

# HYPOGLYCEMIC AND HYPOLIPIDEMIC EFFECTS OF AN AQUEOUS EXTRACT OF *CHAMAEROPS HUMILIS* LEAVES IN OBESE, HYPERGLYCEMIC AND HYPERLIPIDEMIC *MERIONES SHAWI* RATS

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## ABSTRACT

**Ethnopharmacological relevance:** An aqueous concoction made from the leaves of *Chamaerops humilis* (L.) (dwarf fan palm), is used in the Moroccan traditional medicine for the treatment of diabetes, as well as a number of other diseases.

The aim of the study was to experimentally validate the use of *C. humilis* in the folk treatment of diabetes as well as to determine if the aqueous leaf extract of this plant has hypolipidemic properties in an animal model of obesity, hyperglycemia and hyperlipidemia.

The animal model consisted of experimentally induced obesity, hyperglycemia and hyperlipidemia (OHH) in *Meriones shawi* rats. In the acute study, OHH *M. shawi* rats (n = 8) were given a single oral dose (10 mg/kg) of an aqueous extract of *C. Humilis* leaves (plant-extract); taurine (8 mg/kg) was used as the positive control. Plasma glucose levels were determined at 2-, 4- and 6-hr after the dose. In the sub-chronic study, groups of OHH rats (n = 8 for each group) were given daily oral doses of the plant-extract and taurine (at the above doses) for 30 days. Body weight (BW), plasma glucose, total cholesterol and triglycerides were measured at 15 and 30 days of dosing.

The *M. shawi* rats developed OHH when maintained on a hypercaloric diet and forced physical inactivity for 90 days. A single oral dose of the plant-extract decreased plasma glucose levels with the maximum effect occurring at 4-hr after the dose ( $6.88 \pm 1.38$  mmol/L compared to baseline  $12.04 \pm 0.94$  mmol/L;  $P < 0.01$ ). Taurine also decreased plasma glucose (from  $12.26 \pm 1.27$  mmol/L to  $9.15 \pm 1.27$  mmol/L;  $P < 0.05$ ); water treated control group did not show any effect. In normal *M. shawi* (normal) rats, none of the treatments had significant effect on glucose levels.

In the sub-chronic study, daily oral administration of the plant-extract or taurine for 30 days to the OHH rats resulted in a significant decrease in BW (from  $241 \pm 8$  g to  $165 \pm 11$  g;  $P < 0.001$  for the extract, and from  $221 \pm 13$  g to  $189 \pm 11$  g;  $P < 0.05$  for taurine); water treated control rats showed no effect. In normal rats, administration of the plant-extract or taurine for 30 days resulted in an insignificant decrease in BW, while water administration caused a small (normal) increase in the weight. Plasma glucose levels of the OHH rats decreased significantly with daily dosing with the plant-extract [from baseline  $12.04 \pm 0.94$  mmol/L to  $6.10 \pm 0.27$  mmol/L ( $P < 0.05$ ) after 15 days, and to  $4.84 \pm 0.22$  mmol/L ( $P < 0.001$ ) after 30 days]. Taurine was less effective ( $P < 0.05$ ), while water treated control group did not show any effect. In the normal rats, administration of the plant-extract or taurine for 30 days resulted in a small decrease in glycemia.

Administration of plant-extract caused a significant decrease in plasma levels of total cholesterol [from baseline of  $3.46 \pm 0.21$  mmol/L to  $1.05 \pm 0.06$  mmol/L ( $p < 0.01$ ) after 15 days and to  $0.62 \pm 0.02$  mmol/L ( $p < 0.001$ ) after 30 days, and triglycerides [from baseline of  $1.15 \pm 0.17$  mmol/L to  $0.47 \pm 0.04$  mmol/L ( $p < 0.001$ ) after 15 days and to  $0.37 \pm 0.03$  mmol/L ( $p < 0.001$ ) after 30 days]. Taurine was less effective, while water treated control group did not show any effect. There was no effect of these treatments on lipid levels in normal rats.

