Experimentally Challenged Reactivity of the Pituitary-Adrenal-Hematological Axis After *Ruta chalepensis* Administration

Othman Al-Sagair, PhD

Department of Medical Microbiology, Faculty of Science, AlGassim University, AlGassim, Bureidah, Saudi Arabia

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ABSTRACT

After oral treatment with an aqueous extract of *Ruta chalepensis* in male white Wistar rats at doses of 0.5, 1.0 and 2.0 g/kg body weight, a hematological evaluation was performed over a period of 15 days. The evaluated parameters included red and white blood indices, cholesterol, triglycerides, total fat, adrenocorticotropic hormone (ACTH) and cortisol. The results revealed that the extract increased most of the parameters related to the red cells, while on the other hand it decreased most of those parameters related to the white cells and platelets. There was no significant effect on the levels of cholesterol, triglycerides, and total fat. On the other hand the serum concentration of ACTH decreased with an increase of dose while the cortisol increased.

INTRODUCTION

Rue or Herb of Grace, is by far the best known of this genus of sixty species native to the Mediterranean and western Asia and typifies the rue family Rutaceae. Common rue, well known and highly regarded since ancient times, is frequently mentioned in the literature, including the writings of Milton and Shakespeare. It was considered a remedy to all manner of poisons and thought to ward off contagious diseases. It has been discovered that the ethanolic extract of the plant has an anti-inflammatory, antipyretic, analgesic, and CNS depressant activities.[1] Recently, the perinatal toxicity of the plant has been discovered.[2] The present endeavor is intended to elucidate a detailed hematological screening of the impact of *R. chalepensis* aqueous extract with the sole objective of determining its use as a safe phytopharmaceutical-herbal agent.

MATERIALS AND METHODS

Plant Material

The plant material consisted of the leaves of *Ruta chalepensis* collected in 2003 in AlTaif, Saudi Arabia were authenticated by Professor Saleh Bazaid of the Department of Biology, Umm AlQuora University, Saudi Arabia.

Extraction

On arrival, at the laboratory, the sample was ground and soaked in distilled water and kept at 4˚C for 48 hours. The extract was then separated by filtration and subsequently lyophilized and refrigerated at 4˚C until used.

Animals
Forty mature male white Wistar rats weighing 125 to 150 g were divided into 4 groups of 10. One group was kept as a control and given a daily oral dose of saline. The other 3 groups were given an oral dose of 0.5 g, 1.0 g, and 2.0 g of the lyophilized aqueous extract of the *R. chalepensis* for 15 consecutive days. Blood samples, used for clinical hematology and chemistry evaluation, were obtained from the animals via the inner canthus. Hematology and serum chemistry analyses were performed for all animals. At the end of the study during day 15, all animals were killed with approximately 100 mg sodium phenobarbital per kg body weight administered intraperitoneally 24 hours after the last dosing of the respective treatment duration.

Hematological Evaluation
Blood samples were analyzed using a Coulter Counter for the following hematological parameters: white blood cell (WBC) total count, WBC differential counts (including neutrophils [N], lymphocyte [LY], monocyte [MO], eosinophil [EO], and basophil [BA]), red blood cell (RBC) total count, hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count (PLC).

Biochemical Analysis
Serum cholesterol, triglycerides, and total fat were estimated colorimetrically using reagent kits (BioMerieux, Marcy-l'Etoile, France) using a Spectronic 2000 B&L spectrophotometer (Bausch & Lomb, USA).

Hormone Assay
Serum ACTH and cortisol were assayed in a Packard Cobra Gamma Counter Spectrometer (Meriden, Conn) from frozen samples using Coat-a-Count I^{125}–radioimmunoassay kits provided by the Diagnostic Products Corporation (Los Angeles, Calif).

Statistical Analysis
Data were expressed as mean ±SEM. Student *t* test was employed for statistical comparison. $P<0.05$ was considered significant.

RESULTS
The results of the hematological parameters, lipid profile, and hormone concentration are outlined in Tables 1 and 2 respectively. The results of the hematological parameters recorded the fact that *R. chalepensis* aqueous extract given *per oss* significantly decreased ($P<0.05$) all of the indices related to white blood corpuscles, with the exception of the neutrophil count which was increased, at all doses. On the other hand, it significantly increased ($P<0.01$) most of the parameters related to the red blood corpuscles. Yet, the red blood indices (MCV, MCH, and MCHC) were not significantly influenced.

The results indicate that the extract had no effect on cholesterol, triglyceride, and total fat levels. Yet, the results showed that while the extract had an inhibitory effect on serum adrenocorticotropic hormone (ACTH), while in the 3 experimental doses, its influence was stimulatory on the serum cortisol concentration (Table 2).

