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TOXICOLOGICAL EFFECTS OF THE ORAL ADMINISTRATION OF THE AQUEOUS EXTRACT FROM CALLIGONUM COMOSUM TO RATS

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ABSTRACT

Adult male Wistar rats were given daily oral doses of the lyophilized aqueous extract of Calligonum comosum at the rate of 400 mg/kg (b.wt.) for 4 weeks. The biochemical changes included a significant increase in the levels of serum Calcium and SUN ($P < 0.05$). On the other hand, decreased values were noted in the levels of serum albumin ($P < 0.01$). Haematological changes showed increased lymphocyte count ($P < 0.05$), whereas, decreases were noted in the levels of Hb ($P < 0.01$) and total WBCs count ($P < 0.05$), in addition to panleucocytopenia ($P < 0.05$) involving all the granulocytes and the monocytes ($P < 0.001$).

INTRODUCTION

The genus Calligonum (Fam. Polygonaceae) is represented in the flora of Saudi Arabia by the only one species C. calligonum L'Her. It is a glabrous richly branched almost leafless shrub resembling Ephedra. It grows wildly in many areas and is locally known as Arta, Artah or A'bal [1]. The plant has been used in folk medicine, where a decoction of the roots is used for the treatment of toothache, skin diseases and burns [2]. It is also reputed as stimulant and astringent [3].

The plant is extensively used for tanning hides by the people of the Kingdom [4]. Many biological studies dealt with the plant, where it showed moluscicidal activity Against Biomphalaria alexandrina snails and cercaricidal activity against Shistosoma mansoni cercariae [5], and antimicrobial activity which was mainly due to its anthraquinone content[6], and antifungal activity against four Candida species [7].

Concerning chemical study, El-Sayyed and Wagner reported the isolation and identification of kaempterol, quercetin, quercetin-3-0-glucoside (isoquercitrin) and kaempterol 3-0-glucouronide, procyanidine and the carotenoides violaxanthin and neoxanthin [8].

Owing to the fact that the plant is consumed either by man as a medicinal agent, and by animals in range lands as food, this study was undertaken to evaluate the clinical and pathological effects which may accompany the chronic administration of the lyophilized aqueous extract of the plant in rats.

EXPERIMENTAL

Plant Materials :

The plant materials were collected in May 1990 and 1992 from the Qassim area. The plant identification was verified by Dr. H.M. Hassan, College of Science, King Saud University. A vaucher specimen is deposited in the Dept. of Vet. Med., King Saud Univ., Saudi Arabia.

Apparatus :

Serum chemical variables were determined on a Varian DMS-100 UV/visible Spectrophotometer. RBCs and WBCs Counts were determined on a double improved Neubauer chambers. PCV was determined using a microhaematocrit centrifuge. Serum protein fractionation was made using 5 µl samples on agarose gel slabs (5401-001 hydragel protein, LKB-Sebia) Electrophoresis was run for 20 min. using the LKB equipment and the recommended procedures (Sebia 92130) Issy Les Moulineaux, France). Following fixation, staining with amidoblack and destaining in 5% HOAC, the electrophoretograms were scanned to get the % of albumin, alpha, beta and gammaglobulins using a LKB 5300 Preference

Densitometer programmed for protein analysis. A labconco freeze dryer-18 (Model 75018) was used for the preparation of the lyophilized aqueous extract of the plant.

Preparation of the lyophilized aqueous extract of C. comosum :

Air dried leaves were separated from stems, powdered (200 g), then extracted for 5 times with hot distilled water (1 L each). The combined aqueous extract was filtered then lyophilized to give 38 g of yellowish brown powder.

Administration of the extract of C. comosum to rats :

A fresh (10% w/v) solution was prepared in distilled water. Male Wistar rats weighing between 200 and 240 g, divided into two groups, each of 10 were used for the experiment. They were fed on a standard pelleted diet and had free access to water. One group was given daily oral doses of 400 mg/kg.b.wt. of C. comosum lyophilized aqueous extract for a period of 4 weeks.