The results of this study validate the traditional use of the leaves of *C. humilis* in the treatment of diabetes in Morocco. Since, the aqueous leaf extract also decreased total cholesterol and triglycerides, the plant may also be useful in the management of secondary complications of diabetes (dyslipidemia). Furthermore, the plant may become a good source of antidiabetic medication.

**Keywords:** *Chamaerops humilis*; *Meriones shawi* rats; diabetes; obesity; aqueous leaf extract; hypoglycemic activity; hypolipidemic activity; Acute effects; sub-chronic effects.

## INTRODUCTION

There is a worldwide epidemic of obesity, which is associated with a number of pathologies including

dyslipidemia, glucose intolerance, insulin resistance and diabetes mellitus, all of which are risk factors for cardiovascular (CV) disease and mortality (Eckel *et al.*, 2004; Barr *et al.*, 2007; Chu *et al.*, 2007). It is estimated

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by the World Health Organization that the prevalence of diabetes, the most common endocrine disorder, will increase from 171 million in 2000 to 366 million people affected in 2030. Diabetes is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion and/or insulin action. The aim of therapy in diabetes is to achieve normoglycemia to prevent later microvascular complications (retinopathy, nephropathy, neuropathy and microangiopathy), and intensive therapy to achieve glycemic control has been shown to significantly diminish the risk of long-term complications (DCCT, 2002). Since lipid abnormalities, leading to premature atherosclerosis, are the major cause of CV diseases in diabetic patients, ideal treatment for diabetes, in addition to glycemic control, should have a favorable effect on lipid profile. The five types of oral anti-diabetic drugs, currently approved for the treatment of type 2 diabetes do not have a favorable effect on CV disease (Fisman *et al.*, 2008), and some of these drugs are associated with serious adverse effects. Thus, new, relatively non-toxic, therapeutic agents are needed to treat hyperglycemia, which also would correct dyslipidemia to reduce the risk of CV complications of diabetes.

A large number of plants have been used in the traditional medicine to treat diabetes (see Rahman and Zaman, 1989; Marles and Farnsworth, 1994; Jouad *et al.*, 2001; Bnouham *et al.*, 2002; Tahraoui *et al.*, 2007). *Chamaerops humilis* (Labiatae) (dwarf fan palm, European fan palm) belonging to family Arecaceae and subfamily Coryphoideae, locally known as 'doum,' grows in the wild, mainly along the Moroccan coast. An aqueous concoction made from the palm leaves is used in Moroccan traditional medicine for its hypoglycemic effect (Kokwaro, 1976; Bellakhdar *et al.*, 1991; Aliotta *et al.*, 1994). In addition, the bay of this dwarf palm is alleged to have anti-inflammatory, anabolic, urinary antiseptic, antilithic, and diuretic activities (Bellakhdar *et al.*, 1991; Blumenthal *et al.*, 2000; Beghalia *et al.*, 2008).

Despite appreciable progress made in the conventional anti-diabetic management strategies, the search continues for plant-based products for the control of diabetes, which are deemed safe.

*Meriones shawi* rats of Gerbillidae subfamily (also known as Shaw's jird) develop obesity, hyperglycemia and dyslipidemia (OHH) if maintained on a hypercaloric diet and forced physical inactivity (Berrougui *et al.*, 2003), and thus, these rats may serve as an experimental model for the consequences of overeating and sedentary lifestyle in humans.

The objective of the present investigation was to experimentally validate the use of the leaves of *C. humilis* in the folk treatment of diabetes. Since diabetes is often

accompanied by hyperlipidemia, we also investigated if an aqueous extract of *C. humilis* leaves (plant-extract) possesses lipid lowering properties. If administration of the plant-extract caused hypoglycemia as well as hypolipidemia in OHH rats, the plant could become a suitable candidate for further investigations as a source of anti-diabetic agent(s) in humans. The semi-essential amino acid taurine, shown to have hypoglycemic and hypocholesterolemic effects in animal models of diabetes (Mochizuki *et al.*, 1999; Yokogoshi and Oda, 2002; Nandhini *et al.*, 2004; Pari and Venkateswaran, 2004), was used as the positive control.

