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PATHOLOGICAL CHANGES IN EXPERIMENTALLY INDUCED *ASTRAGALUS CEPHALOTES* TOXICITY IN MICE

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There are reports on the pathological and toxicological effects of many *Astragalus* species in domestic and experimental animals (James *et al.* 1981). However, there are no record on the pathological effects of *A. cephalotes* in domestic or experimental animals. Therefore, the pathological effects of *A. cephalotes* a plant which grows in the East Anatolian Region of Turkey is reported.

Materials and Methods

Sixty SWR - Swiss white mice were used in this study. The mice were obtained from the Institute of Veterinary Control and Research in Elazig. The sprouts and roots of *Astragalus* were collected from the pastures of Elazig province of East Anatolian Region of Turkey. The *Astragalus* species were identified by the Department of Biology, Faculty of Science and Literature, Firat University in Elazig, Turkey. Initially mice were fed standard mouse feed for a week. The animals were randomly divided into two groups of 30 (15 female, 15 male mice); experimental sprout (ES) and experimental root (ER) groups. The ES and ER groups were further divided into 5 subgroups each containing 6 mice (3 male, 3 female). Roots and sprouts of *A. cephalotes* were collected daily, chopped in the mixer and added to ground mouse feed at ratios of 5%, 10%, 15% and 20% for experimental subgroups and 0% for control groups. The supplemented groups mouse feed was pelleted again and fed to mice *ad libitum* for 15 weeks. The control groups (0%) were fed on standard mouse pellets. The weight of mice in all the groups were measured weekly. The Independent Samples T-test was performed to analyze the results using the "SPSS for Windows Release" programme. The clinical findings observed in the mice were recorded during the experimental period. At the end of the experimental period all mice remaining in the experimental and control groups were killed by ether anaesthesia and systemic necropsies were performed. Tissue samples were taken from both the mice that died during the experimental period and mice killed at the end of the experiment. Samples from the lung, heart, liver, pancreas, spleen, kidney, stomach, intestines, colon, brain, and cerebellum were fixed in 10% buffered neutral formalin and the samples of testes were fixed in Bouin's solution. Tissues were prepared by routine procedures, embedded in paraffin blocks, cut into sections, 5 µm thick and stained with hematoxylin eosin (H.E.) and the Turnbull Blue Method for heamosiderin. Tissue samples taken from the liver were cut using a frozen microtome, stained with Oil Red O and examined under light microscope.

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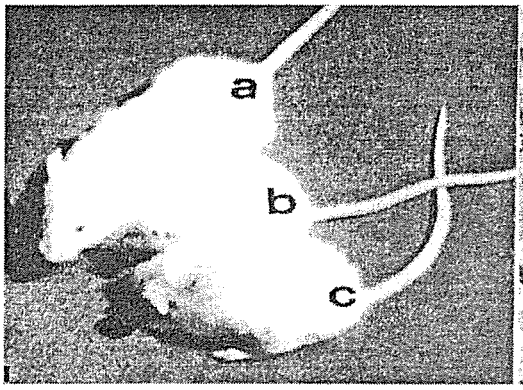


Fig. 1.1 Control group mice (a), sprout group mice 20% (b), root group mice 20% (c),

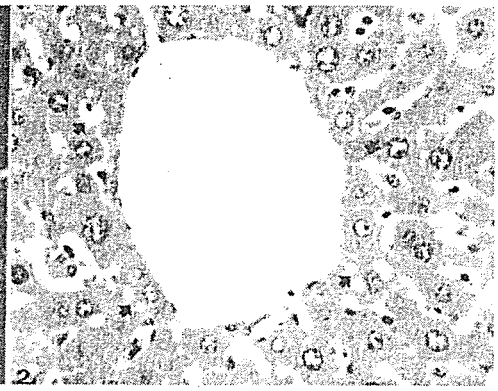


Fig. 1.2. Vacuolations and grannular degeneration in the periacinar region of the liver. HEX400 (20% ES group).

