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Clinicopathological and Toxicological Studies of Oral Administration of *Rhazya stricta* Extract "Harmal" in Rats

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Abstract. *Rhazya stricta* (Apocyanaceae) is abundantly found in Saudi Arabia and is used in traditional medicine for the treatment of rheumatism.

In this study, the lyophilized aqueous extract of the aerial parts of the plant was given orally to mature rats at a dose of 400 mg/kg body weight daily for a period of 8 weeks. This resulted in insignificant changes in the serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, the serum levels of urea nitrogen, total proteins, albumin and inorganic phosphorus. There was, however, significant elevations ($P < 0.01 - P < 0.001$) in serum globulins, and serum calcium together with significant decreases ($P < 0.01$) in serum triglycerides and A/G ratio.

Treatment with the extract also resulted in a significant decrease ($P < 0.05 - P < 0.001$) in the values of RBCs and WBCs counts, Hb%, MCV and MCHC, together with a slight decrease in MCH. The extract was not lethal when given in doses up to 4.0 gm/kg and did not produce any gross or microscopic changes.

Introduction

Rhazya stricta (Apocyanaceae) is a small shrub widely disributed in Western Asia and abundantly found in Saudi Arabia. The leaves and roots of the plant produced more than fifty indole alkaloids, three of which had cytotoxic activity. These were vallesiachotamide [1], sewarine [2, P. 540] and tetrahydrosecamine [3]. Two more alkaloids stricticitine and strictine from the leaves of the plant were also isolated [4]. Moreover, robinin and two glycosides from the leaf extract of the plant were recently isolated by Andersen *et al.* [5].

Rhazya stricta is a bitter tonic and curative for choronic rheumatism [3, 6, P. 222, 7, P. 3911, 8]. The alcoholic extract of *R. stricta* showed a marked leucopenic effect in rats when given orally (20 mg/kg) and that a single i.p. injection of 15 mg/kg significantly reduced the white blood cell count in 7-10 days [9]. The anticancer activity of some of its alkaloids was also reported [10].

The present study was conducted to investigate the clinical and pathological effects of oral administration of the aqueous extract of the plant in rats.

Materials and Methods

The plant material was collected in April 1986 from Qassim area, Saudi Arabia. Its identity was verified by Prof. Hassan Moustafa Hassan, professor of Plant Taxonomy, College of Science, King Saud University, Riyadh, Saudi Arabia. The air dried aerial parts were extracted with hot water. The water filtrate was lyophilized using a labconco freeze dryer – 18 model 75018. The lyophilized aqueous extract was freshly prepared and used as 10% (W/V) solution in normal saline.

Adult male wistar rats weighing between 180-200 gm body weight (b. wt) were used. These were divided into two groups of twenty rats each and were fed on a standard pelleted diet with free access to water. One group was left as control, given normal saline only, whereas, the other group was orally dosed with the aqueous solution of the extract at a rate of 400 mg/kg. b.wt. daily for a period of 8 weeks. After the end of the experiment, all rats were sacrificed and ten whole blood and serum samples were obtained. Whole blood samples were collected in lithium heparinized plastic bottles for haemogram determination, while serum samples were collected in 10 dry and clean bottles and stored at –20°C until analysed.

Animals were dissected for post-mortem examination. Specimens from the liver, kidneys, spleen, intestine, heart and mesentric lymph nodes were fixed in 10% buffered formol saline solution and processed and stained with haematoxyline and eosin for microscopic examination [11, p. 209].

Erythrocytes (RBC) and leucocytes (WBC) counts were estimated using the double improved neubauer chamber [12]. Haemoglobin (Hb%) was estimated using the acid haematin method [13]. Packed cell volume (PCV) was determined in double capillary tube preparations using a microhaematocrit centrifuge, [14]. The formulae of Baker *et al.*, [15, pp. 558-560] were employed for calculations of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

A group of serum chemical variables were determined colorimetrically using commercial kits supplied by BioMerieux (France). These were:

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [16], total proteins [17], serum urea nitrogen [18], triglycerides and inorganic phos-

phate [19, pp. 675, 887], while serum Ca^{2+} was determined using the Boehringer Manheim Calcium kit following the method of Connerty and Briggs [20].

