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### Toxicological effects of *Ambrosia maritima* in Nubian goats

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#### ABSTRACT

Melon Toxicity of Indigenous plant *Ambrosia maritima* was investigated. Twenty fore, 6-8 month old, 12 female and 12 male of Sudanese Nubian goats were used. Dried Leaves powder of *Ambrosia maritima* was mixed with water and given orally by drench to the animals at a dose of 1000 or 2000mg/kg/day for 126 days. Two females drenched the plant at a dose of 1000mg/kg/day, were died at day 8 and 9 post treatment and one female drenched the plant at a dose of 2000/kg/day died at day 13. The results revealed that goats drenched the plant for 126 days, showed chronic toxicity evident by clinical symptoms, pathological and biochemical changes. The main symptoms were watery diarrhea, inappetance, respiratory distress and depression. Later these symptoms disappeared and the animals appeared healthy. The most evident pathological features were hydrothorax, hydroperitonium, hydropericardium, and enlargement of the liver. Histopathological changes were exemplified by degeneration and necrosis of the hepatic cells. Focal necrosis, congestion and haemorrhag of proximal convoluted tubules. Necrosis of intestinal vili with severe infeltration of inflammatory cells. Congestion of alveolar capillaries and pancreatic hyperplasia. These changes were correlated to the activity of Asparate Aminotransferase (AST), Alkaline phosphate (ALP), the concentration of cholesterol, sodium and potassium in the serum, and with the hematological values. The effect of the plant on males was less marked compared to that on female goats. There was gradual increase in mean body-weight of both sexes.

**Key words:** *Ambrosia maritima*, Nubian goats Toxicity, serology, hematology

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## Introduction

Ragweeds (*Ambrosia*) is a genus of flowering plants from the sunflower family (Asteraceae). It grows in temperate regions of the northern hemisphere and South America where dry, sunny grassy plains, sandy soils, along river banks.

There are about forty species worldwide. The plant *Ambrosia maritima* (Damsessa) is a member of this family and is widely used in the Sudan in the folk medicine as antidiabetic (EL Gaazali et al., 1987; Ammar et al., 1993). Also the plant has been reported to have molluscicidal activity against the intermediate hosts of *Schistoma spp.* (El-Magdoub et al., 1977; Vassiliades and Diaw, 1980; El-Sawy et al., 1984; Picman et al., 1986; Delgado et al., 1988; Abou-Basha et al., 1994). El-Hamawi et al. (2004) reported that *A. maritima* had nematocidal effect and it has also been reported to have acaricidal activities against *Hyalomma anatolicum* tick (Ilham, 2012).

The toxicity of *A. maritima* in fish *crustacea* and rats was studied by Alard et al. (1991) and Alard et al. (1991), respectively. They found that *A. maritima* has a very low toxicity to aquatic non-target organisms and it was not toxic when used at the molluscicidal concentrations of 35 to 70 mg/L. No toxic signs could be detected in rats neither after oral administration of 5 mg/kg of dried leaves of the plant as powder or as a methanolic extract, nor after the incorporation of 50,000 ppm. powdered leaves in the feed during 4 weeks. Afaf et al. (2007) reported that a single dose of 2000 mg/kg Body weight of the methanolic extract of *A. maritima* was fatal to rats, but a daily dose of 500 and 250 mg/kg (Bwt) was not fatal. Vassiliades and Diaw, (1980) stated that up to 1000 ppm had no toxic effects in mice. Depression of average body weight and inefficiency of feed utilization were reported in chicks fed a diet containing 10% of *A. maritima* (Bakhiet and Adam, 1996). The toxicity of the plant was also evaluated against *Anopheles stephensi* and *Aedes aegypti* by Geerts et al. (1994). Negligible mortality was observed in both species after application of the dried leaves in the water at concentrations up to 2000 mg/L.

The aim of this study was to investigate the toxic effects of dried powdered leaves of the Sudan local plant, *Ambrosia maritima* in both female and male Nubian goats.

## Materials and methods

### Plant materials

The plant *Ambrosia maritima* was collected from the Nile bank at Atbra, River Nile State/Sudan and authenticated in Medical and Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan. The plant was dried in shade and the dried leaves were collected and grinded into fine powder using blender machine then the leaves powder was kept in dry dark place within Veterinary Research Institute (VRI) till it used.