Determination of haemogram parameters :

At the end of the experiment, all the rats were sacrificed and whole blood and serum samples were collected, whole blood samples were collected in lithium heparinized plastic bottles, while sera samples were separated from another portion of blod collected in a clean and dry McCarteny bottles. Sera were stored at -20°C until analysed. Blood smears were prepared from freshly drawn blood, air dried, fixed in alcohol and stained with leishman's stain. Haemogram and leucogram parameters were determined on the same day of blood collection.

Determination of serum chemical variables :

A selected group of serum chemical variables were determined using commercial kits supplied by BioMerieux (France). Serum total protein [9],

serum urea nitrogen (SUN) [10], triglycerides[11], total lipids [12], bilirubin [13], inorganic phosphorus [14], calcium [15], magnesium [16], ∝-amylase and acid phosphatase [17]. Serum protein fractionation was made using 5 µl samples of the control and treated rats by electrophoresis.

Statistical Analysis :

Data obtained were statistically analysed using Student (t) test [18].

RESULTS

The results of the biochemical and haematological changes are shown in tables (1 and 2) respectively. The administration f the aqueous extract of Calligonum comosum at the rate of 400 mg/kg.b.wt. daily for 4 weeks has shown interesting biochemical and haematological changes. Regarding the biochemical changes, there was significant increase in SUN and calcium levels ($P < 0.05$) and a marked hypoalbuminaemia and depression of µ-amylase activity. A nonsignificant increase in bilirubin, triglycerides, acid phosphatase activity, alpha and beta globulins in addition to a slight decrease in serum total proteins, total lipids, inorganic phosphorus, magnesium, gammaglobulins and A/G ratio was also observed. Regarding the haemogram parameters, there was an increased lymphocyte count ($P<0.05$). Whereas, decreases were observed in the level of Hb ($P<0.01$) and total WBCs count ($P<0.05$). In addition to panleucocytopenia involving all the granulocytes and monocytes ($P<0.001$).

There was also a nonsignificant increase in MCV values and platelets count and its mean value in addition to a slight decrease in the values of RBCs count, PCV, McH and MCHC.

DISCUSSION

The administration of the aqueous extract of Calligonum comosum resulted in hypercalcaemia and slight hypophosphataemia. This is explained on the basis of excessive absorption of calcium from the intestine, possibly stimulated by some factors in the plant notably vitamin D_3 activity. A number of plants were reported to have caused a varying degrees of calcinosis and possibly soft tissue mineralization. Such plants were examplified by Solanum malacoxylon which was found to contain a metabolite of Vit. D. [19-20]. Hypercalcaemia as such could also follow hyperalbuminaemia [21], however, in this experiment there is a decreased level of albumin, which when considered together with hypophosphataemia would suggest renal involvement rather than bone demineralization.

The significant leucocytopenia which included all the granulocytes and the monocytes appear to be due to a significant influence in all leucocyte series in the bone marrow and when erythrocytopenia recorded in this investigation is considered the effect appears to involve the pluripotent cells. Such an influence may be suggestive of steroidal activity of the plant extract. There was significant lymphocytosis presumably due to specific effects on the lymphopoetic organs, however, this finding was associated with decreased levels of gammaglobulins, a finding which suggests a more serious immune defficiency state as the numbers of circulating lymphocytes were unable to produce normal levels of immunoglobulins.

The hypoproteinaemia which could be mainly due to hypoalbuminaemia and partially due to hypogammaglobulinaemia indicates a hepatic dysfunction, due to lew level of synthesis of proteins and albumins [21]. However, some of the albumin could be excreted through a damaged

kidneys which could be imagined from the heightened SUN level in the presence of hypoactive liver. Alternatively, there may be a state of protein malabsorption from the small intestine brought about by the protein precipitant tannins reported to be present in this plant (3).

It is also known that tannins can bring about hepatocellular and renal toxicities which can explain the dysfunctions seen in these organs. the increase in alpha and betaglobulins could also be taken as indicators of acute phase proteins which should be elevated in cases of hepatic and renal necrosis (21).The most important of these globulins are alpha-1 antityrosin, $\propto_1$ acid glycoproteins and $\propto_2$-macroglobin, ceruloplasmin, haptoglobulin and fibrinogen. Although these proteins could have been increased, it is not feasible to verify the fact. Serum triglycerides were also seen to be elevated with a posible explanation on the basis of renal failure, depletion of hepatic glycogen or blood glucose, or necrotizing pancreatitis. The latter condition appear to have been evident by the fact that serum $\propto$-amylase significantly decreased.