DISCUSSION
The reproducible quality of phytopharmaceuticals (herbal medicines) is an essential prerequisite for good efficacy and tolerability in the treatment of functional disorders. In clinical trials and scientific investigations, standardized assessments (ie, validated, internationally recognized and accepted scales) provide the basis for establishing clinical efficacy and tolerability.

Table 1. Effect of the Aqueous Extract of Ruta chalepensis on the Rat Hemogram*

Groups	White Blood Corpuscles					
	WBC	N %	LY %	MO %	EO %	BA %
Control (saline)	12.63±0.39	1.07±0.10	5.90±0.45	0.82±0.04	0.40±0.01	1.07±0.20
Group 1 (0.5g)	8.64†±0.41	1.26†±0.09	4.60†±0.18	0.72‡±0.03	0.37±0.02	0.67±0.05
Group 2 (1.0g)	7.47†±0.27	1.36†±0.03	4.71†±0.07	0.69†±0.05	0.18†±0.01	0.64‡±0.02
Group 3 (2.0g)	7.37†±0.35	1.82†±0.16	4.32†±0.11	0.57†±0.15	0.18†±0.02	0.34†±0.06

*Values are ± SE of mean. n= 10. White blood cell indicates WBC; neutrophils, N; lymphocyte, LY; monocyte, MO; eosinophil, EO; basophil, BA; red blood cell, RBC; hematocrit, HCT; hemoglobin, HGB; mean corpuscular volume, MCV; mean corpuscular hemoglobin, MCH; and mean corpuscular hemoglobin concentration, MCHC.

†$P<0.01$

‡$P<0.05$

Phytochemical screening of the leaves of *Ruta chalepensis* showed the presence of alkaloids, flavonoids, coumarins, tannins, volatile oil, sterols and/or triterpenes.[1]

Several plant extracts were recently hematologically evaluated and were found to be inactive and did not reveal any abnormalities.[3-8] Yet, the extract of *Allium ascalonicum* decreased most of the parameters relating to red cell and increased most of those parameters relating to white cells,[9] a result contrary to the results recorded in the present investigation, and quite clearly demonstrates the decrease in total WBC, lymphocytes, eosinophils, basophils, and platelets.

Previous hematological studies on the extract of *Ruta chalepensis* failed to significantly change the prothrombin time and fibrinogen level.[1] Yet, a significant fall in RBC level of *R. chalepensis* was recorded in the treated animals,[10] a result which does not coincide with the recorded results observed in the increased total RBC count and its indices.

The findings show significant Pituitary-Adrenal-Hematological (PAH) axis responsiveness after *R. chalepensis* treatment. It appears that the PAH axis activity causes a significant level of cortisol release, which makes it influential in increasing the release of neutrophils from bone marrow, and in contrast, decreases the number of circulating lymphocytes, eosinophils, and monocytes.[11,12]

Stem cells and later precursor cells are stimulated by various chemical messengers called cytokines, known as interleukins. The *R. chalepensis* extract or/and the cortisol could have released these messengers. The interleukins influence the production of blood cells and promote proliferation white cells.[13,14] A

Table 2. Effect of the Aqueous Extract of *Ruta chalepensis* on the Rat Plasma Lipogram, ACTH and Cortisol Levels*

Groups	Cholesterol Mmol/L	Triglycerides Mmol/L	Total lipids Mmol/L	ACTH pg/mL	Cortisol μg%
Control (saline)	3.75±0.01	1.39±0.05	2.62±0.07	11.68±0.54	0.26±0.01
Group 1 (0.5 g)	3.73±0.02	1.39±0.06	2.90±0.18	10.70±0.18	0.30±0.03
Group 2 (1.0 g)	3.53±0.10	1.43±0.03	2.46±0.06	9.97†±0.37	0.54†±0.07
Group 3 (2.0 g)	3.42±0.04	1.69±0.07	2.56±0.07	8.29†±0.42	0.66†±0.07

*Values are ± SE of mean, n = 10

†P<0.01

Red Blood Corpuscles						Platelets
RBC	HGB	HCT	MCV	MCH	MCHC	
8.41±0.10	67.2±0.60	15.3±0.23	80.24±0.51	18.20±0.17	22.70±0.26	1150.0±28.71
8.92†±0.13	72.06†±0.19	16.23†±0.09	79.04±0.42	17.74±0.15	22.39±0.10	845.0†±4.08
9.22†±0.10	73.26†±0.31	16.4†±0.06	79.60±0.86	17.86±0.22	22.39±0.16	825.6†±37.33
9.28†±0.06	74.7†±0.35	16.6†±0.23	80.50±0.61	17.96±0.21	22.23±0.15	816.6†±17.84

negative correlation was found between cortisol levels and interleukins,[15-17] which could have thus reflected its action on the present findings.

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