### Animals and experimental designs

Two experiments were conducted, males and females. Twenty-four 6-9 month old (12 female and 12 male) goats were purchased from Omdurman market, and were housed in clean disinfected pens within the (VRI.). The animals were constantly fed sufficient amount of sorghum (Dura) and concentrate ration and provided drinking water ad lib., and they were allowed 15 days preliminary period during which they were given prophylactic doses of albendazole, coccistop and cypermethrin spray for the control of internal and external parasites, respectively.

At the end of the preliminary period, each female and male goats were divided randomly into 3 groups each of 4 animals. F1, F2 and F3 were the female groups, M1, M2 and M3 male groups.

The leaves powder was mixed with water and was given orally by drench.

Groups (F2 and M2) and (F3 and M3) were given *A. maritima* by drench at a dose rate of 1000 and 2000 mg/kg/day, respectively for 18 weeks. Groups F1 and M1 were kept as untreated control groups, they given water only.

Animals on each group were weighed and bled from the jugular vein before the experiment was started and then in 2-week intervals for 18 weeks. The terminal blood samples were taken immediately before slaughter of the animals. The blood was collected either on EDTA for hematological studies or on centrifuge tubes to separate the serum by centrifugation of blood at 5000 rpm for 5 min. Sera were stored at -20 °C for biochemical analysis.

### Laboratory analysis

Hemoglobin (HB) concentration, Packed Cell Volume (PCV), Red and White Blood Cells (RBC and WBC) were determined according to Schalm (1975). Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated from RBC, PCV and HB values.

**Table 1: Detail of goats treated with *A. maritima* at doses of 1000 and 2000/mg/kg/day for 126 days**

Group	No.	Age (month)	Daily dose(mg/kg/day)	First body weight (kg)	Last body weight (kg)	Results (day)
F1 (♀control)	1	6	Nil	11	17.75	Slaughtered (126)
	2	6	Nil	9	13.5	Slaughtered (126)
	3	6	Nil	11	19.5	Slaughtered (126)
	4	6	Nil	11	15.5	Slaughtered (126)
F2	5	6	1000	11.2	-	Died (8)
	6	6	1000	11	-	Died (9)
	7	7	1000	11.2	21	Slaughtered (126)
	8	7	1000	13	20.5	Slaughtered (126)
F3	9	7	2000	10	-	Died (13)
	10	6	2000	10	12.5	Slaughtered (126)
	11	6	2000	12	18	Slaughtered (126)
	12	7	2000	11	16	Slaughtered (126)
M1 (♂control)	13	6	Nil	9	14.5	Slaughtered (126)
	14	6	Nil	13	17	Slaughtered (126)
	15	6	Nil	12	16	Slaughtered (126)
	16	6	Nil	11	14.5	Slaughtered (133)
M2	17	6	1000	9	12	Slaughtered (133)
	18	8	1000	10	15	Slaughtered (133)
	19	6	1000	12.1	18.5	Slaughtered (133)
	20	6	1000	14	19.5	Slaughtered (133)
M3	21	6	2000	9.5	13.0	Slaughtered (133)
	22	6	2000	12.1	19.5	Slaughtered (133)
	23	6	2000	13.8	20.0	Slaughtered (133)
	24	7	2000	10	17.5	Slaughtered (133)

F= female, M= male

Sera were analyzed for the activities of Aspartate transaminase (AST) and Gammaglutamyl transferase (GGT) using commercial kits (Spinreact, Santa Coloma, Spain). Alkaline phosphate (ALP), and cholesterol concentration using commercial kits Chemie (1972) and Richmond (1973), respectively. Calcium and phosphorus were determined according to Trinder (1960) and Varley (1967), respectively. Bilirubin, and Urea concentrations were determined using commercial kits (Linear Chemicals, Barcelona, Spain). Serum sodium and potassium was measured by flame photometry as described by Varley (1967).

### Pathological examinations

Necropsies were made on all goats immediately after humane slaughter or death to see gross lesions and specimens of intestines, liver, kidneys, spleen, heart, pancreas, brain, lung and spinal cord were fixed in 10% neutral buffered formalin and processed for histopathological examination.

### Results

Details of goats dosed with *A. maritima* are given in table 1. In all treated goats symptom appeared 48 hours post the first dose. The first symptom was diarrhea which was more pronounced in 3 females number 5, 6 and 9. These animals also showed inappetence, weakness of the hind limbs and respiratory distress and were died on day 8, 9 and 13 respectively. Male goats in groups M2 and M3 and the rest of treated females showed watery diarrhea during the first week then they recovered and appeared healthy. No signs of toxicity recognized in control groups during the study period.

