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The potential protective effects of *Centaurium umbellatum* on hyperglycemia and lipid profile in streptozotocin-induced diabetic rats

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ABSTRACT

The aim of the present study was to explore the anti-diabetic activity of *Centaurium umbellatum* (CU) in streptozotocin (STZ)-induced diabetic rats. Diabetes was induced by a single intraperitoneal injection of STZ (65 mg/kg bw). Normal and diabetic rats were treated with CU aqueous leaf extract, at a dose of 200 mg/kg bw, for 4 weeks. Serum glucose and insulin concentrations, hepatic glycogen content, serum lipid profile and glucose tolerance test were performed. CU treatment significantly decreased serum glucose levels and improved glucose homeostasis in STZ-induced diabetic rats by stimulating the few surviving beta cells to release more insulin. The hepatic glycogen content was markedly increased by CU treatment in STZ-induced diabetic rats. The hypolipidemic action of CU leaf aqueous extract was confirmed by a significant decrease in the levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and an increase in high-density lipoprotein cholesterol (HDL-C) in (STZ + CU) group compared to STZ-induced diabetic rats. The present data suggested that *C. umbellatum* leaf aqueous extract has both hypoglycemic and hypolipidemic effects which can help the cure and management of diabetes.

KEYWORDS

Centaurium umbellatum;
Diabetes;
Biochemical parameters;
Rat.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder that constitutes a major public health problem throughout the world. Current estimation indicates that approximately 4% of the global population suffer from DM, a percentage which is expected to reach 5.4% in 2025[1]. This disease is a multifactor disorder associated with chronic hyperglycemia and some disruptions in carbohydrate, fat, and protein metabolisms emanating from defects in reactive oxygen species scavenging enzymes[2], high oxidative stress impairing pancreatic beta cells[3,4] and deficiencies or disruptions in insulin secretion[5].

The major role of insulin in diabetes is to maintain the whole body glucose homeostasis via glucose transporter 4 (GLUT4), expressed in adipose tissue, skeletal and cardiac muscles[6,7]. During insulin stimulation, intracellular vesicles that store GLUT4, translocate to the plasma membrane and facilitate glucose uptake[8,9].

Under diabetic condition, reduced expression of GLUT4 causes impairment of insulin signaling and stimulates glucose production in the liver. These alterations lead to high glucose concentrations in blood[10].

Several therapeutic strategies are currently available for the treatment of this chronic metabolic disorder, including the stimulation of endogenous insulin secretion, enhancement of insulin action at the target tissues, inhibition of dietary starch and lipid degradation and treatment with oral hypoglycemic agents[11]. The limitations associated with those therapeutic strategies have led to a determined search for more efficient and cost-effective alternatives. This trend has been further intensified by increasing doubts surrounding current dietary and other lifestyle behaviors together with growing interests in functional foods and nutraceuticals[12]. Complementary and alternative medicine applications have attracted special attention in recent research which they offer new promising opportunities for the development of efficient, side effect-free, and lower cost alternatives than synthetic hypoglycemic agents[13-15].

Of particular relevance to this argument, *Centaurium umbellatum* (CU), belonging in Gentianaceae family, is a medicinal plant used in numerous countries combined with other plants. As preliminary experiment, this medicinal plant (CU) has been reported to contain phenolic acids, natural drugs[16]. Studies in experimental animals have shown that plant mixture extract containing *Centaurium umbellatum* and other plants like *Vaccinium myrtillus* L. and *Taraxacum officinale* have a variety of pharmacological functions including antihyperglycemic, free radical scavenging and antioxidant activities[17-19].

To our knowledge, the potential protective effect of *C. umbellatum* on hyperglycemia and lipid profile in diabetes has not been explored. Based on the above information, we hypothesize that CU can reduce diabetic disruptions. The aim of the present work is to study the protective effects of CU in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Plant material and extracts preparation

Centaurium umbellatum (CU) plants (family: *Gentianaceae*) were collected from North Tunisia during May and June (2009). According to the flora of Tunisia botanical identification was carried out by Doctor Abdelhamid Nabli, Professor Emeritus at the Faculty of Sciences, University of Tunis El Manar. Voucher specimen of *C. umbellatum* was deposited at the Faculty of Pharmacy (Monastir, Tunisia). Leaves of the plant (CU) were washed quickly in running water, dried in an oven at 40°C and then finely powdered in a Willey mill. The powder was extracted with distilled water (50 g powder/500 ml water) by boiling under reflux for 20 min. The decoction obtained was centrifuged, filtered, frozen at -20 °C and then lyophilised (Free Zone® Dry 4.5, USA) to yield approximately 10% (w/w) of the tansy extract, and was stored at -20 °C until used.