Serum protein fractionation was made by using 5 l samples from treated and control rats. These were applied to agarose gel slabs (5401-001 Hydragel protein, LKB-Sebia). Electrophoresis was run for 20 min. using LKB equipment and methods (Sebia 91130 Issy Les Moulineaux, France).

Following staining with amidoblack and destaining in 5% acetic acid, the electrophoretograms were scanned for the percentages of albumin, α, β and γ-globulins fractions and the A/G ratio in LKB-5300 "preference" densitometer programmed for protein analysis.

Toxic effects

The LD_{50} for the extract was determined in rats [21, p. 60]. Rats were given oral doses ranging between 400-4000 mg/kg.b.wt., and were kept under observation for 24 hr. during which symptoms of toxicity and mortality were recorded.

Statistical analysis

Data were statistically analysed using paired student "t" test [22].

Results

Table 1 shows that the daily oral administration of *Rhazya stricta* aqueous extract to mature rats in a dose of 400 mg/kg.b.wt. for 8 weeks produced an insignificant increase in the activity of serum ALT, AST and serum urea concentration. There was however, a significant increase ($P < 0.01$-$P < 0.001$) in serum calcium α, β and γ-globulins together with a significant decrease in serum triglycerides, and A/G ratio. A slight decrease in serum total proteins, albumin and inorganic phosphorus were also observed. Table 2 shows that total RBCs, WBCs counts, Hb%, PCV, MCV and MCHC were significantly decreased ($P < 0.05$-$p < 0.001$) as a result of treatment with the plant extract, however, MCH is slightly decreased.

Toxic effects

The LD_{50} experiment revealed that *Rhazya stricta* aqueous extract in doses ranging from 400-4000 mg/kg.b.wt. of rats produced no deaths or even toxic symptoms.

Gross and histopathological findings

No post-mortem or histopathological changes or lesions were found.

Table 1. Effect of oral administration (Means ± SE) of an aqueous extract from, *Rhazya stricta* to rats at a dose rate of 400.0 mg/kg.b.wt. daily for a period of 8 weeks.

Variables	Control n = 10	Treated n = 10	Variables	Control n = 10	Treated n = 10
ALT (unit/ml)	158.5 ± 9.67	164.8 ±16.3	Total proteins (gm/l)	71.7 ±4.88	70.26 ±2.5
AST (unit/ml.)	283 ± 6.82	293.4 ± 7.85	Albumin (gm/l)	46.6 ±1.06	42.13 ±3.0
Urea Nitrogen (mg/l)	397.6 ±10.48	410.5 ±19.74	α-globulins (gm/l)	15.87 ±0.15	16.8 ±0.23 **
Triglycerides (gm/l)	63.0 ±5.95	33.0 ± 2.93 ***	β-globulins (gm/l)	5.86 ±0.05	7.17 ±0.14 ***
Calcium (mg/100 ml)	14.62 10.6	18.6 ± 0.83 **	γ-globulins (gm/l)	2.71 ±0.015	3.85 ±0.066 **
Inorganic phosph (mg/100 ml.)	20.22 ± 1.67	19.12 ± 0.83	A/G ratio	1.9 ±0.035	1.5 ±0.12 **

** $P < 0.01$

*** $P < 0.001$

Table 2. Effect of oral administration (Means ± S.E.) of an aqueous extract from *Rhazya stricta* to rats given daily in doses of 400 mg/kg. b.wt. for 8 weeks on blood characteristics.

