Persistent Hyperinsulinemia as a Result of Infusion with *Ruta Chalepensis*

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ABSTRACT

Objective: The possible glycemic effect of *Ruta chalepensis* was evaluated on mature and immature rats over a period of 6 hours (acute phase) and 15 days (chronic phase) of oral administration.

Methods: Eighty mature and 40 immature male Wistar rats were divided into groups of 10, three groups were controls and 9 treated groups. In experiment 1, three of the mature rat groups received different doses of the aqueous extract of the leaves of *Ruta chalepensis* as a single dose. The fourth group acted as control and received saline. In experiment 2, the same treatment was carried out on immature rats. Glycemia was measured after the first 30 minutes and thereafter every other hour. In experiment 3, three experimental groups of mature rats were given *R. chalepensis* extract orally for 15 consecutive days and the effect of the plant extract was evaluated in the liver, cardiac and skeletal muscles glycogen, blood glucose, insulin, and prolactin.

Results: Under the experimental conditions employed, administration of the *Ruta chalepensis* infusion caused hyperglycemia during the first 3 hours to return to normal levels after the fifth hour. On continuation of the infusion for 15 successive days, the blood glucose level remained normal, but blood insulin levels increased by two fold. This increase was dose dependent. At the same time, there was an increase in the level of glycogen deposition in the liver, heart, and skeletal muscle.

Conclusions: Improving the insulin-secretory capacity of pancreatic □-cells and/or improving the action of insulin through the administration of the water extract of *R. chalepensis* and demonstrating the existence of insulinomimetic compounds in the extract, would be of great interest and could prove useful as a natural treatment of diabetes.

INTRODUCTION

Ruta or Herb of Grace is by far the best known of this genus of 60 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 literature, including the writings of Milton and Shakespeare.

Ruta chalepensis has long been used in folk medicine. Its use as an abortifacient and uterine stimulant has been reported in ancient Turkish[1,2] and Chinese[3] literature. In folk medicine in the Middle East, infusions of this plant are used as an abortifacient. Since we know of no previous research on the

glycemic properties of this plant, experiments were carried out in order to test whether the aqueous extract of *R. chalepensis* impacts blood glucose levels.

MATERIALS AND METHODS

Test Material

Leaves of *Ruta chalepensis* were collected from AlTaif region on the west coast of Saudi Arabia during 2002. The leaves were macerated and extracted with distilled water (1:2 weight/volume) for 48 hours at 4˚C. The crude filtrate was lyophilized and the required doses were made up in double distilled water.

Experimental Animals

Colony bred mature (150-200 g) and immature (25-30 g) Wistar rats, maintained in air conditioned quarters (22 ± 1˚C) under uniform husbandry conditions, were used in this study. General procedures for animal care and housing were in accordance with the United States Department of Agriculture through the *Animal Welfare Act (7USC 2131) 1985* and *Animal Welfare Standards* incorporated in *9 CFR Part 3, 1991*.

Animals were kept in groups of 10 and given an unlimited supply of pelleted food and tap water. The plant material was macerated with an equal amount of distilled water and suspended for 24 hours at 4˚C. The suspension was filtered and the extract was lyophilized.

Dose and Duration of Treatment

Experiment 1: Forty of the mature rats were divided into four groups, ten in each. Three of which were orally treated with the crude aqueous extract at dose regimens of 0.5 g (Group I), 1 g (Group II) and 2 g (Group III) per animal. After the oral administration of the aqueous extract of *R. chalepensis*, hourly blood samples were obtained. Control animals received saline. Sodium fluoride tubes were used for glucose estimation.

Experiment 2: The forty immature rats were also divided into four groups, ten in each. Three of the four groups were orally treated with the crude aqueous extract at dose regimens of 0.5 g (Group I), 1 g (Group II) and 2 g (Group III) per animal. After the oral administration of the aqueous extract of *R. chalepensis*, hourly blood samples were obtained. Control animals received saline. Sodium fluoride tubes were used for glucose estimation.

Experiment 3: The crude aqueous extract was administered orally at dose regimens of 0.5 g (Group I), 1 g (Group II) and 2 g (Group III) per animal every day for 15 days. Control animals received saline. After 15 days of daily oral administration of the aqueous extract of *R. chalepensis*, the animals were weighed and autopsied under pentobarbital anesthesia (50 mg/kg given intraperitonially), 24 hours after the last dosing of the respective treatment. Blood samples were collected from the inner canthus in heparinized tubes and sodium fluoride and the plasma were immediately separated and stored at –20˚C until used for hormonal assays and blood glucose estimation respectively. Tissue samples from the heart and skeletal muscles were immediately weighed and introduced into 30% potassium hydroxide for glycogen analysis.

Biochemical Analysis

Plasma glucose was estimated using BioMerieux Kits (Marcy-l'Etoile, France), while the liver and muscle glycogen were estimated using a Spectronic 2000 B&L spectrophotometer.[4]

Hormonal Assays

The collected plasma was assayed for insulin and prolactin. The hormones were measured by means of a radioim-

Table 1. Effect of an Aqueous Extract of *Ruta chalepensis* on Plasma Glucose Levels After Oral administration in Normal Mature Male Rats

Groups	Treatment	30 (min)	60 (min)	2 hr	3 hr	4 hr	5hr
Control	Saline	102					
Group 1	0.5g	175*	185*	176*	161*	126†	108
Group 2	1.0g	182*	190*	185*	170*	133*	110
Group 3	2.0g	195*	210*	198*	185*	145*	118†

*$P<0.01$
†$P<0.05$

Table 2. Effect of an Aqueous Extract of *Ruta chalepensis* on Plasma Glucose Levels After Oral Administration in Normal Immature Male Rats

Groups	Treatment	Plasma glucose level (mg/100mL)						
		30 (min)	60 (min)	2 hr	3 hr	4 hr	5 hr	6 hr
Control	Saline	70						
Group 1	0.5g	90*	94*	122†	120†	110†	90*	72
Group 2	1.0g	95*	100†	128†	130†	125†	99*	70
Group 3	2.0g	102†	110†	139†	139†	128†	102†	75

*$P<0.05$
†$P<0.01$

munoassay Coat-A-Count kit (Diagnostic Products Corporation, Los Angeles, Calif) using a Packard Cobra gamma counter (Meriden, Conn).

