

Studying the effects of *Ruta chalepensis* on blood glucose, cholesterol and triglycerides levels in rats.

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Key wards: *Ruta chalepensis*, blood glucose, cholesterol, triglycerides

ABSTRACT

Ruta chalepensis is a native plant to Eurasia and North Africa, it has been used in traditional herbal medicine for various purposes. This includes For example, as abortifacient, digestive, anti-inflammatory, analgesic and anti- parasites. In folk Libyan medicine it is believed to be helpful for diabetes and dyslipidemia. Water and alcoholic extracts of *Ruta chalepensis* were tested in rats for potential lowering effects on blood glucose, cholesterol and/or triglycerides. In this study, the water and alcohol extracts was administrated to normal rat and blood glucose, cholesterol and triglycerides were estimated with urine output, food and water intake as biological markers for blood glucose. In unexpected manner, neither water nor alcoholic extracts decrease blood glucose, cholesterol and/or triglycerides in rats. However, high concentrations of alcoholic extract (15 and 20%w/v) resulted in a significant elevation of blood glucose levels. Administration of alcoholic extract 7.5%w/v daily for 7 days produced increase in urine output on every day. The results point to a potential risk of using the plant as an anti-diabetic remedy, also indicate to the presence of material(s) that might have diuretic effect.

INTRODUCTION

Ruta Chalepensis is a species of citrus family commonly named as fringed rue. It is native to Eurasia and North Africa. *Ruta Chalepensis* is extensively used throughout the world as a herbal remedy for various illnesses. For example, it is used as emmenagogue and abortifacient, digestive, analgesic and anti-inflammatory and anti-parasites (Miguel 2009). It was also reported to be used as laxative, anti-spasmodic and anti-epileptic (Aouadhi et al. 2013). *Ruta Chalepensis* contains various bioactive secondary metabolites such as furanocoumarines, alkaloids (Günaydin a and Savci b † 2005), essential oils and rutin (Terkmane et al. 2017). Locally for many years *Ruta Chalepensis* was used as traditional herbal therapy for Diabetes mellitus and hyperlipidemia.

Diabetes and hyperlipidemia are a growing medical problem in Libyan's population as they effecting a large number of people (Albrki, Elzouki, and Tashani 2007) and are contributed to serious health complications (Purnell et al. 2013). Natural products are major source of drugs for various illnesses and about 80% of people still depend on tradition medicine (Verma and Singh 2008). In Libya *Ruta Chalepensis* grows along Mediterranean coast (Louhaichi et al. 2011) where most of Libya's population are clustered. Therefore, this plant is commonly used as a traditional remedy in this area, it is believed to be useful for diabetes and high blood cholesterol levels. It has been also reported that *Ruta*

chalepensis reduces oxidative stress and decreases hyperglycemia and enhance lipid profile in experimental diabetic animals (Hamdiken, Bouhalit, and Kechrid 2017). In addition quinoline isolated from *Ruta chalepensis* was found to exert a potent inhibitory activities against α -glucosidase and α -amylase which probably leads to decrease glucose levels (Park and Lee 2015). On contrary, it was found *Ruta chalepensis* result in hyperglycemia accompanied with elevation in insulin levels in rats (Al-sagair 2004). Therefore we promoted to investigate the effects of this plant on some metabolic parameters; blood glucose, cholesterol, triglycerides, urine output and water intake.

Materials and methods

Collection and preparation of the plant

Ruta Chalepensis was collected during the flowering season as whole plant from Geriaan area 90km South of Tripoli. The taxonomic identification of the plant was done in the department of Botany University of Tripoli, Libya. The whole plant was shade-dried for 10 days, then ground to rough powder.

Plant water extraction was carried out by The decoction (Azwanida 2015) procedure like how it was prescribed in Libyan folk medicine. The powder was boiled in distilled water (according to the required concentration) for 5 minutes, the mixture was cooled to room temperature and then filtered. The filtrate was used for different treatments. The plant alcoholic extraction was undertaken

by maceration (Seidel 2012) by soaking the powder in ethanol (95%) for 5 days. The resulted residue after evaporation was prepared in the desired concentration as a suspension in 5%w/v gum acacia.

Animals and test plant administration

Male Albino rats were used for different experiments. Rats were bred in the animal house of Tripoli University where each group was housed separately in a cage. Standard food pallet diet (Beeky company, Austria) and water were available ad lib. The animals were kept at constant room temperature (20-25°C), with 12 hours dark/light cycle. The institutional animal ethical committee (IAEC) has approved the protocol to conduct experiments on these animals. *Ruta Chalepensis* extracts or vehicle control were administrated by oral gavaging, blood for biochemical parameters investigation was collected 2 hours post single dose treatment for acute effect. For sub chronic effect animals were treated once daily for 7 days at day 7 blood was collected for investigation. The volume of administration was 2.5 ml/100g (Turner et al. 2011). Blood for examination was collected directly from animal heart by cardiac puncture technique.