There were gradual increases on mean body weight of both female and male goats, drenched *A. maritima*, and the rate of growth is faster in females given 1000mg/ kg than those given 2000mg/ kg body weight

At necropsy, the most prominent features observed in both treated female and male goats, were hydrothorax, hydropericardium, and hydroperitonium. The liver was enlarged and showed pale yellow color and congestion (fig.1). Kidneys, lung, brain and heart were also congested in some treated animals.

Histopathological changes in all treated groups were almost similar in nature but varied in extend and severity between groups and even within the same group. The most prominent features of most vital

organs including the heart and brain were congestion and haemorrhage. Degeneration and necrosis and vacuolated cytoplasm of the liver hepatocytes were recognized in goats treated with *A. maritima* at dose 2000mg/kg/day (Fig.2). Renal damage was severe showed by focal necrosis of cortical convoluted tubules and extended to medullary tubules. Severe congestion and haemorrhage were also seen in proximal convoluted tubules and medullary tubules (Fig.3). Erosive enteritis and necrosis of intestinal villi with sever infiltration by inflammatory cells were detected in a goats dosed with 2000mg/kg /day of the plant (Fig. 4) and congestion of alveolar capillaries and pancreatic hyperplasia were also detected (Fig 5)

Hematological and serological changes were illustrated in tables 2 and 3, respectively. The values of PCV and RBC were significantly decreased ( $p < 0.01$ ) and WBC were significantly increased ( $p < 0.05$ ) in female goats drenched 2000mg/ kg /day of the plant. There were no significant changes on the values of the hematological parameters between the control and tested males and female treated with 1000mg/ Kg/day

The values of ALP and cholesterol were significantly increased ( $p < 0.01$ ). Sodium concentration was increased, while potassium was significantly decreased ( $p < 0.05$ ) in female goats drenched *A. maritima* at 2000mg/kg/day. No changes in serum constituents of either treated males or females dosed with 1000mg/ kg/day

### Discussion

The Nubian goat was selected here as model experimental animal due to its mounting role in the economy of the simple traditional Sudanese farmer. No research has been done to investigate the effect of the long-term drenching of the leaves powder of *A. maritima* on female and male Nubian goats. In the present study, the toxicological effects of the plant *A. maritima* on female and male goats was studied

*Ambrosia maritima* was found to be fatal to 3 out of 8 treated female goats. These animals showed severe watery diarrhoea, inappetence, respiratory distress, recumbency and death. The rest of the female and male groups also showed watery diarrhoea, inappetence and weakness and the symptoms disappeared within one week and the animals appeared healthy. This might have been brought about by development of intestinal erosions and catarrhal enteritis or inhi-

**Table 2: Hematological results of Ambrosia maritima-treated goats.**

Group	Daily dose (mg/kg/ day)	Haematological Results (mean $\pm$ SE)					
		Hb (g/dl)	PCV (%)	RBC ( $\times 10^6$ mm)	MCV (m <sup>3</sup> )	MCHC (%)	WBC ( $\times 10^3$ mm <sup>3</sup> )
F1♀ (control)	0	9.2 $\pm$ 0.3	29.5 $\pm$ 0.4	13.3 $\pm$ 0.6	22.18 $\pm$ 1.0	31.19 $\pm$ 0.7	5.64 $\pm$ 0.4
F2	1000	10.7 $\pm$ 0.6 <sup>Ns</sup>	30.3 $\pm$ 3.2 <sup>Ns</sup>	12.4 $\pm$ 2.8 <sup>Ns</sup>	24.44 $\pm$ 1.3	35.31 $\pm$ 1.6 <sup>Ns</sup>	7.05 $\pm$ 1.5 <sup>Ns</sup>
F3	2000	9.4 $\pm$ 1.5 <sup>Ns</sup>	20 $\pm$ 4.5 <sup>**</sup>	8.0 $\pm$ 1.8 <sup>**</sup>	21.67 $\pm$ 2.1 <sup>Ns</sup>	34.62 $\pm$ 3.4 <sup>Ns</sup>	8.85 $\pm$ 0.6 <sup>*</sup>
M4 (♂control)	0	8.48 $\pm$ 0.4	24.4 $\pm$ 2.5	13.3 $\pm$ 0.4	18.4 $\pm$ 3.1	34.7 $\pm$ 0.9	8.71 $\pm$ 0.4
M5	1000	8.1 $\pm$ 0.1 <sup>Ns</sup>	22.9 $\pm$ 1.3 <sup>Ns</sup>	13.2 $\pm$ 2.1 <sup>Ns</sup>	17.3 $\pm$ 1.3 <sup>Ns</sup>	34.2 $\pm$ 2.2 <sup>Ns</sup>	7.42 $\pm$ 1.3
M6	2	8.1 $\pm$ 0.9 <sup>Ns</sup>	22.9 $\pm$ 5.2 <sup>Ns</sup>	13.2 $\pm$ 2.2 <sup>Ns</sup>	17.3 $\pm$ 4.1 <sup>Ns</sup>	34.2 $\pm$ 3.1 <sup>Ns</sup>	7.42 $\pm$ 3.1