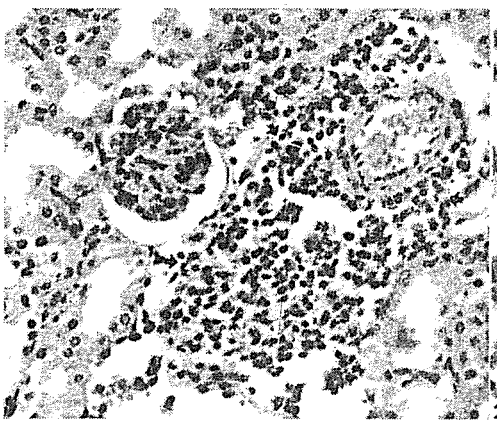


Fig. 2.1 : Kidney : Focal lymphocyte infiltration in the periglomerular and perivascualr region and tubular degeneration.

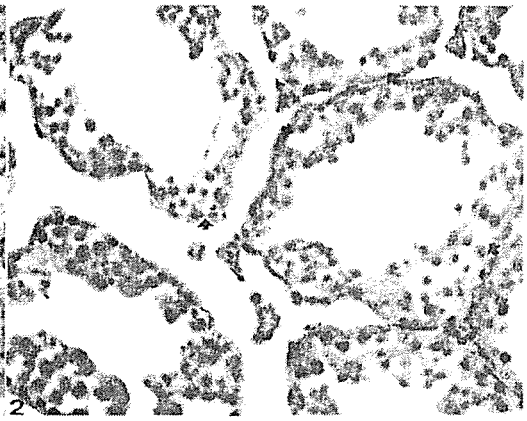


Fig. 2.2. Testis : Vacuolations and reduced spermatogenesis in the seminiferous tubules HEX400 (20% ES group).

Results and Discussion

In the 8th week of the experiment, weight loss (Fig. 1.1), lethargy, fatigue, tremor and sudden jerky movement reactions to light, sound and physical stimulants were noticed in the 10,15 and 20% ES and ER subgroups. Cattle, horses, goats, sheep and deer are known to be susceptible to *Astragalus toxicity*. Acute or chronic toxicity are seen in domestic and experimental animals according to the amount of the plant ingested (James *et al.*, 1981; Wolfe and Lance, 1984). Clinical symptoms in *Astragalus* toxications occur within a minimum of 2 to 3 weeks and a maximum of 7 to 8 weeks (James *et al.*, loc. cit). In this study, clinical manifestations were observed after the 8th week in the 10, 15 and 20% ES and ER subgroups, which was consistent with the literature.

Accumulation of jelly-like yellow-colored fluid (2-5 ml) in the abdominal cavity was observed in all the treated ES and ER

subgroups. Congestion and gray-white colored foci, 1-2 mm in diameter, were observed on the visceral surface of the liver and they were especially evident in the 15 and 20% ES subgroups. Pin-Point haemorrhagic lesions were observed in the lungs of both experimental subgroups. Prominent subcapsular hyperemia and haemorrhage were observed in the kidneys of the 15 and 20% ES subgroups with similar but milder findings in the 15 and 20 % ER subgroups. No macroscopic lesions were detected in the testes of the 5 and 10% ES and ER subgroups. The testes in the 15 and 20% ES and ER subgroups had a swollen appearance and were softer in consistency. No abnormal macroscopic findings were detected in the other organs.