## MATERIALS AND METHODS

### *Plant material*

About 1 kg of the whole plant, locally known as 'doum' was collected in March 2002 from Taounate, a Moroccan province, and authenticated by Prof. M. Fennane of the Scientific National Institute, Rabat, and a voucher specimen (CH 18) was deposited in the herbarium of the Faculty of Sciences Dhar El Mehraz, Fez, Morocco.

### *Preparation of an aqueous extract of the leaves of C. humilis*

The plant leaf extract (plant-extract) was prepared as is used in the traditional way in Morocco. Briefly, the plant was dried in the dark at room temperature, and the dried leaves were ground to a coarse powder in an electric grinder. Then 50 g of the powder was suspended in 1 L of distilled water and heated to boil under reflux for 30 min. The decoction obtained was centrifuged, filtered, frozen at -20°C, and then lyophilized (FreeZone® Dry 4.5) to give a residue (yield = 25% w/w).

For each experiment, the lyophilized plant-extract was carefully prepared under the same conditions throughout the studies (time, temperature and the amount of plant material and water used for extraction under reflux and lyophilization), and each time the quality of extraction was checked by the yield of the lyophilized material. For assuring stability, the lyophilized material was stored at -20°C until used. The residue of the aqueous extract was freshly reconstituted with distilled water (plant-extract) prior to administration to rats.

### *Experimental animals: management and treatment protocol*

Investigations using experimental animals were approved by the institutional committee, and were conducted in accordance with the internationally accepted principles for laboratory animal use and care for animals following the French Technical Specifications for the Production, Care and Use of the Laboratory Animals.

*M. shawi* rats were collected in the wild in the Moroccan province of Boulemane, and bred in our animal facility.

Adult male rats, 12-14 weeks old weighing between 120 and 150 g were selected for the experiments, and housed in metal cages and maintained at uniform temperature of  $22 \pm 2^\circ\text{C}$ , 12 h/12 h light/dark cycle. The rats were maintained on laboratory chow and water *ad libitum*.

#### Development of pathology in *M. shawi* rats

For the development of OHH, *M. shawi* rats were fed a hypercaloric diet (Berrougui *et al.*, 2003) having the following composition: protein 14%, complex carbohydrates 60%, sugar 10%, and fat 16%, with added salts (4%) and vitamin mix (1%), and were restrained to be physically inactive for a period of up to 90 days. They had free access to water. For comparison, a group of the *shawi* rats (normals) were maintained on regular rat chow and normal physical activity. Body weight (BW) of rats was determined every 30 days. Blood was collected by retro-orbital sinus puncture (rats were fasted for 12 hr and anesthetized with diethyl ether) on Day 0, Day 30, Day 60, and Day 90 from both normal and OHH rats, for measurement of plasma levels of glucose, total cholesterol and triglycerides.

#### Plant leaf extract treatment protocol

##### Acute dose study

For the acute dose study, groups (8 in each group) of OHH *M. shawi* rats were administered orally single doses (by gavage) of the plant-extract (10 mg/kg), taurine (8 mg/kg), or water (10 mL/kg). Groups of normal (non-obese) rats ( $n = 8$  in each group) were given the same treatment as the OHH rats. Blood was collected (as above) before dosing and then at 2-, 4- and 6-hr after the dose for the measurement of glucose, total cholesterol and triglycerides.

##### Sub-chronic study

For the sub-chronic study, groups of OHH rats (8 in each group) received daily doses of the plant-extract (10 mg/kg), taurine (8 mg/kg) or water (10 mL/kg) for 30 days. The same doses of the plant-extract, taurine and water were administered to groups (8 in each group) of normal rats. The rodents were weighed and blood was collected as above (for measurement of glucose, total cholesterol and triglycerides) before daily dosing and then 15 and 30 days after repeated dosing.

