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Some Pharmacological Studies on *Teucrium mascatense:* Effect on Glucose Homeostasis in Normal and Streptozotocin Diabetic Rats and Antimicrobial Activity

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ABSTRACT. The effect of *Teucrium mascatense* water extract on glucose homeostasis, at different doses, was studied in normal and streptozotocin diabetic rats. *T. mascatense* had no effect on fasting glucose levels and on the oral glucose tolerance test on either normal or diabetic rats. Chronic administration of *T. mascatense* in drinking water at a concentration of 0.25 % for 37 days did not have any effect on the parameters of glucose homeostasis studied (plasma glucose and fructosamine, body weight and feed and fluid intake) during the normal and diabetic phase of the experiment. When administered for a similar time at a concentration of 1.0 % in drinking water, however, *T. mascatense* significantly reduced food and fluid intake, body weight and at times plasma glucose concentration. At both concentrations used, *T. mascatense* appeared to be non-toxic.

The antimicrobial activity of the chloroform and methanol extract of *T. mascatense* indicated that the latter extract showed a wide range of high activity against the microbes tested.

Phytochemical screening showed that *T. mascatense* contains appreciate amounts of saponins and moderate amounts of terpenes and/or sterols and flavonoids.

The use of plants in folk medicine is practiced extensively worldwide. It has been estimated that about 25% of all prescription drugs in industrialized countries contain active components obtained from higher plants (Farnsworth and Bingel 1977, Jaroszewski 1984). It is paradoxical however, that despite the use of plants remedies as tradition antidiabetic treatments since ancient times (Ajgaonkar 1979.) and despite the fact that over 400 plants are reported to posses antidiabetic effect (Ajgaonkar 1979, Bailey and Day 1989, Ivorara *et al.* 1989) yet no specific compound has been isolated and dispensed as a hypoglycaemic drug.

Type 2 (NIDDM, non-insulin dependent diabetes mellitus) is among the most common disorders in developed and developing countries (Zimmet 1982). For this reason The World Health Organization has recently recommended that the use of traditional plant treatments for diabetes mellitus should be encouraged especially in developing countries where conventional treatment of diabetes mellitus is not always available (1980).

Teucrium mascatense Boiss. is a dense compact perennial herb. It is fairly widespread at the upper mountain elevations especially in Khor Fakann area (U.A.E.). The plant is a member of the Labiatae, a large family of some 3000 species arranged in about 200 genera. The genus *Teucrium* is made up of approximately 300 species of herbs and shrubs of varying habits, native to the temperate and warmer parts of the world.

The plant is used by people of the region for diabetes mellitus, however it has not received any scientific or medical scrutiny that we are aware of. On the other hand *T. polium*, and *T. oliverianum* were reported to posses hypoglycaemic effects (Gharaibeh *et al.* 1988, Ajabnoor *et al.* 1984). Similarly, there is no antimicrobial or phytochemical studies on *T. mascatense*. Iridoids (Fikenscher and Hegnauer 1969), triterpenes (Anderson *et al.* 1979), falvonoids (Brieskorn and Biechele 1969, Oganesyan and Mnutaakanyan 1985), volatile oils (Despina and Beasiere 1985) and diterpenes, (Node *et al.* 1974) were , however, reported from other *Teucrium* spp. In the present study, normal and streptozotocin diabetic rats were used to evaluate the acute and chronic effect of *T. mascatense* water extract on glucose homeostasis. The extract was administered to experimental animals at a dose comparable to that used by diabetics in the region. In addition, phytochemical and antimicrobial activity of T. mascatense were also evaluated.

Materials and Methods

Animals

Adult male Wistar rats weighing 220 ± 20 g (mean ± S.D.) were used. They were housed in an airconditioned room (25 ± 2 °C) and supplied with standard pellet diet (Abu Dhabi Flour and Animal Feed Factory, Abu Dhabi, U.A.E.) and water ad lib.

Plant Material

T. mascatense used in this study was collected from Khor Fakann area (U.A.E.). The plant was botanically authenticated and herbarium specimens were deposited at the National Herbarium, Desert and Marine Environment Reserch Center, United Arab Emirates University.