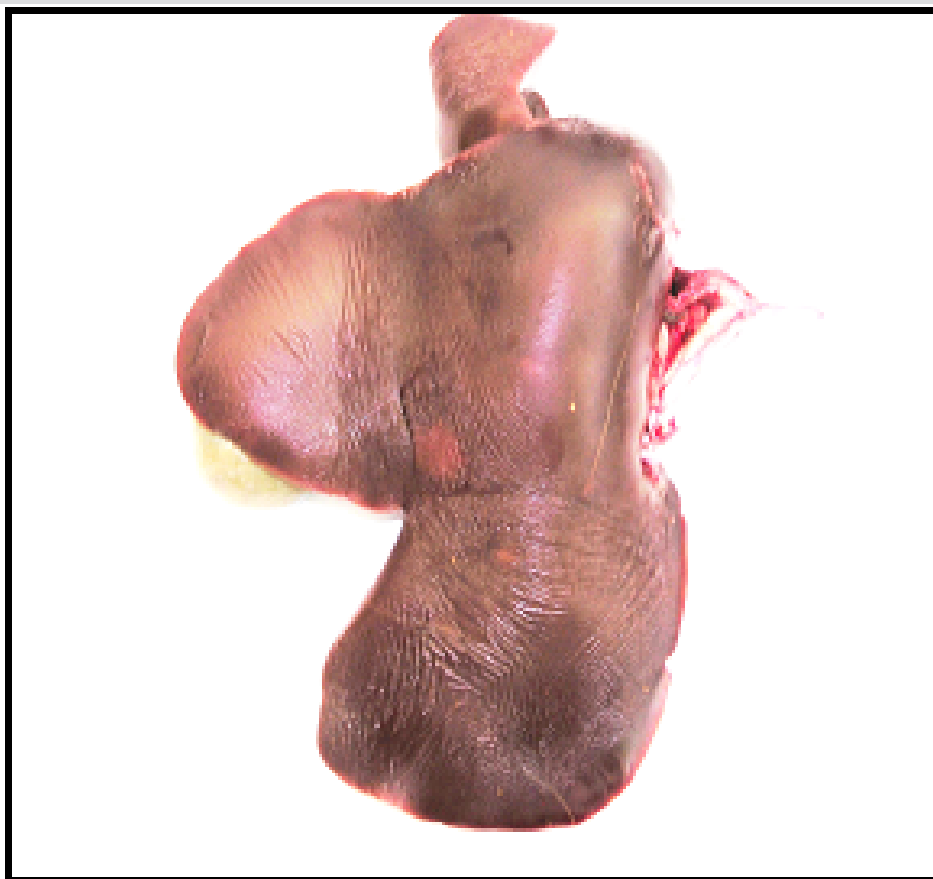
Ns = Not significant, \* = p< 0.05; \*\* = p< 0.01

**Table 3: Result of serum constituent analysis in goats drenched Ambrosia maritima at 1000 and 2000mg/kg/day for 18 weeks**