**Table 1:** Effect of hypercaloric diet and forced physical inactivity (A) and normal diet and physical activity (B) for up to 90 days on the body weight, and plasma levels of glucose, total cholesterol and triglycerides of *M. Shawi* rats

#### A. Hypercaloric diet with forced physical inactivity

Parameter	Days of Treatment			
	Day 0	Day 30	Day 60	Day 90
Body weight (g)	132 $\pm$ 5	151 $\pm$ 4*	189 $\pm$ 7** 2	241 $\pm$ 8***
Fasting plasma glucose (mmol/L)	4.85 $\pm$ 0.26	5.93 $\pm$ 0.27*	7.72 $\pm$ 0.36**	12.82 $\pm$ 0.72***
Total cholesterol (mmol/L)	1.29 $\pm$ 0.02	1.86 $\pm$ 0.06**	2.59 $\pm$ 0.09**	3.45 $\pm$ 0.21**
Triglycerides (mmol/L)	0.56 $\pm$ 0.03	0.69 $\pm$ 0.02*	0.89 $\pm$ 0.04**	1.20 $\pm$ 0.17**

#### B. Normal diet and physical activity

Parameter	Days of Treatment			
	Day 0	Day 30	Day 60	Day 90
Body weight (g)	123 $\pm$ 5	131 $\pm$ 2	141 $\pm$ 4	142 $\pm$ 5
Fasting plasma glucose (mmol/L)	4.66 $\pm$ 0.05	5.27 $\pm$ 0.12	5.33 $\pm$ 0.31	5.61 $\pm$ 0.35
Total cholesterol (mmol/L)	1.29 $\pm$ 0.02	1.35 $\pm$ 0.04	1.23 $\pm$ 0.11	1.33 $\pm$ 0.08
Triglycerides (mmol/L)	0.52 $\pm$ 0.01	0.45 $\pm$ 0.03	0.49 $\pm$ 0.14	0.54 $\pm$ 0.23

Values represent mean  $\pm$  SEM of eight rats; for the composition of the hypercaloric diet see Text  
(\* ) P<0.05; (\*\* ) P<0.01; (\*\*\*) P<0.001; when compared to baseline values (Day 0)

**Table 2:** Effect of a single oral dose of *C. humilis* extract, taurine or water on plasma levels of glucose, total cholesterol and triglycerides in obese-hyperglycemic-hyperlipidemic *M. Shawi* rats

Treatment Group	Dose	Plasma glucose levels (mmol/L) at time after dose			
		0 hr	2 hr	4 hr	6 hr
Water (control)	10 mL/kg	12.80 $\pm$ 1.72	13.59 $\pm$ 0.80	12.43 $\pm$ 0.44	14.15 $\pm$ 0.66
Taurine	8 mg/kg	12.26 $\pm$ 1.27	11.15 $\pm$ 0.83	9.15 $\pm$ 1.27*	9.54 $\pm$ 1.16*
<i>C. humilis</i> extract	10 mg/kg	12.04 $\pm$ 0.94	8.99 $\pm$ 0.72	6.88 $\pm$ 1.38**	7.27 $\pm$ 1.77**

Doses: plant-extract (10 mg/kg); taurine (8 mg/kg); water (10 mL/kg), Values represent mean  $\pm$  SEM of eight rats  
(\* ) P<0.05; (\*\* ) P<0.01; when compared to pre-treatment values (0 hr)

**Measurement of biochemical parameters**

Blood samples were collected (from rats as described above) using capillary tubes containing sodium fluoride-oxalate for the measurement of glucose and in tubes containing ethylenediamine tetraacetic acid for plasma lipids. Plasma was obtained by centrifugation (3500 rpm for 15 min) of blood, and glucose levels were determined by a reflective glucometer using the glucose oxidase kit, while levels of total cholesterol and triglycerides were determined by enzymatic methods using kits (Biosystems, S.A., Spain).