Group	RBCs $\times 10^{-6}$	WBCs $\times 10^{-3}$	Hb %	PCV %	MCV Cu mm	MCHC %	MCH u ug
Control n = 10	5.702 ± 0.158	7.3 ± 0.084	9.44 ± 1.13	40.0 ± 2.9	23.5 ± 1.93	77.9 ± 7.43	20.14 ± 1.55
Treated	4.758 ± 0.316 *	5.63 ± 0.482 **	6.24 ± 0.411 *	29.2 ± 2.03 *	8.24 ± 0.77 ***	23.56 ± 1.98 ***	16.25 ± 2.73

* $P < 0.05$

*** $P < 0.001$

Discussion

The experiment showed that the oral administration of *R. stricta* aqueous extract to mature rats resulted in insignificant changes in serum enzyme activities of ALT, AST, serum urea nitrogen, total proteins, albumin, inorganic phosphorous and MCH. There were however, significant increases in serum globulins, and calcium levels together with significant decreases in serum triglycerides, A/G ratio, RBCs and WBCs counts, Hb%, PCV% MCV and MCHC when compared with the control values.

Increased serum globulins and decreased A/G ratio obtained in this study might be due to stimulation of the liver to synthesize α and β-globulins on one hand and to stimulation of the lymphocytes to produce γ globulins on the other, especially when the total WBCs count was low in the treated group, indicating the presence of immunopotentiating factor in the plant extract.

The significant increase in serum calcium level in the presence of slightly lowered phosphate concentration may be attributed to increased intestinal absorption of calcium. This could be due to increased formation of calcium binding proteins, as a result of some factors(s) present in *R. stricta* having an action similar to vitamin D_3.

The significant ($P < 0.001$) decrease in serum triglycerides level could be attributed to decreased availability of conjugated bile salts. Farah *et al.* [23], noted a decrease in the level of serum total bilirubin in sheep treated with *R. stricta* extract. This could be due to a selective increase in the activity of the liver to conjugate bile components including bile acids and their subsequent loss through the intestines.

Moreover, it could be speculated that some components in *R. stricta* reduced the rate of esterification of fatty acids to triglycerides. The significant decrease in RBCs and WBCs counts with the subsequent decrease in Hb%, PCV%, MCV and MCHC might be due to suppression of the bone marrow. This finding is in accordance with Siddiqui and Bukhari [9] who reported that *R. stricta* extracts showed a marked leucopenic effect in rats when given orally in a dose of 20 mg/kg.b.wt. They also added that a single intraperitoneal injection of 15 mg/kg. b. wt. significantly reduced the WBCs count for a period of 7-10 days. This marked leucopenic effect might be most probably due to the cytotoxic effect of the extract on bone marrow as was observed by Mukhopadhyay *et al.* [10] who reported anticancer activity of some alkaloids of *R. stricta*.

From the obtained results, we may conclude that the aqueous extract of *R. stricta* is nontoxic to rats at 4.0 gm/ kg.b.wt. with hypolipidaemic and immunopotentiative effects.

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دراسات إكلينيكية مرضية وسمية لتناول خلاصة نبات الحرمل عن طريق الفم في الفئران

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ملخص البحث. يوجد نبات الحرمل بوفرة في المملكة العربية السعودية وهو يستخدم شعبيًا كعلاج لبعض الأمراض مثل الروماتيزم. ولقد تم في هذه الدراسة إعطاء الخلاصة المائية عن طريق الفم للفئران البالغة في جرعة مقدارها ٤٠٠مجم/ كجم من وزن الفئران يوميًا ولمدة ثمانية أسابيع متصلة. ولقد أدى ذلك إلى حدوث تغيرات غير معنوية في نشاط إنزيمي ALT and Ast وكذا في بولينا المصل والبروتينات الكلية ـ والزلال والفسفور غير العضوي. كما كان هناك زيادة معنوية في جلوبيولينات المصل والكالسيوم. كما صاحب ذلك نقص معنوي في الجليسيريدات الثلاثية ونسبة الزلال للجلوبيولينات. كذلك أدى العلاج بخلاصة الحرمل إلى حدوث نقص معنوي في عدد خلايا الدم الحمراء والبيضاء وكذا في نسبة الهيموجلوبين وحجم الخلايا المضغوطة ومتوسط حجم الكريات، وكذا متوسط محتوى الكريات من الهيموجلوبين.

لم يؤد تعاطي خلاصة نبات الحرمل إلى حدوث وفيات في حيوانات التجارب حتى عندما أعطى في جرعات مقدارها ٤جم/ كجم إضافة إلى عدم حدوث أي تغيرات مرضية.