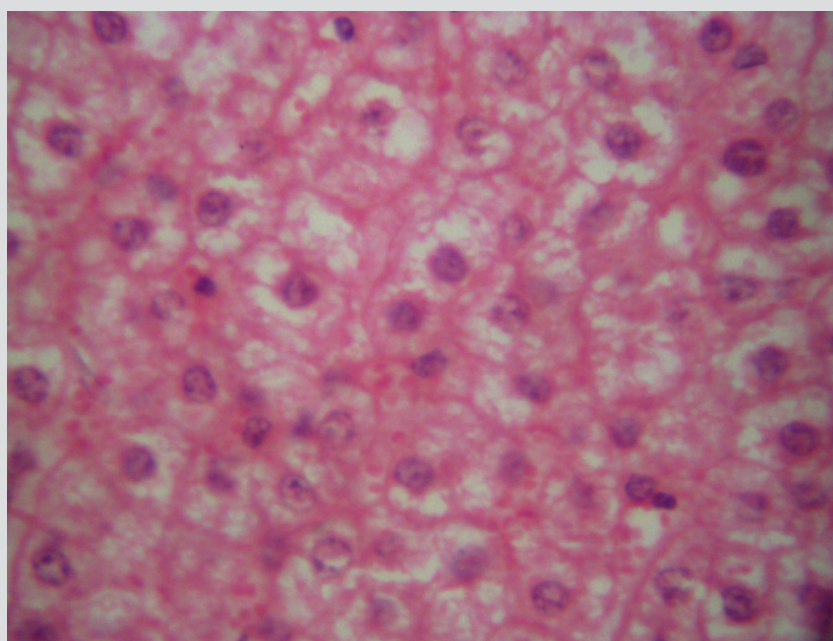
Group	AST (i.u)	GGT (i.u%)	ALP (i.u)	Cholestrol (mg/dl)	Bilirubin (μmol/L)	Urea (mg/dl)	Ca (mg%)	Na (mg%)	K (mg%)
F1	26.9 $\pm$ 0.2	9.9 $\pm$ 0.8	45.4 $\pm$ 0.3	139.4 $\pm$ 0.9	0.24 $\pm$ 1.1	27.5 $\pm$ 1.1	8.6 $\pm$ 0.9	143.5 $\pm$ 1.2	4.4 $\pm$ 0.3
F2	26.9 $\pm$ 2.9	9.9 $\pm$ 0.9	65.1 $\pm$ 0.9	138.4 $\pm$ 0.9	0.2 $\pm$ 2.5	28.1 $\pm$ 1.9	8.6 $\pm$ 0.9	144.1 $\pm$ 0.8	4.4 $\pm$ 0.9
F3	58.2 $\pm$ 2.1 <sup>*</sup>	10.8 $\pm$ 0.6	85.3 $\pm$ 0.8 <sup>**</sup>	177.7 $\pm$ 3.1 <sup>**</sup>	0.2 $\pm$ 2.5	28.0 $\pm$ 1.4	8.6 $\pm$ 0.5	175.8 $\pm$ 0.6 <sup>*</sup>	2.0 $\pm$ 1.0 <sup>*</sup>
M1	26.3 $\pm$ 1.5	9.9 $\pm$ 4.2	83.5 $\pm$ 0.9	139.8 $\pm$ 0.9	0.2 $\pm$ 0.8	28.3 $\pm$ 0.9	8.6 $\pm$ 0.6	145.2 $\pm$ 1.3	4.5 $\pm$ 1.5
M2	26.5 $\pm$ 0.8	9.7 $\pm$ 5.2	84.2 $\pm$ 0.8	137.9 $\pm$ 0.6	0.2 $\pm$ 3.0	28.6 $\pm$ 0.6	8.6 $\pm$ 0.8	142.5 $\pm$ 1.3	4.6 $\pm$ 0.7
M3	26.1 $\pm$ 0.5	9.8 $\pm$ 3.1	85 $\pm$ 1.6	140.3 $\pm$ 1.4	0.2 $\pm$ 0.8	27.1 $\pm$ 1.3	8.6 $\pm$ 1.0	142 $\pm$ 0.9	4.4 $\pm$ 0.3

\* = p< 0.05; \*\* = p< 0.01





**Fig. 1: Liver enlargement, pale yellow color or congestion of female goat treated with 1000mg/kg/day *A. maritima* for 126 days**