**STATISTICAL ANALYSIS**

Results are presented as mean  $\pm$  standard error of mean (SEM) for body weight, fasting plasma glucose, triglycerides and plasma total cholesterol for eight observations. Within group comparisons were performed by the analysis of variance using ANOVA test (Graph Pad Prism Version 3.00, USA). Significant differences between groups were assessed by the Student's *t*-test; a probability level of less than 5% ( $p < 0.05$ ) was considered significant.

**RESULTS****Induction of obesity, hyperglycemia and hyperlipidemia in *M. shawi* rats**

The hypercaloric diet and forced physical inactivity for 90 days induced obesity, hyperglycemia and hyperlipidemia (table 1-A) in the rats. The BW of these rats increased gradually from  $132 \pm 5$  g to  $241 \pm 8$  g ( $p < 0.001$ ), as well as plasma levels of glucose [from  $4.85 \pm 0.26$  mmol/L to  $12.82 \pm 0.72$  mmol/L ( $p < 0.001$ )], total cholesterol [from  $1.29 \pm 0.02$  mmol/L to  $3.45 \pm 0.21$  mmol/L ( $P < 0.01$ )] and triglycerides [from  $0.56 \pm 0.03$  mmol/L to  $1.20 \pm 0.17$  mmol/L ( $P < 0.01$ )] in 90 days (table 1-A). In contrast, rats maintained on regular rat chow and normal physical activity (normal rats), showed a small (normal) increase in body weight, but no change in glucose, total cholesterol and triglyceride levels (table 1-B).

**Acute dose study: effect of a single oral dose of *C. humilis* leaf extract and taurine on fasting plasma glucose in control and obese hyperglycemic *M. shawi* rats**

In the OHH rats, plasma glucose levels started to decline only 2 hr after the single oral dose (10 mg/kg) of the plant-extract. Maximum hypoglycemia was achieved at 4 hr after the dose (glucose levels decreased from  $12.04 \pm 0.94$  mmol/L to  $6.88 \pm 1.38$  mmol/L;  $P < 0.01$ ) (table 2). Taurine (8 mg/kg) also decreased plasma glucose (from  $12.26 \pm 1.27$  mmol/L to  $9.15 \pm 1.27$  mmol/L;  $P < 0.05$ ), but to a lesser extent, while water treated control group showed no effect (table 2). Plasma levels of glucose at 6-hr after dose were similar to those at 4-hr. In the normal animals, a single dose of the plant-extract or taurine had no effect on plasma glucose levels (data not shown).

**Sub-chronic dose study: effect of daily oral doses of *C. humilis* leaf extract and taurine for 30 days on body weight, glycemia, total cholesterol and triglycerides of normal and OHH *M. shawi* rats****Effect on body weight**

In the sub-chronic study, oral administration of the plant-extract (10 mg/kg) or taurine (8 mg/kg) daily for 30 days to the OHH resulted in a significant decrease in BW (from  $241 \pm 8$  g to  $165 \pm 11$  g;  $P < 0.01$  for the plant-extract, and from  $221 \pm 13$  g to  $189 \pm 11$  g;  $P < 0.05$  for taurine); water treated control group did not show any effect (table 3). In normal rats, administration of the plant-extract or taurine for 30 days resulted in an insignificant decrease in BW, while water administration in control animals caused a small (normal) increase in the weight (table 4).

**Effect on glycemia**

Daily oral administration of the plant-extract to OHH rats decreased plasma glucose levels significantly after 15 days (from  $12.04 \pm 0.94$  mmol/L to  $6.10 \pm 0.27$  mmol/L;  $p < 0.05$ ); rats became normoglycemic (levels =  $4.84 \pm 0.22$  mmol/L;  $P < 0.001$ ) after 30 days of daily dosing (table 3). Taurine also caused a reduction in glycemia, but less than produced by the plant-extract [levels decreased from  $12.26 \pm 1.72$  mmol/L to  $10.32 \pm 0.77$  mmol/L (NS) at 15 days, and to  $8.42 \pm 0.50$  mmol/L ( $p < 0.05$ ) at 30 days]; water treated control group did not show any effect (table 3). In the normal rats (table 4), administration of the plant-extract or taurine for 30 days resulted in an insignificant decrease in glycemia [from  $4.94 \pm 0.98$  mmol/L to  $4.33 \pm 0.43$  mmol/L (NS) for the extract, and from  $5.44 \pm 1.12$  mmol/L to  $3.88 \pm 0.56$  mmol/L (NS) for taurine]; water treated control group did not show any effect.