Biochemical analysis of blood and urine samples

Values of blood glucose levels were determined in mg/dl by enzymatic colorimetric (GOD-PAP) test which based on Triender reaction (Lott and Turner 1975) was applied using serum or plasma. Enzymatic colorimetric (CHOD-PAP) (Herrmann, Schütz, and Reuter 1983) test was used to measure the values of blood cholesterol levels using blood serum. Triglycerides blood levels were also assessed colourimetrically by enzymatic colorimetric (GPO-PAP) test (Seftel et al. 1988).

Glucose and ketone bodies in urine were directly measured by insertion of the urine test strip in the collected urine and the developed colour was compared with the standards.

Results and discussion

Neither acute nor sub chronic administration of water extract of *Ruta chalepensis* alter rat blood glucose, cholesterol and triglycerides levels.

Ruta Chalepensis 15%w/v water extract acute effect (2 hours post oral treatment) on blood glucose, cholesterol and triglycerides was investigated (figure.1). Also the sub chronic (7 days) effect for the extract was studied by treating animals daily for 7 days (figure.2). We used decoction to extract the plant because we wanted the mimic the traditional medicine recipe for *Ruta chalepensis* preparation as a remedy. The decoction in water is used for the extraction of water-soluble and heat-stable constituents since the method involves boiling the plant material with water for a given period of time.

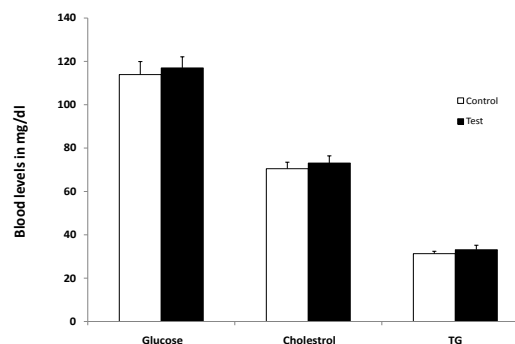


Figure. 1. acute effect of 15% water extract of *Ruta Chalepensis* (test) on rat blood glucose, cholesterol and triglycerides.

Ruta chalepensis water extract (15%w/v) showed no effect on blood glucose, cholesterol or triglycerides levels either with acute or sub-chronic administration (figures 1 and 2). This may be due to the possibility of *Ruta chalepensis* having no active constituents that may affect these parameters. Water is not suitable to extract the lipophilic substances in *Ruta chalepensis* which would have effect on blood glucose levels. It may also be that the concentration of the *Ruta chalepensis* extract is insufficient to produce an effect on blood glucose.

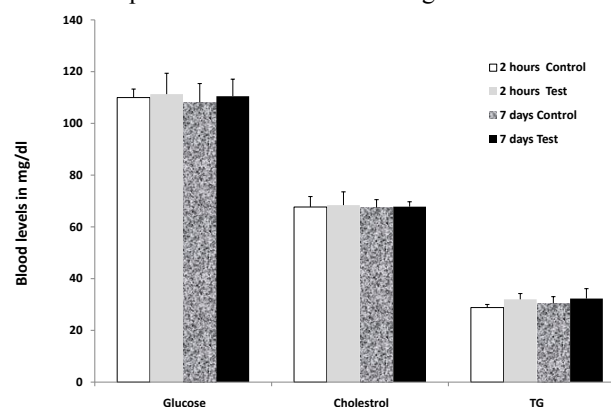


Figure. 2. The sub chronic effect of 15% water extract of *Ruta Chalepensis* (test) on rat blood glucose, cholesterol and triglycerides.

Chronic administration of alcoholic extract of *Ruta chalepensis* induces blood glucose level rather than decrease it.

Alcohol is a good all-purpose solvent for preliminary extraction of various chemical compounds, so *Ruta chalepensis* macerated in alcohol to extract potential biologically active compounds and also to avoid thermal decomposition. The acute effects of various concentrations of *Ruta chalepensis* extract residue on blood glucose (figure 3) was contrary to what has been suggested in Libyan traditional medicine. As at concentrations; 15 and 20%w/v the levels of blood glucose were raised significantly. In addition upon sub chronic administration of lower concentration (7.5%w/v) (figure 6), the blood glucose levels were also increased,

although this increases was not statistically significant, even though it has been mentioned that *Ruta chalepensis* decrease streptozotocin induced hyperglycemia in rats (Hamdiken et al. 2017).