Animals

Adult male rats of Wistar strain, weighing about 240 g, were obtained from the Central Pharmacy (SIPHAT, Tunis, Tunisia). The animals were maintained at 22 ± 3 °C, 45–75% humidity, 12 h light–dark cycle, and kept to acclimate for 1 week before the onset of experiment. They were fed a commercial standard pellet diet (SICO, Sfax, Tunisia) and provided with water *ad libitum*.

The experimental protocol was approved by the Local Animal Care Committee at Sfax University. All the experimental procedures were carried out in accordance with international guidelines for Care and use of laboratory animals[20].

Induction of diabetes mellitus

Rats were made diabetic by a single intraperitoneal injection of streptozotocin (STZ) at a dose of 65 mg/kg bw diluted immediately before injection in citrate buffer (0.1 M, pH 4.5). Three days after STZ injection, diabetes was confirmed in the overnight-fasted rats by measuring blood glucose concentration. The rats with glycemia above 250 mg/dl were considered to be diabetic and they were included in the study. Treatment with plant extracts was started on the third day after STZ injection and continued for 30 days.

Experimental design

The rats were randomly divided into six groups of seven animals each as follows: control rats (C) received vehicle only; diabetic rats (STZ) received a single dose of STZ (65 mg/kg bw); CU-treated diabetic rats (STZ

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+ CU) received daily CU (200 mg/kg bw) for a period of 30 days; (C + CU) CU-treated control rats received daily only CU extract (200 mg/kg bw) for a period of 30 days. All injections were made by intraperitoneal way.

On the last day of the experiment, animals were sacrificed by decapitation to avoid stress. Blood samples were collected without heparin for biochemical estimations. Liver tissue was removed, cleaned and washed in ice-cold saline solution. The median portions were drawn, weighed and homogenized with phosphate buffer (0.1 M, pH 7.4), centrifuged at 3000 × *g* and the supernatants were collected for biochemical assays.

Biochemical assays

(a) Determination of glucose and hepatic glycogen levels

Serum glucose levels were assayed by an enzymatic method, using commercial reagent kit (ref. 20121) purchased from Biomaghreb (Ariana Tunis, Tunisia). Hepatic glycogen content was determined by Orthotoluidine reagent and measured at 620 nm using a spectrophotometer[21].

(b) Estimation of insulin concentration

Serum insulin level was determined using rat Insulin enzyme-linked immunosorbent assay (ELISAT) kit Ref. RIT-461 N_ AKRIN-010T (Shibayagi Co., Ltd., Japan).

(c) Analysis of lipid parameters

Serum lipid parameters such as total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) levels were determined using commercial reagent kits from Biomaghreb Diagnostic (Ariana, Tunisia) (ref. 20111; 20131; 20113, respectively). The low-density lipoprotein cholesterol (LDL-C) fraction was determined according to the Friedewald equation[22]: LDL-C = TC - (Triglycerides/5 + HDL-C).

(d) Glucose tolerance test (GTT)

GTT was conducted in control and treated rats, 24 h before sacrifice day. Blood samples were collected from the tail vein of control and experimental rats, which were fasted overnight to obtain baseline blood glucose levels. Subsequently, rats of both control and experimental groups were injected intraperitoneally with glucose (2 g/kg bw). Blood was collected at an interval of 30 min up to 2 h for glucose estimation using a glucometer (Esprit 2, BAYER, France).

Statistical analysis

The data were analyzed using the statistical package program Stat view 5 Software for Windows (SAS Institute, Berkley, CA). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) test as a post hoc test for comparison between groups [(STZ, STZ + CU, C + CU) vs. (C)] and [STZ + CU] vs. [STZ]. All values were expressed as means ± S.E. Differences were considered significant if $p < 0.05$.

RESULTS

Serum glucose levels and water consumption

As shown in TABLE 1, serum glucose levels of the diabetic rats remained high throughout the experiment period compared to those of control group. The administration of *C. umbellatum* to diabetic rats reduced serum glucose levels by 59% without reaching the normal value after 30 days. Treatment with CU alone did not show any change in this parameter, when compared to control group.

One week after STZ injection, water consumption significantly increased by 58% in diabetic rats compared to that of control group. It continued to be high throughout the experiment period. The administration of *C. umbellatum* countered the rise of water consumption. This effect was more pronounced at one week of treatment. Treatment with CU alone had no effects on this parameter when compared to that of control group (TABLE 1).