**Effect on lipids**

The effect of sub-chronic administration of *C. humilis* leaf extract on total cholesterol levels in the OHH rats was very significant in that the levels decreased from  $3.46 \pm 0.21$  mmol/L to  $1.05 \pm 0.06$  mmol/L ( $p < 0.01$ ) after 15 days and to  $0.62 \pm 0.02$  mmol/L ( $p < 0.001$ ) after 30 days (table 3). Taurine also decrease cholesterol levels but to a lesser extent: from  $3.06 \pm 0.21$  mmol/L to  $1.98 \pm 0.11$  mmol/L (NS) after 15 days and to  $1.52 \pm 0.11$  mmol/L ( $p < 0.05$ ) after 30 days; water administration in control animals caused insignificant reduction in cholesterol levels. The plant-extract also caused a very significant decrease in the triglyceride levels [from  $1.15 \pm 0.18$  mmol/L to  $0.47 \pm 0.04$  mmol/L ( $p < 0.001$ ) after 15 days and to  $0.37 \pm 0.03$  mmol/L ( $p < 0.001$ ) after 30 days]. The effect of taurine was much less pronounced [levels decreased from  $1.20 \pm 0.11$  mmol/L to  $1.09 \pm 0.15$  mmol/L (NS) after 15 days and to  $0.77 \pm 0.13$  mmol/L ( $p < 0.05$ ) after 30 days]; water treated control group did not show any effect (Table 3). The effect of treatment with the plant-extract, taurine and water on cholesterol and triglyceride levels in normal rats was negligible (data not shown).

## DISCUSSION

Feeding a hypercaloric diet to *M. shawi* rats with forced physical inactivity for up to 90 days caused significant metabolic changes resulting in the development of obesity (83% increase in body weight), hyperglycemia (164% increase in glucose levels), hypercholesterolemia (levels increased by 167%), and hypertriglyceridemia (levels increased by 114%). In humans, obesity caused by overeating and sedentary lifestyle can lead to diabetes, which is often accompanied by dyslipidemia. Thus, *M.*

*shawi*, a rat of the gerbilladeae family, appears to be an excellent model for studying obesity, diabetes and dyslipidemia induced by overeating and physical inactivity.

Results of this study demonstrated that an aqueous extract of the leaves of *C. humilis* induced significant decrease in glycemia in OHH *M. shawi* rats, both after single and sub-chronic oral dosing. In the acute study, plasma glucose levels of the OHH decreased by 43% ( $P < 0.01$ ) at 4-6 hr after the dose; taurine was less effective (-25%,  $P < 0.05$ ).

**Table 3:** Effect of daily oral doses of aqueous extract of *C. humilis* leaves, taurine or water for up to 30 days on body weight, plasma levels of glucose, total cholesterol and triglycerides in obese hyperglycemic-hyperlipidemic *M. Shawi* rats.