Table (1) : Serum chemical variables and protein fractions in serum of rats given daily oral doses of aqueous extract of C. comosum in a dose of 400 mg/kg.b.wt. for 4 weeks.

(Mean ± SE) n = 10					
Variable	**Control**	**Treated**	**Variable**	**Control**	**Treated**
Total proteins gm/L	79.13±6.80	73.26±6.35	Calcium mmol/L	2.12±0.05	2.36±0.08 *
SUN mmol/L	2.58±0.14	3.02±0.15 *	Magnesium mmol/L	1.14±0.05	1.00±0.32
Bilirubin mmol/L	0.90±0.001	1.05±0.18	Acid phos-phatase U/L	6.03±0.01	6.78±0.39
Total lipids gm/L	9.79±0.20	9.34±0.11	Albumin gm/L	44.23±1.91	33.66±1.37 ***
Triglycerides mmol/L	4.44±0.13	5.34±0.92	$\propto$- + ß - globulins gm/L	22.30±4.70	29.03±4.40
Inorganic phosphorous mmol/L	3.31±0.02	2.54±0.28	Gamma-globulins gm/L	14.08±2.19	7.36±2.88
$\propto$-Amylase µ/L	8.65±0.83	4.09±0.55 **	A/G ratio	1.14±0.85	1.00±0.25

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Table (2): Haemogram and weights of selected organs shown as percentages of total body weight of rats treated with the lyophilized aqueous extract from C. verbascifolia in daily oral dose of 400 mg/kg.b.wt. for 4

(Mean ± SE) n = 10					
Variable	**Control**	**Treated**	**Variable**	**Control**	**Treated**
RBC x 10^{12}/L	3.93±0.84	3.86±0.22	Liver	3.2±0.19	4.75±1.95
WBC x 10^{9}/L	8.13±0.27	3.79±0.5***	Kidneys	0.7±0.03	0.76±0.04
Hb gm/dL	9.08±0.215	9.2±11.2	Heart	0.24±0.03	0.29±0.08
PCV L/L	0.26±0.05	0.26±0.04	Spleen	0.32±0.02	0.22±0.06
MCV fl	78.39±16.92	35.66±3.86	Pancreas	0.36±0.24	0.45±0.03
MCH Pg	25.78±10.89	74.83±9.55**	Adrenals	0.024±0.001	0.03±0.003
MCHC gm/L	34.92±6.84	26.0±4.37	Brain	0.69±0.07	0.53±0.1
Eosinophils No/µL	189.4±42.28	83.38±16.58***	Total body	244.4±49.8	241.0±34.1
Neutrophils No/µL	2872.3±352.84	1197.6±181.5***	weight		
Basophils No/µL	121.95±28.3	37.9±6.91*			
Lymphocytes No/µL	4458.5±278.04	2372.5±238.5***			
Monocytes No/µL	487.8±35.77	98.54±16.4***			

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

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التأثيرات السمية الناتجة عن تعاطى الخلاصة المائية المجففة لنبات الكاليجونم كوموزم (القرطة) عن طريق الفم للفئران

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فرع القصيم - بريدة ص . ب ١٤٨٢ المملكة العربية السعودية

تم فى هذا البحث إعطاء الخلاصة المائية المجففة لنبات القرطة لذكور الفئران عن طريق الفم يوميا ولمدة ٤ اسابيع فى جرعة قدرها ٤٠٠ مجم/كجم من ورن الجسم .

ولقد شملت التغيرات الكيميائية الحيوية وجود زيادة ملحوظة فى مستوى الكالسيوم واليوريا فى مصل الدم . على الجانب الآخر كان هناك نقصا ملحوظا فى مستوى الزلال . وأظهرت صورة الدم وجود زيادة واضحة فى عدد كرات الدم الليمفاوية بيما كان هناك انخفاضا واضحا فى هيموجلوبين الدم والعدد الكلى لكرات الدم البيضاء على اختلاف انواعها .