Preparation of Plant Extract

a. Coarsely powdered aerial parts of *T. mascatense* (200 gm) were macerated with distilled water (600 ml) for 24 hours with occasional shaking. The extract was filtered and freeze dried using Christ LDC-2 freeze dryer (yield 4.38 %).

b. Extracts used as drinking water were prepared using maceration technique (10 % concentration). The extract was filtered. The filtrate was freeze dried till use which was diluted to the desired concentration with drinking water.

c. For antimicrobial activity, 10 gm of the coarsely powdered aerial part of the plant was extracted successively with chloroform and methanol for 24 hours with a soxhlet extractor. The extracts were separately evaporated under vacuum and residue was weighed. Each residue was then dissolved or suspended in the same extracting solvent to a final concentration of 0.1 gm/ml.

Induction of Experimental Diabetes

Streptozotocin (Sigma Chemical Company, Poole, Dorset) (20 mg/ml) was dissoloved in citrate buffer (0.01 M, pH 4.5) immediately before administration. The drug was administered in a dose rate of 50 mg/kg body weight via a lateral tail vein under light ether anaesthesia. The animals were used 48 hours later for the acute experiments.

The effects of T.mascatense on fasting glucose levels:

The acute effects of *T. mascatense* on fasting glucose levels was studied in normal and diabetic rats. Animals were fasted for 15-18 hours but were allowed access to water. Initial blood samples from tail-tips were taken from each rat by heparinised capillary tubes for determination of basal glucose levels. Blood samples were then taken at 1, 2, 3 and 4 hours following dosing with *T. mascatense* at different doses vize; 2, 100 and 200 mg/ 200 gm body weight or with an equivalent volume of noramal saline for glucose analysis.

The Effect of *T.mascatense* on Oral Glucose Tolerance Test (OGTT)

Normal and diabetic rats were fasted for 15-18 hours but were allowed access to water. *T.mascatense* 100 and 200 mg/200 gm body weight dissolved in 1.0 ml distilled water or an equivalent amount of normal saline was given orally simultaneously with the glucose load (1.0 gm/kg body weight). Blood samples were taken as in previous experiment before dosing and every half hour for two hours and was treated as in previous experiment for glucose analysis.

Chronic Effects of *T.mascatense*

In this experiment, two groups of 8 rats each were used. After a 5 day run-in period, one group received a decoction of *T.mascatense* as 0.25% of plant material in lieu of drinking water. Treatment was maintained through out the experiment (37 days). The other group received ordinary tap water. Food was allowed ad lib. Diabetes was induced on day 8 by administration of streptozotocin. Body weight, food and fluid intake were recorded daily. Blood samples were obtained from the tail-tips at regular intervals for the determination of plasma glucose, aspartate amino transferase (GOT), urea, creatinine, total cholesterol and fructosamine. The same experiment was repeated in two groups of rats, but *T. mascatense* was given as a 1.0 % decoction.

Toxic Effects of *T.mascatense*

At the end of the experimental periods of the chronic experiments, animals were anaesthatised with ether, blood was collected from the posterior vena cava in plain vacutainers. It was allowed to clot and serum was separated by centrifugation at 3000 g for ten minutes and were stored in a deep freezer at - 20°C. Sera were analyzed within 3 days for the activities of GOT, alnanine aminotransferase (GPT), gamma glutnyl transferase (GT), creatinine kinase (CK), alkaline phosphatase (ALP), and for the concentrations of glucose, creatinine, urea, total protein, toal bilirubin (TB), Ca, Mg and P.

Blocks of livers, intestines, spleen, kidneys, lungs, heart, spinal cord and brain were fixed in 10 % formal saline and paraffin sections 6 um thick were stained with haematoxylin and eosin.

Antimicrobial Activity

Laboratory strains of *Staphyloccocus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Aspergillus terreus and A. flavus* were used in this study. Antimicrobial activity was determined by Reeves modified method (1989); where plates of brain hearts infusion agar (oxoid) and Sabouraud dextrose agar (oxoid) were selected to be used with bacteria and fungi respectively. Cups were made on each plate using a sterile sharp cork borer. The plates were inoculated with 0.2 ml of the plant extract. Triplicates of each extract were carried out.