Treatment (Groups & Dose <sup>#</sup> )	Treatment Period		
	Baseline	15 Days	30 Days
Body weight (g)			
Control (water; 10 mL/kg)	235 ± 12	220 ± 8	225 ± 1
Taurine (8 mg/kg)	221 ± 13	211 ± 13	189 ± 11*
<i>C. humilis</i> extract (10 mg/kg)	241 ± 8	201 ± 12*	165 ± 11**
Plasma glucose levels (mmol/L)			
Control (water; 10 mL/kg)	12.82 ± 1.72	11.72 ± 0.61	12.21 ± 0.49
Taurine (8 mg/kg)	12.26 ± 1.27	10.32 ± 0.77	8.42 ± 0.50*
<i>C. humilis</i> extract (10 mg/kg)	12.04 ± 0.94	6.10 ± 0.27*	4.84 ± 0.22***
Plasma cholesterol levels (mmol/L)			
Control (water; 10 mL/kg)	3.10 ± 0.21	3.01 ± 0.17	2.89 ± 0.18
Taurine (8 mg/kg)	3.06 ± 0.21	1.98 ± 0.11	1.52 ± 0.11*
<i>C. humilis</i> extract (10 mg/kg)	3.46 ± 0.21	1.05 ± 0.06 **	0.62 ± 0.02***
Plasma triglyceride levels (mmol/L)			
Control (water; 10 mL/kg)	1.03 ± 0.13	1.08 ± 0.10	1.08 ± 0.07
Taurine (8 mg/kg)	1.20 ± 0.11	1.09 ± 0.15	0.77 ± 0.13*
<i>C. humilis</i> extract (10 mg/kg)	1.15 ± 0.18	0.47 ± 0.04***	0.37 ± 0.03***

Values represent mean ± SEM. of eight values.

# Rats (n = 8 in each group) were treated for 30 days with daily oral doses of plant-extract (10 mg/kg), taurine (8 mg/kg) or water (10 mL/kg), (\*)  $P < 0.05$ ; (\*\*)  $P < 0.01$ ; (\*\*\*)  $P < 0.001$  compared to the baseline values.

**Table 4:** Effect of daily oral doses of aqueous extract of *C. humilis* leaves, taurine or water for up to 30 days on body weight and plasma levels of glucose in normal (non-obese) *M. Shawi* rats

Treatment (Groups & Dose <sup>#</sup> )	Treatment Period		
	Baseline	15 Days	30 Days
Body weight (g)			
Control (water; 10 mL/kg)	132 ± 5	137 ± 5	142 ± 3
Taurine (8 mg/kg)	143 ± 2	141 ± 5	134 ± 6
<i>C. humilis</i> extract (10 mg/kg)	126 ± 3	121 ± 12	118 ± 4
Plasma glucose levels (mmol/L)			
Control (water; 10 mL/kg)	6.11 ± 0.54	5.45 ± 0.82	5.44 ± 1.20
Taurine (8 mg/kg)	5.44 ± 1.12	4.38 ± 1.05	3.88 ± 0.56
<i>C. humilis</i> extract (10 mg/kg)	4.94 ± 0.98	4.72 ± 0.32	4.33 ± 0.43

Values represent mean ± SEM. of eight values.

# Rats (n = 8 in each group) were treated for 30 days with daily oral doses of plant-extract (10 mg/kg), taurine (8 mg/kg) or water (10 mL/kg).

There was no effect of the plant extract or taurine in control (non-obese) rats. Sub-chronic oral administration of the plant-extract (daily for 30 days) decreased glycemia by 60% ( $P < 0.001$ ) and caused a significant reduction in BW (-32%,  $P < 0.01$ ). The plant extract also produced a significant reduction in the levels of total cholesterol (-82%,  $P < 0.001$ ) and triglycerides (-70%,  $P < 0.001$ ) in these OHH rats. These effects were similar to those produced by taurine, the reference compound, which was less potent than the plant-extract, at the doses studied.

The relatively rapid onset of hypoglycemic action of a single oral dose of the plant-extract suggests any one or more of the following modes of action, some in common with taurine: a) direct insulin-mimetic effect, b) enhanced secretion of insulin from the  $\beta$ -cells of the pancreas, and c) increased tissue uptake of glucose by enhancement of insulin sensitivity (Nandhini et al., 2004; Ogawa et al; 2005; Kim et al., 2006). Some of these mechanisms have also been proposed for the hypoglycemic action of *Mormodica charantia* fruit (Miura et al., 2001; Rath et al., 2002).