**Fig.2: Degeneration, necrosis and vacuolated cytoplasm of the liver hepatocytes of a goat treated with 2000mg/kg/day *A. maritima* For 126 days (X 40 H & E)**

bition of the enzyme cholinesterase or both correlations. Similar result have been reported by Afaf et al. (2007) who reported that a single intramuscular dose of 2000 mg/kg body weight of the methanolic extract *A. maritima* was found to be fatal to rats but a daily doses of 500 mg/kg (B.wt.) caused inappetance, decreased activity, lameness and even paralysis, while at the dose 250mg /kg/(Bwt) no clinical signs were observed. *A. maritima* plant was found to enhance the growth of both female and male goats and the rate of growth was faster in female drenched 1000mg/kg than ones given 2000mg/kg/day. This might have been due to the nutritive value of the plant and this agreed with the results of Bakhiet and Adam (1998) who reported improvement in body weights in chickens fed a diet mixed with 2% of *A. maritima*, but when *A. maritima* was increased to 10% the body weight was decreased. Contrary to Afaf et al. (2007) who reported that there was no effect on body weights of rats received water extract of *A. maritima* at a doses of 1000 and 500 mg/kg (B. wt.). However diarrhoea was the most prominent sign were observed. All treated goats showed hydrothorax, hydro pericardium and hydroperitonium. This may be due to increased permeability of endothelial cells and may be attributed to the changes observed in the kidneys and heart. Enlargement of the liver was detected in a goat drenched the plant at 2000mg/kg this confirmed the finding of Afaf et al. (2007) who found that 500mg/kg of water extract of *A. maritima* increased the liver weight of rats.

The liver abnormalities were congestion, degeneration and necrosis and fatty cytoplasmic vacuolation of the hepatocytes. These changes may explain the elevated level of AST and ALP in female goats treated with 2000mg/kg/day of *A. maritima* this suggested hepatic dysfunction (Cornelius and Kaneko, 1963). Liver damage seen as fatty change was also manifested by the raised cholesterol level in female goats drenched with 2000mg/kg of the plant. These results were similar to that found on goats and rats treated with other plant, *Guiera senegalensis* (Ilham, 2011 and Azza et al. 2008, respectively)

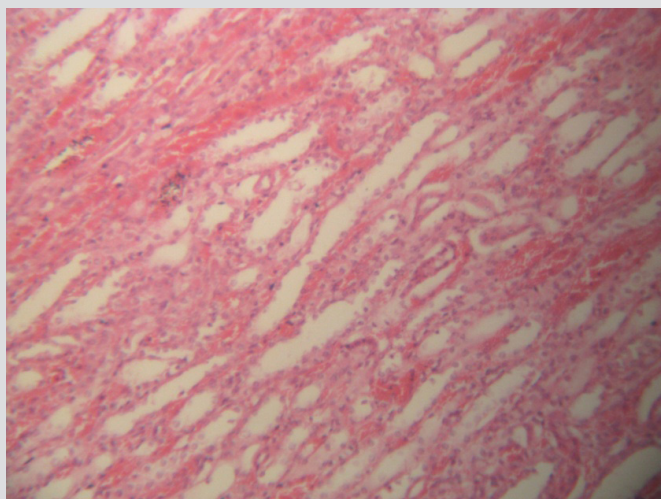
Renal injury was demonstrated by degeneration of tubular epithelium, congestion and haemorrhage of proximal convoluted tubules. The decreased values of RBC in treated female goats may be attributed to renal damage which might have affected erythropoietin production. Anemia is defined as deficiency of erythrocytes or haemoglobin per unit volume of blood. The clinical signs of anaemia do not appear

until the haemoglobin level of the blood falls below 50% of the normal level and RBCs count and PCV are usually depressed (Radostits *et al.*, 1995). When there are decreases in RBC, Hb and PCV and an increase in MCV, the anaemia is of a macrocytic type. In the present study, goats drenched 2000mg/kg/day leave powder of *A. maritima* showed decreases in Hb, PCV and RBC and an increase in MCV. This type of anaemia has been observed in Najdi sheep fed with *Rhazya stricta* (Adam, 1998) and in Bovan chicks fed with *Casia italica* (Bakhiet and Adam, 1996). Leukocytosis was detected in treated female goats

