

SUBCHRONIC EFFECTS OF THE ORAL ADMINISTRATION OF THE LYOPHILIZED AQUEOUS EXTRACT OF CHROZOPHORA VERBASCIFOLIA IN RATS

Fathalla M. Harraz, Mohamed O. Farah and Sayed A. Abdel-Aziz

DEPT. OF VET. MED., COLLEGE OF AGRIC. AND VET. MED., KING SAUD UNIV., QASSIM
BRANCH, BUREIDAH, P.O.BOX 1482, SAUDI ARABIA

ABSTRACT :

Daily oral doses (400 mg/kg.b.wt.) of the lyophilized aqueous extract of C. verbascifolia were given to adult Wistar rats for a period of 4 weeks. The results revealed a significant decrease in cholesterol level ($P < 0.05$) and inorganic phosphorus ($P < 0.001$), in addition to a marked increase ($P < 0.001$) in serum albumin and calcium. The haemogram showed a marked leucocytopenia, lymphocytopenia, decreased neutrophils, monocytes ($P < 0.001$) and basophils counts ($P < 0.05$). Moreover, a significant increase ($P < 0.01$) in MCH was also recorded. Changes in body and organ weights as well as histopathological findings were also discussed.

INTRODUCTION

Chrozophora verbascifolia (Willd) A-Juss. grows wild in many different regions of the Kingdom of Saudi Arabia and is locally known as Tannoum (1). Plants of the genus Chrozophora are monocious herbs or shrubs, often covered with stellate indumentum (1,2). Other species of Chrozophora viz. C. plicata is reported to be used as a laxative, a blood purifier and in cases of leprosy (3,4). To the best of our knowledge, this plant has not subjected to any chemical or biological studies except our recent phytochemical study (5). The fact that the plant is naturally available for range animals on the one hand and the use of other species of Chrozophora in folk medicine has stimulated us to investigate the effects of the lyophilized aqueous extract of the plant on the blood picture

and on various serum biochemical constituents in experimental rats.

MATERIALS AND METHODS

Plant Material :

C. verbascifolia was collected during October 1989 and May 1991, in the flowering and fruiting stages from the Qassim province, Saudi Arabia. The plant identification was verified by Dr. H.M. Hassan, College of Science, Riyadh, Saudi Arabia. A vaucher specimen is deposited in the Department of Vet. Med., King Saud University, Qassim, Saudi Arabia.

Apparatus :

A Labconco freeze dryer-18 (Model 75018) was used for the preparation of the lyophilized aqueous extract of the plant.

Serum chemical variables were determined on a Varian DMS-100 U/V 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 slabo (5401-001 hydragel protein, LKB-Sebia). Electrophoresis was run for 20 min. using the LKB equipment and recommended procedures (Sebia 92130) Issy Les Moulineaux, France). Following fixation, staining with amidoblack and destaining in 5% HOAC, electrophoretograms were scanned to get the percent of albumin, alpha-, beta- and gammaglobulins using LKB 5300 Preference Densitometer programmed for protein analysis.

Preparation of the lyophilized aqueous extract of C. verbascifolia :

The air-dried powdered parts of the plant (200 gm) were extracted

with hot distilled water for 5 times. The combined purple aqueous extract was filtered through a sintered glass funnel, then lyophilized in a Labconco freeze dryer-18 model 75018) to give a shining purple residue (38 gm).

Experiment :

A fresh (10% w/v) solution of the extract was prepared in distilled water. Male Wistar rats weighing between 200-250g, divided into two equal groups, each of ten were used. They were kept 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 the plant extract for 4 weeks while the other group was kept as control.

Determination of the haemogram parameters :

At the end of the experiment, all the rats were sacrificed and whole blood and serum samples were collected. Serum samples 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 chemical kits supplied by (BioMerieux, France). Serum total protein (6), serum urea nitrogen (SUN) (7), triglycerides (8), total lipids (9), cholesterol (10), inorganic phosphorous (11), uric acid (12), total iron (13), calcium (14), and magnesium (15). Serum protein fractionation was made using 5 µl samples of the control and treated rats by electrophoresis.

Statistical analysis :

The obtained data were statistically analysed using Student (t) test⁽¹⁶⁾.

RESULTS

The results of biochemical analysis, haematological variables and changes in body weights are shown in tables (1 and 2). Treatment with the aqueous extract of *C. verbascifolia* for 4 weeks in a dose of 400 mg/kg.b.wt. resulted in some interesting findings.

Biochemically, there were decreases ($P < 0.05$) in the level of cholesterol and ($P < 0.001$) in inorganic phosphorus, in addition to a marked increase in serum albumin and calcium levels ($P < 0.001$). Whereas a nonsignificant increase in the levels of serum total proteins, globulins and A/G ratio, total lipids, triglycerides and magnesium. And a slight decrease in the levels of serum urea nitrogen, uric acid and total iron was also recorded.

Result of the haemogram revealed a significant decrease in leucocytes, neutrophils, lymphocytes, monocytes ($P < 0.001$) and basophils count ($P < 0.05$). A marked increase ($P < 0.01$) in MCH value, and a slight decrease in RBCs count, PCV, eosinophils count and MCHC was also noticed, in addition to a slight increase in Hb content.

Changes in body and organ weights included a slight increase in the weights of liver, kidney, heart, pancreas and adrenals as well as a slight decrease in spleen, brain and total body weight.

Post-mortem findings :

No obvious P.M. lesions were detected.

Histopathological findings :

The main changes were mild to moderate and include scattered centrilobular coagulative degeneration of hepatocytes with foci of cell necrosis. There are areas of mild to moderate interlobular haemorrhage in the pancreas and few areas of subcapsular haemorrhages in the adrenal gland. The ovaries showed cellular degeneration in one area and hyperplastic proliferation in the fibromuscular stroma. Other organs which were also examined were normal.