The hypocholesterolemic activity of the extract after sub-chronic administration may be due to a number of mechanisms, some in common with taurine, including a) inhibition of HMG-CoA reductase, b) stimulation of cholesterol-7-alpha-hydroxylase (CYP7A1), which converts cholesterol into bile acids, and/or c) inhibition of cholesterol absorption from the intestine due to formation of complexes with compounds such as glycosides and saponins (Amin Riyad et al., 1988; Yokogoshi and Oda, 2002; Chen et al., 2004). A reduction in triglyceride levels may be due to decreased lipogenesis, increased lipolytic activity by inhibition of hormone-sensitive lipase (Al-Shamaony et al., 1994) or the lipogenic enzymes (Pari and Venkateswaran, 2004), and/or activation of lipoprotein lipase (Ahmed et al., 2001) as has been proposed for taurine (Yokogoshi and Oda, 2002; Chen et al., 2004) and some anti-diabetic plants (*Mormodica charantia* (Ahmed et al., 2001), *Artemisia herba alba* (Al-Shamaony et al., 1994) and *Ceasalpinea bondecella* (Sharma et al., 1997)] exhibiting hypolipidemic activity.

The active constituents of the *C. humilis* leaf extract responsible for the hypoglycemic and hypolipidemic actions are not known. However, these activities may be mediated by one or more of the following compounds identified in *C. humilis*: steroidal saponins [dioscin, methyl protodioscin, parisaponin Pb (Hirai et al., 1986)], flavones and flavonoids [tricin rutinoside, epicatechin (Harborne et al., 1974; Hirai et al., 1986; Harborne et al., 1998; Polya, 2003)], cyclitols [quercitol, 2-deoxy-D-chiroinositol (Polya, 2003)], and polysaccharides (Moyna and Fabio, 1984). Among the identified constituents of *C. humilis*, the flavonoid-polyphenol antioxidant epicatechin,

shown to have insulin-like activity (Ahmad et al., 1989), produced hypoglycemia in streptozotocin-induced diabetic rats (Quine and Raghu, 2000); it also exhibited antiatherosclerotic properties (Chyu, 2004). The flavonoid tricic has been shown to have antilipidemic and antioxidant activity (Duarte-Almeida et al., 2007). The cyclitol quercitol has glucosidase inhibitor activity which blocks the absorption and metabolism of carbohydrates (Ogawa et al., 2005). Some polysaccharides have hypoglycemic and hypolipidemic activities (Aларcon-Aguilar et al., 2000; Li et al., 2006).

The results of the study show that an aqueous extract of *C. humilis* leaves caused hypoglycemia in OHH *M. shawi* rats, thus, validating the traditional use of *C. humilis* in the treatment of diabetes in Morocco. The fact that continuous treatment for 30 days with the plant-extract caused a significant decrease in blood glucose levels of the hyperglycemic rats but not in the normoglycemic rats, is significant in that continuous use of the extract or the accidental overdose may not result in hypoglycemic shock, unlike with insulin or sulfonylurea drugs, which cause severe hypoglycemia when taken in excessive doses (Ferner, 1988).

Since, many antidiabetic drugs do not correct dyslipidemia, the observed hypocholesterolemic and hypotriglyceridemic effect of the plant-extract in these OHH rats, makes *C. humilis* quite important in the management of diabetes, since the plant-extract or component(s) of the plant may also reverse dyslipidemia associated with diabetes, and prevent the CV complications (Onat et al., 2006). Further investigations are needed to elucidate the mechanism(s) of action of *C. humilis*, especially bioactivity-guided fractionation, isolation and identification of the constituents of the plant extract responsible for the observed pharmacological activities. It is possible that *C. humilis* may become the source of valuable antidiabetic compound(s).

## ABBREVIATIONS

BW = body weight; *C. humilis* (*Chamaerops humilis*); *M. shawi* (*Meriones shawi*); SEM (standard error of measurement); CV = cardiovascular

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