.1-50.9)	**9.4 $\pm$ 5.2** ^{b} (78.6-102.3)
5 **500mg/kg (Orally)** (*)	**90.4 $\pm$ 3.6** ^{ab} (81.7-99.2)	**41.4 $\pm$ 2.4** ^{ab} (35.6-47.2)	**120.5 $\pm$ 1.02** ^{b} (118.0-122.9)
6 **(Control)** (*)	**29.4 $\pm$ 2.1** ^{c} (24.6-34.3)	**30.2 $\pm$ 1.4** ^{c} (27.0-33.4)	**138.5 $\pm$ 31.8** ^{a} (65.1-211.8)

* = 95% Confidence interval for the mean. Means in the same vertical row with different superscripts are significantly different. ($P < 0.05$), M $\pm$ S.E = mean $\pm$ Standard error.

DISCUSSION

It is found that *A. mexicana* seeds, seed oil, and seed extracts repeated at doses used caused inappetance and decreases in body weight of rats, are toxic and fatal to some rats. This result is in agreement to that reported by (Kausal *et al.,* 1989) who found retardation in growth and food consumption in rats fed diets containing 5% seed oil for 8 weeks.

Ranvir and Chaterjee, (1989) studied the toxicity of *A. mexicana* seeds to rats and observed significant reduction in body weight, significant increase in blood glucose, BUN and SGOT and lesions indicative of hepatonephropathy. (Kausal *et al.,* 1988) suggested that the hepatic microsomal enzymes as well as the mitochondrial membranes are vulnerable to the peroxidative attack of *A. mexicana* oil and may be instrumental in leading to the hepatotoxicity symptoms noted in *A. mexicana* poisoned victims.

The intermittent diarrhea may attribute to gastroenteritis or to the parasympo-pathomimtic cholinergic effect of the plant constituents (El Gamal, 1995).

The involvement of the nervous system in the plant toxicosis especially in epidemic dropsy is controversial. (Sachdev *et al.,* 1989) concluded that *A. mexicana* or its toxins do not have any significant effect on neuron system in human. However, (El Gamal, 1995) found that prostaglandin F_2 like activity is significantly increased in brain tissue extracted from albino rats fed on *A. mexicana* seed for 31days. (Moncada *et al.,* 1978) pointed to the implication of the prostaglandin system in the inflammatory changes, which characterize *A. mexicana* toxicity through the extensive membrane damage favouring release of endogenous fatty acid substrate.

Hyperaesthesia, depression and weakness of the limbs may be attributed to hepatornal insufficiency or significant reduction of the cardiac muscles which may lead lately to congestive heart failure and/or the involvement of the nervous system.

The present study has shown that the characteristic features of *A. mexicana* seed and seed extract toxicity in rats are more of neuro-enterohepatonephropathy.

For future work, it is highly recommended to carry out a bioactivity, directed fractionation to detect which chemical compounds among the secondary metabolites is responsible for each of the pharmaco/toxicological manifestations associated with this taxon.

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