The plates were incubated at 37°C for 24 hours for bacteria and for 3 days for fungi. Diameters of the inhibition zones developed around the cups were measured and considered as a criterion for antimicrobial activity. The mean

diameter of the inhibition zones were calculated. Solvents used to dissolve or suspended extracts were used as control.

Phytochemical Screening

The aerial part of the plant was phytochemically screened for terpenes and/or sterols, alkaloids, saponins, anthraquinones, flavonoids, tannins and cardiac glycosides using the methods described by El-Tawil (1983).

Chemical Methods

The concentrations of glucose, fructosamine, creatinine, urea, total protein, TB, Ca, Mg, P and the activities of GOT, GPT, CK, GT and ALP were measured by Cobas Fara II autoanalyser (Roche, Switzerland) using kits supplied by the manufacturer. All chemical analysis were carried out in serum or plasma after appropriate calibration of the instrument. Statistical significance was assessed by Student's t− test (Brownlee 1965).

Results

T. mascatense had no effect on fasting glucose levels in either normal or diabetic rats at all doses tested (Table 1). Similarly, at both doses tested, *T. mascatense* failed to significantly improve the OGTT in normal or diabetic rats (Table 2).

Chronic administration of *T. mascatense* did not alter any of the parameters measured (basal glucose, body weight, food and fluid intake) during the 8 days of administration to normal rats (Fig. 1 A). Administration of streptozotocin on day 8 resulted in the characteristic symptoms of diabetes viz; hyperglycaemia, hyperphagia, polydipsia and loss of body weight. (refer to values before and after streptozotocin administration, Fig. 1 A & B). Treatment with *T. mascatense* for 31 days did not significantly affect the parameters of glucose homeostasis when compared to the control group. In the group receiving *T. mascatense* at a concentration of 1.0 % , however, there was a significant reduction in food intake when compared to control values (Fig. 1 B), Which was paralleled by significant reductions in body weights and fluid intakes. In the treated group, glucose values was significantly lower at days 12 and 15 when compared to control values.

The serum concentration of fructosamine on the 5 th week was significantly higher than their corresponding values in week 1. (Table 3). There was no significant difference of fructoseamine between the two groups in either week 1 or week 5.

Table 1. The effect of *T. mascatense* on fasting Glucose levels in normal and streptozotocin diabetic rats:

Glucose (mg/dl)
Hours after dosing

	N	Dose mg/200/gbw	0	1	2	3	4
Normal Rats							
Control	8	0	107 ± 9	110 ± 5	106 ± 4	110 ± 5	104 ± 6
T. mascatense	7	2	95 ± 4	100 ± 5	95 ± 6	95 ± 4	99 ± 3
	8	100	133 ± 8	135 ± 6	129 ± 7	137 ± 6	130 ± 7
	8	200	106 ± 8	104 ± 9	106 ± 5	105 ± 6	108 ± 6
Diabetic Rats							
Control	8	0	450 ± 22	465 ± 24	457 ± 30	471 ± 25	461 ± 27
T. mascatense	8	100	479 ± 30	467 ± 33	433 ± 29	433 ± 24	438 ± 18
	8	200	469 ± 19	484 ± 21	442 ± 23	432 ± 19	433 ± 18

Values are means ± S.E.M. Streptozotocin was administered 48 hours before experiment at an I/V dose of 50 mg/kg body weight (see text). No significant difference was seen for *T. mascatense* or control group at any time after dosing when compared to its zero time value.

Administration of *T. mascatense* for 37 days did not have any affect on the activities of GOT, GPT, GT, CK, ALP or in the serum concentrations of glucose, urea, creatinine, cholesterol, total protein, T.B., Ca and Mg (data not shown).

Histological sections of all organs studied did not show any detrimental effects of *T. mascatense*.

The antimicrobial activity of the chloroform and methanol extract are shown in Table 4. The methanol extract showed a wide range of high activity against the microbes tested.

Phytochemical screening showed that *T. mascatense* contains appreciable amounts of saponins and moderate amounts of terpenes and/or sterols and flavonoids.