DISCUSSION

As a result of treatment with the extract of Chrozophora verbascifolia, the serum total proteins and albumin were slightly and significantly increased presumably because of the enhanced liver activity in synthesis and the fact that an additional amount of proteins was dietary, i.e. supplied by the plant extract. An interesting biochemical finding is the highly elevated serum calcium which was accompanied by a marked hypophosphataemia. Increased calcium level could have been brought about through the influence of some factors present in the plant extract, notably vitamin D₃ activity which either increases calcium absorption from the gastrointestinal tract, or causes increased mobilization of calcium from its depots in the bones.

There are a number of calcinogenic plants in the literature notable among which are Solanum malacoxylon and Cestrum diurnum. The activities of these plants were attributed to their contents of the glycoside, 1,25-dihydroxy-vitamin D₃ (Calciferol) (17-19). Hypercalcaemia can also

arise as a result of some effects on other organs suffering from conditions such as : hyperparathyroidism, pseudohyperparathyroidism or thyrotoxicosis, in which cases it is not possible to make comments on their involvement due to shortage of data. However, hypercalcaemia which was associated with hypophosphataemia suggests a renal involvement rather than mobilization of calcium from bones. This further suggests that the hypercalcaemia could result from an increased absorption of calcium from the alimentary tract. It is also known that hypercalcaemia follows hyperalbuminaemia (20). Enhanced renal excretion of phosphates as may be evidenced by hypophosphataemia could also explain the hypouricaemia on the same basis. Moreover, the plant extract caused a significant decrease in serum cholesterol pointing to a further evidence of the liver damage evidenced in this study by the coagulative necrosis leading to disturbance of phospholipids and fat metabolism, in addition to the possible involvement of the thyroid gland.

However, the slight increase in total lipids and triglycerides considered together with the slight hyperproteinaemia and marked hyperalbuminaemia also suggests a dietary origin in the plant extract. Hyperlipidaemia usually occurs due to lack of lipoprotein lipase (20). The actual cause may not be easy to trace because of its diverse origin viz. hypothyroidism, diabetes mellitus, pancreatitis and renal dysfunction.

The second interesting finding was the occurrence of panleucocytopenia, where the levels of WBCs, neutrophils, eosinophils, basophils, lymphocytes and monocytes counts were depressed. The reason for such changes could be due to adrenal hyperactivity or the presence of some steroids in the plant that affected directly or indirectly these events. However, it is also interesting to note that despite the significant

Table (1): Serum chemical variables and protein fractions in serum of rats given daily oral doses of *C. verbascifolia* aqueous extract 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	53.31±8.35	63.25±3.61	Total iron mmol/L	55.34±3.39	52.1±0.73
SUN mmol/L	8.82±0.137	8.36±0.49	Calcium mmol/L	2.08±0.15	3.11±0.09 ***
Cholesterol gm/L	1.73±0.07	1.49±0.05 *	Magnesium mmol/L	0.71±0.01	0.72±0.03
Total lipids gm/L	10.2±0.13	11.48±0.58	Albumin gm/L	26.5±1.53	38.5±3.58 **
Triglycerides mmol/L	2.87±0.07	3.93±0.72	α_2 + β - globulins gm/L	13.37±0.45	16.49±2.41
Inorganic phosphorus mmol/L	0.42±0.02	0.29±0.02 ***	Gamma-globulins gm/L	14.52±0.78	17.75±2.61
Uric acid mmol/L	357.9±0.67	354.61±5.91	A/G ratio	0.61±0.2	1.44±0.37

* 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 weeks

(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	0.031±0.003
MCHC gm/L	34.92±6.84	26.0±4.37	Brain	0.69±0.07	0.53±0.1
Eosinophils No/ μ L	189.4±42.28	83.38±16.58***	Total body weight	244.4±49.8	241.0±34.1
Neutrophils No/ μ L	2872.3±352.84	1197.6±181.5***			
Basophils No/ μ L	121.95±28.3	37.9±6.91*			
Lymphocytes No/ μ L	4458.5±278.04	2372.5±238.5***			
Monocytes No/ μ L	487.8±35.77	98.54±16.4***			

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

lymphocytopenia, gamma-globulins were slightly increased suggesting that the slight hypergammaglobulinaemia appear to have an alternative origin.

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التأثيرات تحت المزمعة الناشئة عن تعاطي الخلاصة المائية المجفدة لنبات الكروزوفورا فيرباسيفوليا (تانوم) في الفئران

فتح الله محمد حراز ، محمد عمر فرح ، السيد احمد عبدالعزيز
قسم الطب البيطري بكلية الزراعة والطب البيطري - جامعة الملك سعود
فرع القصيم - بريدة ص.ب ١٤٨٢ المملكة العربية السعودية

تم في هذا البحث اعطاء الخلاصة المائية المجفدة لنبات الكروزوفورا عن طريق الفم للفئران البالغة في جرعة قدرها ٤٠٠ مجم/كجم من وزن الجسم يوميا ولمدة اربعة اسابيع. أظهرت النتائج وجود انخفاض معنوي في نسبة الكولستيرول وعنصر الفوسفور الغير عضوي في مصل الدم. بالاضافة الى حدوث زيادة ملحوظة في الزلال والكالسيوم. بالنسبة للتأثير على صورة الدم تسببت الخلاصة المائية للنبات في احداث نقص ملحوظ في كرات الدم البيضاء بجميع انواعها علاوة على حدوث زيادة معنوية في متوسط الهيموجلوبين في كريات الدم. كما تم في هذا البحث مناقشة التغيرات التي حدثت في اوزان الجسم والاعضاء علاوة على التغيرات المرضية.