Congestion of the liver veins was observed in the 5 and 10% ES and ER subgroups. Vacuolation in the periacinar hepatocytes, focal coagulation necrosis, perivascular lymphocytic infiltration, haemorrhage and hemosiderosis were observed in the 15 and 20% ES and ER subgroups (Fig. 1.2). Mild hyperemia and haemorrhage were observed in the cortex and medulla of the kidneys in the 5 and 10% ES and ER subgroups. Vacuolation and degeneration in the proximal convoluted tubular epithelium together with perivascular and periglomerular lymphocytic infiltration were observed in the 15 and 20% ES and ER subgroups, which were more severe in the 15 and 20% ES subgroups (Fig. 2.1). Perialveolar capillary hyperemia, haemorrhage and thickening of the interaveolar septum were observed in the 15 and 20% ES and ER subgroups. No significant histopathologic changes were observed in the testes of the 5 and 10% ES and ER subgroups. The basal membranes of the testicular seminiferous tubules were thin and vacuolations were evident in the tubular epithelial cells of the 15 and 20% ES and ER subgroups (Fig. 2.2). No significant vacuolation and no gross lesions were observed in any organs, of the control mice. Macroscopically, edema and congestion in the lungs, subepicardial petechial hemorrhage, hepatomegaly and discoloration of the liver have been reported (El Hamidi and Leipold, 1989). Microscopically, vacuolation in the hepatocytes, renal tubular epithelial cells and neurons and degeneration of the tubular epithelial cells of the testes have been described (Romero *et al.*, 2004).

The principal microscopic finding of *Astragalus* genus was vacuolation of the parenchymatous cells such as hepatocytes, renal epithelial cells and neurons (Romero *et al., loc. cit*). In this study vacuolations caused by *A. cephalotes* in the cytoplasm of hepatocytes, tubular epithelial cells of the kidney and testes as seen in other *Astragalus* genus were observed. These vacuolations were stained with Oil Red O to distinguish them from fat vacuoles, but no fatty tissue was observed in the liver. In a study on sheep (El Hamidi and Leipoid, *loc. cit*), no neuronal vacuolations were observed, but this was considered to be due to accumulation of mannose - rich oligosaccharides in the cell cytoplasm that was insufficient to form a vacuole. Likewise, vacuolations in the cytoplasm of neurons was not observed in this study.

Studies report weight loss in livestock due to *Astragalus* toxication (James *et al., loc. cit*; Oehme and Bailiew, 1968). In this study, there was no statistically significant difference in the weights of mice between experimental groups, but a significant difference was observed between the weights of the control group and ER groups and also between the control group and ES groups.

Degeneration in the tubular epithelial

cells of the testes and reduction in the spermatogenesis have been reported after chronic intoxication of rams and bulls by experimental dosing of *Astragalus* species (Panter *et al., loc. cit*). In this study, a decrease in birth and an increase in still birth rates were noticed in the female mice in the 15 and 20% ES and ER subgroups. Macroscopic softening and swelling and also degeneration of the epithelium of the seminiferous tubules together with reduced spermatogenesis in the testes of male mice in the 15 and 20% ES and ER subgroups was observed. This may be the reason for reduced birth rates among the female mice in the same subgroups after the 10th week of the experimental period.

Astragalus lentiginosis and *Astragalus pubentissimus* given to sheep on the 25th, 30th and 120th days of pregnancy (Pfister *et al.*, 2005), and cattle on the 120th day of pregnancy (James *et al.*, 1967) caused abortion and fetal malformations in sheep after 58th day and in cattle after 145th day of pregnancy. In this study, *A cephalotes* was given to white mice *ad libitum* for 105 days, but no fetal malformation was observed.

Summary

In this study, an experimental toxicity was induced in SWR - Swiss white mice by feeding standard pellets supplemented with *Astragalus cephalotes*. Clinically, lethargy, tremors, sudden jerks in response to stimuli, loss of weight and decreased birth rate were observed. Macroscopically, there was yellowish gel - like fluid within the abdomen in all the treated ES and ER groups. White focal areas 1-2 mm diameter were observed in the liver of the treated mice in the 15 and 20% ES and ER group. There were vacuolations in the parenchyma cells of the liver, kidneys and pancreas as well as congestion and haemorrhages in the lungs and heart. In addition, there was focal coagulation necrosis and perivascular lymphocyte infiltrations in the liver and perivascular and periglomerular lymphocyte infiltrations in the kidneys of the 15 and 20% ES and ER groups. It was observed that *A. cephalotes* sprouts were more toxic than *A. cephalotes* roots.

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