Discussion

The present study has investigated the possible hypoglycaemic effect of *T. mascatense* using the streptozotocin diabetic rat, which at the dose tested (50 mg/kg body weight), serves as a model of hypoglycaemia and insulin resistance (Chang *et al.* 1983). The results of the current investigation indicated lack of effect

of *T. mascatense* on the parameters of glucose homeostasis. This was true for the acute experiments (effect on fasting glucose and OGTT) or with the chronic experiment (lower dose; 0.25 % in drinking water) which suggest lack of extrapancreatic effect of *T. mascatense* as the streptozotocin diabetic rat is non-responsive to insulin secretagouges like tolbutamide and RM-11894 (Tutwiler *et al.* 1978). Although it can not be completely ruled out, it is highly unlikely that

Table 2. The effect of *T. mascatense* on OGTT in normal and streptozotocin diabetic rats

Glucose (mg/dl)
Minutes after dosing

	N	Dose mg/200/gbw	0	30	60	90	120
Normal Rats							
Control	5	0	98 ± 4	181 ± 5	134 ± 7	115 ± 4	108 ± 3
T. mascatense	7	100	119 ± 8	163 ± 10	151 ± 6	130 ± 7	121 ± 5
	7	200	111 ± 9	164 ± 4	159 ± 5	151 ± 4	134 ± 3
Diabetic Rats							
Control	7	0	437 ± 14	614 ± 15	566 ± 17	517 ± 21	485 ± 18
T. mascatense	7	100	447 ± 17	580 ± 14	556 ± 19	510 ± 21	503 ± 22
	7	200	379 ± 13	558 ± 14	567 ± 15	451 ± 17	507 ± 17

Values are means ± S.E.M. Treatment was given simultaneously with glucose load (1g/kg). Streptozotocin (50 mg/kd) was given 1/V 48 hours before experiment. No significant effect was seen for *T. mascatense* when compared with Control group.

Table 3. The effect of *T. mascatense* as a decoction (0.25 and 1.0 %) in place of drinking water on plasma fructosamine and glucose concentrations in non-diabetic (week 1) and diabetic (week 5) rats.

Concentration of *T. mascatense*	Week 1		Week 5	
	Glucose (mg/dl)	Fructosamine (ug/dl)	Glucose (mg/dl)	Fructosamine (ug/ml)
0.25 %	120 ± 5	237 ± 18	627* ± 41	486* ± 29
Control	122 ± 4	228 ± 21	615* ± 25	436* ± 24
1.0 %	120 ± 7	188 ± 19	602* ± 36	340* ± 33
Control	125 ± 8	191 ± 17	625* ± 31	334* ± 27

Values are mean ± SEM. At each week there was no significant difference between the *T.mascatense* treated group with its own control group. Significant differences (*, $P < 0.005$) was observed between week 5 and week 1 corresponding values.