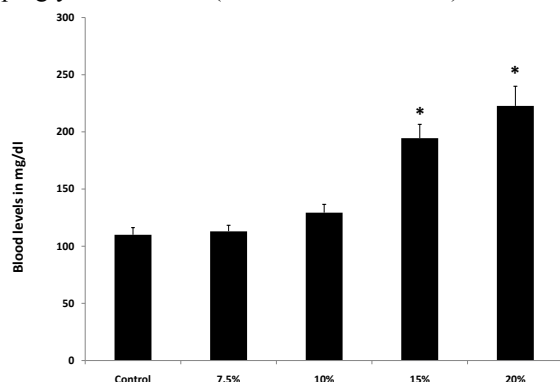


Figure 3. Acute effect of alcoholic extract residue of *Ruta chalepensis* on rat blood glucose

On the other hand The furanocoumarines in *Rutaceae* family including *Ruta chalepensis* such as scopoletin inhibit glucose-6-phosphate dehydrogenase (Adamska-Szewczyk, Glowniak, and Baj 2016) causing increase in blood glucose levels. Furthermore, quinoline isolated from *Ruta chalepensis* was found to exert a potent inhibitory activities against α -glucosidase and α -amylase (Park and Lee 2015) which could lead to increase in glucose levels. Moreove, it was found *Ruta chalepensis* result in increase in hyperglycemia accompanied with increase in insulin levels in rats (Al-sagair 2004). This may explain the noticed increases in blood glucose levels after gavaging of *Ruta chalepensis* extract to rats.

Ruta chalpensis alcoholic extract showed no significant effect on serum cholesterol or triglycerides in either acute and sub chronic administration (figures 4, 5 and 6). The plant contains rutin, furanocoumarines, alkaloids and possibly other bioactive substances which can be extracted by ethanol. However, rutin which is a constituent of this plant (Terkmane et al. 2017) has been shown to decrease serum triglycerides (Ganeshpurkar and Saluja 2017) and serum cholesterol (Ziaee et al. 2009). The absence of any effect on serum cholesterol and triglycerides levels excludes the lipid lowering effect of plant at the used concentrations of extracts.

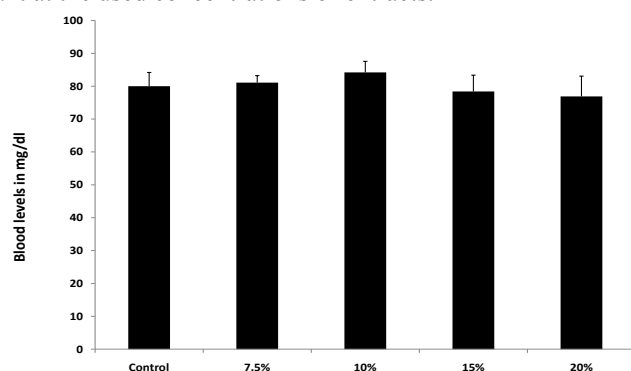


Figure 4. acute effect of alcoholic extract residue of *Ruta chalepensis* on rat blood cholesterol levels.

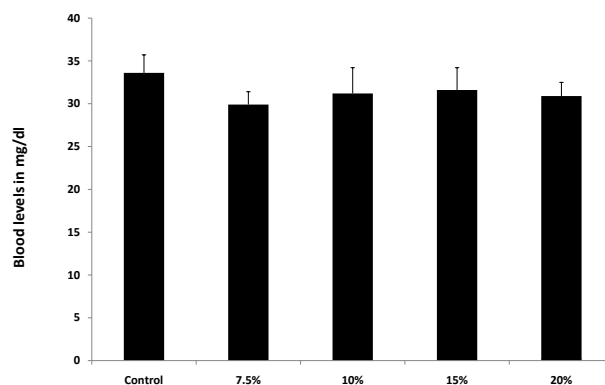


Figure 5. acute effect of alcoholic extract residue of *Ruta chalepensis* on rat blood triglycerides levels.

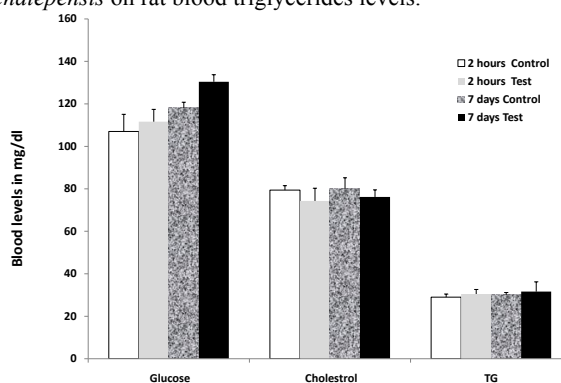


Figure 6. Sub chronic effect of 7.5% alcoholic extract residue of *Ruta chalepensis* on rat blood glucose, cholesterol and triglycerides.