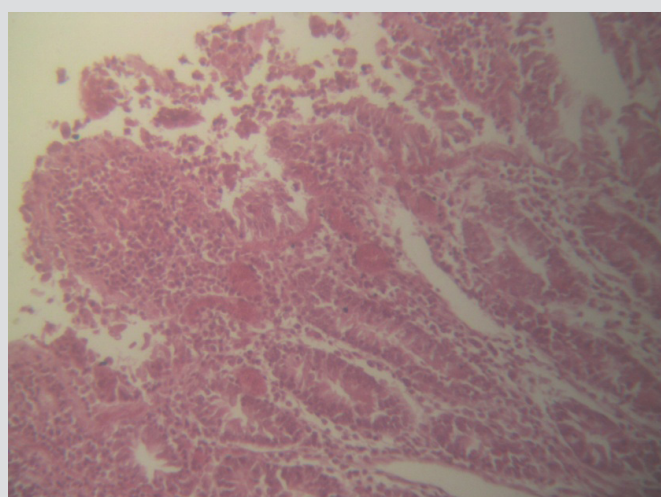
Renal damage is also reflected by the raised level sodium and decreased potassium in female goats. Respiratory distress is also reflected by congestion of alveolar capillaries, alveolar emphysema and thickening of alveolar septa of the lung. Elevation of sodium and decrease in potassium may be due to the loss of electrolytes through the watery diarrhea.

Generalized congestion and haemorrhages were recognized in many organs including heart and brain of treated animals. These may be due to influence of the plant constituents on the endothelium leading to injury and hence affecting blood vessels permeability. This points to endotheliotoxicity. Pancreatic haemorrhage and hyperplasia were observed. This may enhance insulin secretion and may explain the folk use of the plant as a hypoglycemic agent. Ahmed (1999) pointed out that 2% *A. maritima* leaves extract produced a notable reduction in blood glucose level in mice and rabbits. Ammar *et al.* (1993) reported that different extracts of the herb *A. maritima* significantly reduced post-prandial and fasting blood glucose after 1.5 hours after glucose ingestion and in others after 2 hours. Also they found neither alcohol nor petroleum ether extract has a significant effect on serum insulin level after 1.5 or 2 hours from glucose ingestion. They suggested that the hypoglycemic effects may be exerted through the inhibition of glucose absorption, increase sensitivity of receptors to insulin, insulinase inhibiting effect, stimulating cells of pancreas to secrete insulin or stimulation of peripheral tissues uptake of the glucose.

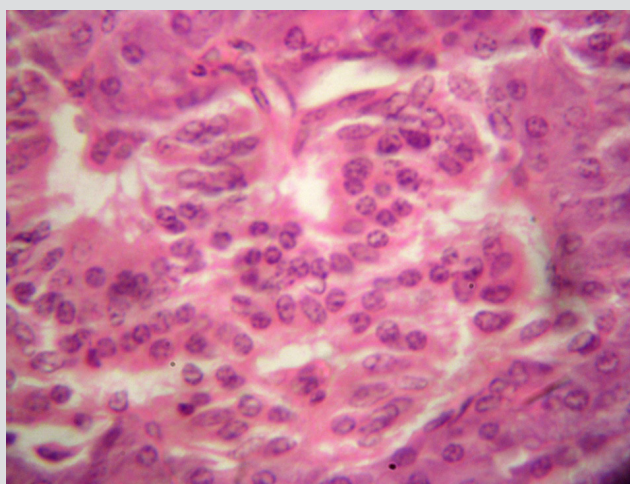
Contrary to Hassieb *et al.* (1993) who stated that *A. maritima* was not toxic even at the concentration of 1:500 in drinking water and Vassiliades and Diaw (1980) who stated that up to 1000 ppm had no toxic effects in mice and Alard *et al.* (1991) who concluded that *A. maritima* has a very low toxicity to aquatic non-target organisms and it is not toxic when used



**Fig.3: Kidney Congestion and hemorrhage in proximal convoluted tubules and medullary tubules in a goat drenched *A. maritima* at a dose of 2000mg/kg/day for 126 days (X 40 H & E)**



**Fig.4: Intestine: Sloughing of epithelial cells and necrosis of intestine villi with lymphocytic infiltration of a goat drenched *A. maritima* for 126 days (X 40 H E).**



**Fig.5: Pancreas Hyperplasia of Islet of Langerhans in male goat treated with *A. maritima* at a dose of 1000 mg/kg/day for 126 days (H & E x 400)**



at the molluscicidal concentration of 35 to 70 mg/litre. The present study stated that *A. maritima* was toxic to both female and male goats at 2000mg/kg/day, but the female goats are more sensitive to the plant toxicity than the males, and the plant was found to have renal and hepatic chronic toxicity.

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