Statistical Analysis

Statistical analysis of the difference of the means was calculated using a Student *t* test. Data are expressed as mean ± standard error of the mean (SE).

RESULTS

The glucose levels of the controls and the glucose levels of plant-treated mature and immature animals, after the administration of the aqueous extract of *R. chalepensis* into the oral cavity through an oral catheter without anesthesia, are shown in Tables 1 and 2.

As shown in Table 1, plasma glucose levels in the mature rats reached a maximum significant level after 60 minutes, while in the immature rats maximum significant hyperglycemia was reached after 3 hours and in both immature and mature rats hyperglycemia started to decrease with time (Table 2). Yet, in both experiments, normal glycemic levels were reached after 5 hours in the mature and immature animals.

The *R. chalepensis* aqueous extract was ingested orally for 15 consecutive days revealing no effect on the glucose level in the blood (Table 3). Yet, the level of insulin was strikingly elevated, rising 180 %, however, no effect was seen in the prolactin level.

Consequently, it was interesting to look at the influence of the *R. chalepensis* aqueous extract on the liver and muscle glycogen; there was a 40 to 50 % increase of glycogen deposition in the liver, heart, and skeletal muscle (Table 3).

The increase in the insulin level in the blood, and in glycogen deposition in the liver and muscles was dose dependent.

DISCUSSION

Phytochemical screening of the leaves of *Ruta chalepensis* showed the presence of alkaloids, flavonoids, coumarins, tannins,

Table 3. Effect of an Aqueous Extract of *Ruta chalepensis* on Tissue Glycogen, Plasma Glucose, Insulin and Prolactin Levels After Oral Administration for 15 days in Normal Mature Male Rats

Groups	Glycogen (μg/ 100 mg tissue)			Glucose (mg/dL)	Insulin (μIU/mL)	Prolactin (ng/dL)
	Liver	Cardiac muscle	Skeletal muscle			
Control (saline)	40370±2208	77530±608	48721±2065	91.3±1.58	3.05±0.42	1.07±0.02
Group 1 (0.5g)	60095*±1223	78348*±4763	54156*±1947	88.8±1.72	5.62*±0.6	1.15±0.04
Group (1.0g)	264790*±4161	84236*±344	53396*±3358	93.7±1.38	7.39*±0.46	1.16±0.03
Group 3 (2.0g)	67248*±4846	86921*±2121	57555*±1429	80.7±8.7	8.26*±0.54	1.00±0.00

*$P<0.01$

volatile oil, sterols and/or triterpenes[5]. It is well known that blood glucose level is regulated by the interaction of hormones such as, insulin, glucagon, prolactin, and cortisol.

In this study, we investigated the effects of the aqueous extract of *R. chalepensis* on blood glucose tissue glycogen and blood hormones in normal fed rats. The recorded results indicated a consistent increase in blood glucose levels, in mature and immature normal fed rats, which occurred within 30 minutes and was short lived, lasting for 5 hours, after which the blood glucose level returned to normal. The reasons for such an instant and transient impact is yet to be explained, although the seeds of *M. charantia* have induced a similar effect, which has been attributed to □, □-trehalose, present in the seeds.[6]

After administration of the aqueous extract for 15 consecutive days, the *R. chalepensis* extract inhibited cortisol or a prolactin-mediated increase in blood glucose and the normoglycemia was reached by modulating the blood insulin level. A similar mechanism has been proposed to explain the glycemic effect in rabbits fed other indigenous plants such as *Eriobotrya japonica.* This diet has been found to produce a glycemic effect through the release of insulin from the pancreatic □-cells, an action seen with tormentic acid, glibenclamide, and sulfonylureas.[7,8] Yet, the longer duration of action of the *R. chalepensis* extract when compared to *Eriobotrya japonica* is significant.

An increase in cortisol resulted in increased glucose release from the liver, increased glycogenesis and augmented gluconeogenesis from amino acids, and lead to glycogen deposition in the liver and a decrease in muscular utilization of glucose.[9] Hyperinsulinemia could have been induced through the action of the increase in the cortisol level and which could occur without hyperglycemia.[10]

Although, the possible existence of insulinomimetic compounds in *R. chalepensis* have an effect, the compounds alone are unlikely to account for the oral normoglycemic effect of the plant, which has also been demonstrated in other plants.[6]

CONCLUSION

The observations recorded in the present investigation suggest that *Ruta chalepensis* possesses normoglycemiant properties by raising serum circulating insulin levels through stimulation of insulin release from the pancreas without any counterregulatory factors and metabolic bias. Further studies are needed to clarify the detailed mechanism of action of the water extract of *R.*

chalepensis causing normoglycemia and persistent hyperinsulinemia. If the administration of the water extract of *R. chalepensis* improves the insulin-secretory capacity of the pancreatic □-cells and/or improves the action of insulin and demonstrates the existence of insulinomimetic compounds in the extract, it would be of great interest as a natural source for treatment of diabetes.

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