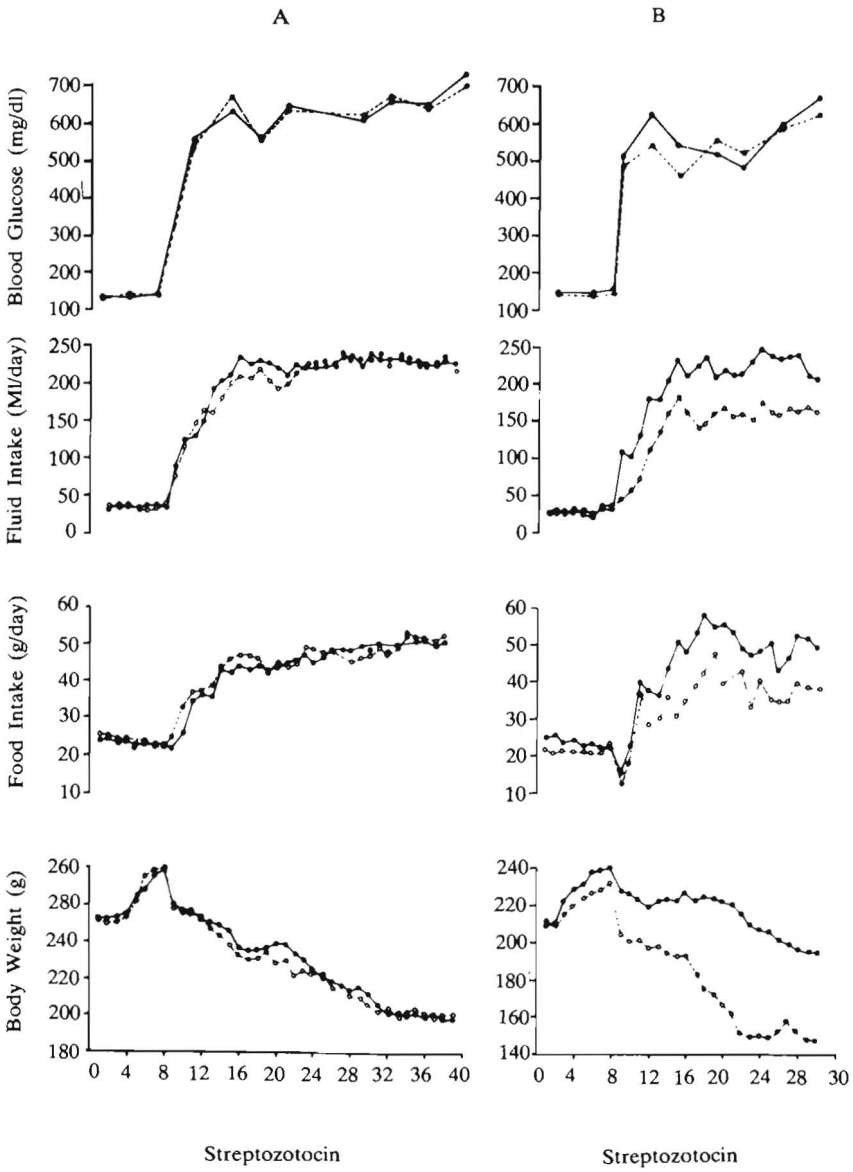

Fig. 1. Effect of *T. mascatense* as a decoction 0.25 % (A) and 1.0 % (B) w/v in place of drinking water on plasma glucose, body weight, food and fluid intake in non-diabetic (day 0-8) and streptozotocin diabetic (day 8-37) rats. Control , *T.mascatense* values are mean ± SEM of group of 8 rats (A) and group of 5 rats (B).

Table 4. Antimicrobial activity of the chloroform and methanol extract of *T. mascatense*

Extract	*S.aureus*	*B.subtilis*	Diameter of inhibition zone (m.m.)			*A.terreus*	*A.flavus*
			E.coli	*P.aeruginosa*	*C.albicans*		
Chloroform	–	–	14	13	11	–	–
Methanol	12	22	14	19	15	12	–

These values are the mean of three determinations.

T. mascatense would be effective in animals with partially functioning β cells of the pancreas since *T. mascatense* was ineffective in normal rats. Oral hypoglycaemic agents like tolbutamide and glibenclamide are very effective in reducing fasting glucose levels in normal rats.

It is of interest that when *T. mascatense* was allowed in drinking water at a concentration of 1.0 % it had a significant anorexic effect which was accompanied by significant weight loss and fluid intake. Indeed at times, the blood glucose levels was also lower than control values. This anorexic effect might explain the claimed beneficial effect of the plant in diabetics. It is well established that dietary control is the cornerstone of the management of diabetes, irrespective of the severity of the symptoms. Treatment regimens that have been proved to be effective include diet in combination with exogenous insulin or orally effective hypoglycaemic drugs. The reduction of glucose which we have observed in the rats, although statistically significant, is unlikely of any physiological significance. This is based on the fact that fructosamine concentration, a parameter which is accepted to reflect the overall metabolic control of diabetes over the preceding weeks, was not significantly reduced in the treated group when compared with the control group (Table 3) which suggests erratic control of diabetes. Indeed when glucose levels in the treated group were significantly lower than control values, the values still exceeded 450 mg/dl. Another important fact which undermine the beneficial effect of *T. mascatense* in diabetic rats is that there was continuous body weight loss a phenomenon which usually reflects poor control of diabetes.