Effect of *Ruta chalepensis* on food, water intake and some urine parameters

The administration of 15%w/v water and 7.5%w/v alcoholic extracts of *Ruta chalepensis* daily for 7 days had no significant effect on water or food intake on any day of the administration. The daily examination of the 24 hours collected urine sample did not show any presence of glucose or ketone bodies **Table.1 and 2**. However there was a consistence increase in the urine volume on all the days of treatment, which points to a potential diuretic effect.

Table. 1. effect of 15% water extract on food, water intake and some urine parameters.

Days	Water intake (ml)		Food intake (g)		Urine output (ml)		Glucose in urine		Ketone in urine	
	Control	15% water extract	Control	15% water extract	Control	15% water extract	Control	15% water extract	Control	15% water extract
1	23.10±1.02	22.10±1.25	10.30±0.29	10.00±0.29	10.20±0.99	11.90±0.50*	-ve	-ve	-ve	-ve
2	23.00±0.99	22.80±1.10	10.42±0.31	10.40±0.33	09.60±0.53	12.30±0.54*	-ve	-ve	-ve	-ve
3	23.90±1.02	22.60±0.90	10.42±0.50	09.90±0.62	09.10±0.15	11.02±0.06*	-ve	-ve	-ve	-ve
4	24.20±0.55	23.40±3.00	11.10±0.23	10.40±1.20	09.20±0.53	12.90±0.4*	-ve	-ve	-ve	-ve
5	23.50±0.68	22.80±1.98	10.12±0.42	10.30±0.34	09.20±0.50	12.00±0.34*	-ve	-ve	-ve	-ve
6	22.30±1.20	22.70±1.33	9.06±0.38	10.10±0.51	09.10±0.22	11.30±0.85*	-ve	-ve	-ve	-ve
7	21.90±.98	22.50±1.11	10.82±0.41	10.10±0.30	09.30±0.58	11.90±0.4*	-ve	-ve	-ve	-ve

The diuresis effect might be produced by rutin which is present in *Ruta chalepensis* and has been shown to have a diuretic action (D'avigdor et al. 2014). However, It is too premature to point out any active ingredient which may be responsible for the this diuretic effect.

Table. 2. Effect of 7.5% alcoholic extract on food, water intake and some urine parameters.

Days	Water intake (ml)		Food intake (g)		Urine output (ml)		Glucose in urine		Ketone in urine	
	Control	Alcoholic extract 7.5%	Control	Alcoholic extract 7.5%	Control	Alcoholic extract 7.5%	Control	Alcoholic extract 7.5%	Control	Alcoholic extract 7.5%
Day 1	20.80±1.77	22.30±1.2	10.32±0.53	12.23±0.71	08.9±0.24	10.3±0.57*	-ve	-ve	-ve	-ve
Day 2	21.20±1.67	21.13±0.54	11±1.21	11.23±1.3	8.20±0.33	10.90±0.35*	-ve	-ve	-ve	-ve
Day 3	19.00±0.32	20.08±1.03	12.02±1.0	13.58±2.55	8.10±0.13	11.2±0.42*	-ve	-ve	-ve	-ve
Day 4	22.20±0.88	23.30±1.1	13.30±0.8	14.04±0.32	8.10±0.20	10.30±0.70*	-ve	-ve	-ve	-ve
Day 5	19.90±1.41	21.10±1.30	12.33±1.11	13.01±1.60	9.61±0.44	11.60±0.77*	-ve	-ve	-ve	-ve
Day 6	20.30±2.47	22.30±3.2	13.20±0.82	14.00±0.63	8.50±0.33	11.9±0.43*	-ve	-ve	-ve	-ve
Day 7	21.20±3.55	22.44±2.1	16.53±0.43	17.75±1.3	8.33±0.62	12.1±0.31*	-ve	-ve	-ve	-ve

In few wards, most of discovered drugs are from the plant sources, *Ruta chalepensis* was used in Libyan folk medicine in treatment of diabetes and hyperlipidemia. As it well known most herbs used in traditional medicine are lack the confirmed experimental and clinical trail studies. In purpose of this, water and alcohol extract of *Ruta*

chalepensis was studied in rat, neither of both extract altered the blood glucose, cholesterol nor triglycerides. Instead of lowering blood glucose level chronic administration of alcoholic extract of *Ruta chalepensis* increases the blood glucose level.

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