Other species of Teucrium were reported to posses antidiabetic effect. Recently Gharaibeh *et al.* (1988) reported a hypoglycaemic effect of *T. polium* in streptozotocin diabetic rats given intravenously or intraperitoneally 5 ml of a 20 % decoction. The authors suggested that the effect could be explained on a peripheral enhancement of glucose metabolism rather than to increased insulin secretion from the pancreas. *T. polium* was also ineffective when given orally to normal or diabetic rats. it is interesting that they have also observed an anorexic effect of *T. polium* 24 hours after the intraperitoneal administration.

T. oliveranium has also been reported to posses a dose dependent hypoglycaemic effect in alloxan diabetic mice, but not in normal mice, with a decoction or an alcoholic extract when administered orally (Ajabnoor *et al.* 1984). These differences in results obtained by us when compared to others could be attributed to the species of the plant used and/or to the experimental design and animal model. It is interesting that *T. scorodonia* was reported to accelerate intestinal movements and to reduce bile secretion in experimental animals (Neumann 1965). These effects will clearly shorten transit time of food and reduce fat absorption from the small intestine respectively. If these effects were to occur with *T. mascatense*, they might alos contribute to the weight loss observed in our animals.

The high antimicrobial activity of the methanol extract could be related to the presence of terpenes and/or flavonoids since the latter two, among other compounds, were reported to posses antimicrobial activity (Rios *et al.* 1987, Habtemariam *et al.* 1990).

It is concluded that *T. mascatense* lacks a direct effect on fasting glucose levels and on the OGTT in normal and diabetic rats but it has an anorexic effect which might explain its claimed antidiabetic potential. The apparent lack of toxic effect of *T. mascatense* justifies further investigations on its anorexic and antimicrobial activity of the methanol extract.

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بعض الدراسات الفارماكولوجية لنبات الجعدة *Teucrium mascatense* وتأثيره على مستوى جلوكوز الدم في الجرذان السليمة والمصابة بداء السكر التجريبي وفعاليته ضد بعض الميكروبات

ابراهيم عبدالرحمن وصفي و أحمد خضر بشير و عبدالله أحمد عبدالله و محمد هادي أميري

مركز بحوث الصحراء والبيئة البحرية ـ جامعة الامارات العربية المتحدة
ص . ب : ١٧٧٧٧ ـ العين ـ دولة الامارات العربية المتحدة

تم دراسة المستخلص المائي لنبات الجعدة *(T. mascatense)*، بعدة جرعات، على مستوى جلوكوز الدم في جرذان سليمة وأخرى تم اصابتها بمرض السكر التجريبي بواسطة ستربتوزوتوسين (٥٠ ملغم / كلجم).

في التجارب الحادة لم يكن للمستخلص المائي لنبات الجعدة بجميع الجرعات التي جربت أي أثر معنوي على مستوى الجلوكوز في الدم أو على تحمل الجلوكوز في الجرذان السليمة أو المصابة بمرض السكر.

في التجارب المزمنة، تم تقديم المستخلص المائي لنبات الجعدة لمجموعة جرذان بنسبة ٠,٢٥ و٠,١ ٪ بدل ماء الشرب، بالاضافة إلى مجموعة ضابطة، ولمدة ٣٧ يوماً وتم إحداث مرض السكر التجريبي في اليوم الثامن من التجربة. هذا ولم يوجد أي تأثير فعال لنبات الجعدة حين قُدِّم بنسبة ٠,٢٥ ٪ على مستوى جلوكوز وفركتوس أمين الدم، ووزن الجسم وإستهلاك الطعام والماء. إلا أنه حين قُدِّم النبات بنسبة ٠,١ ٪ فقد لوحظ إنخفاض معنوي على كمية إستهلاك

الطعام، الماء ووزن الجسم وفي بعض الأحيان مستوى الجلوكوز في الدم أيضاً. كما لم توجد أي دلالات سمية للنبات في التجارب الحادة والمزمنة.

ولقد أشارت التجارب على فعالية مستخلص الميثانول على عدة ميكروبات تم دراستها، كما وأفادت نتيجة المسح الفايتوكيمائي على وجود كميات معقولة من السابونين وكميات متوسطة من التيربينات و/أو استيرولات وفلافينويدات.