Insulin level and hepatic glycogen content

Insulin levels in streptozotocin-induced diabetic rats were reduced significantly by 65% compared to those of controls. The administration of *Centaurium umbellatum* aqueous extract to diabetic rats increased significantly serum insulin levels by 44%. No significant changes were observed between control and (C + CU)-treated groups (TABLE 2).

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TABLE 1 : Blood glucose level (mg/dl) and water consumption (ml/day/rat) at 2, 7, 14, 21 and 30 days after daily administration of CU aqueous extracts in rats control and experimental groups.

Groups	Control (C)	Diabetic (STZ)	Diabetic + CU (STZ + CU)	Control + CU (C + CU)
Blood glucose [a]				
2nd day (initial state)	91 ± 6	255 ± 4 ***	259 ± 5 ***	87 ± 3
7th day	87 ± 2	285 ± 1 ***	188 ± 3 ***++	90 ± 4
14th day	94 ± 4	302 ± 7 ***	129 ± 5 +++	97 ± 1
21th day	110 ± 1	341 ± 5 ***	141 ± 6 *+++	98 ± 3
30th day (final state)	98 ± 3	344 ± 4 ***	139 ± 5*+++	97 ± 2
Water consumption [b]				
2nd day (initial state)	21.34 ± 2.61	40.67 ± 3.24 ***	31.52 ± 52 ***	26.63 ± 7.84
7th day	24.15 ± 2.29	57.11 ± 2.14 ***	37.08 ± 2.80 **++	24.13 ± 1.07
14th day	22.33 ± 2.70	60.21 ± 5.69 ***	45.03 ± 3.17 ***++	21.74 ± 3.44
21th day	19.65 ± 0.80	64.34 ± 2.60 ***	47.05 ± 1.90 ***++	23.08 ± 4.13
30th day (final state)	20.23 ± 1.13	67.12 ± 1.44 ***	49.11 ± 2.30 ***++	25.34 ± 2.13

Data are expressed as mean ± S.D. (n = 7); Treated groups (STZ); (STZ + CU); (C + CU) *vs* control group (C): $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; (STZ + CU) group *vs* (STZ) group: $^{++}p < 0.01$; $^{+++}p < 0.001$; [a]: (mg/dl); [b]: (ml/day/rat).

After STZ treatment, we found a reduction in liver weight by 34% and hepatic glycogen content by 71%. The Administration of CU to diabetic rats significantly reversed these changes to near-normal values. Control rats treated alone with *C. umbellatum* did not show any change in these parameters when compared to those of control group (TABLE 2).

TABLE 2 : Liver weight (g), liver glycogen content (mg/g) and serum insulin level (ng/ml) in adult rats (control and experimental groups) after 30 days of treatment.

Groups	Control (C)	Diabetic (STZ)	Diabetic + CU (STZ + CU)	Control + CU (C + CU)
Liver weight [a]	12.34 ± 1.07	8.05 ± 3.10 ***	10.23 ± 2.03 *++	12.03 ± 0.74
Liver glycogen content [b]	30.71 ± 3.45	4.15 ± 0.83 ***	28.15 ± 1.22 +++	29.13 ± 3.60
Serum insulin [c]	1.21 ± 0.07	0.42 ± 0.03 ***	0.75 ± 0.06 ***++	1.18 ± 0.05

Data are expressed as mean ± S.D. (n = 7); Treated groups (STZ); (STZ + CU); (C + CU) *vs* control group (C): $^{*}p < 0.05$; $^{***}p < 0.001$; (STZ + CU) group *vs* (STZ) group: $^{++}p < 0.01$; $^{+++}p < 0.001$; [a]: (g); [b]: (mg/g); [c]: (ng/ml).

Lipid profile

The effects of *Centaurium* leaf extracts on lipid parameters were presented in TABLE 3. Our results showed that the administration of STZ increased serum triglycerides (TG) and total cholesterol (TC) levels by 56 and 43% respectively, in comparison to control rats. The administration of *Centaurium umbellatum* extracts countered the significant rise in the levels of the parameters cited above.

There were also a significant decrease in serum HDL-C levels by 64% and an increase in serum LDL-C levels by 34% in diabetic rats when compared with those of control group. Administration of *C. umbellatum* leaf extracts to diabetic rats restored partially the levels of HDL-C and totally LDL-C when compared to those of controls. No significant changes were observed between control and (C + CU)-treated groups.

TABLE 3 : Triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels in the serum of adult rats (controls and experimental groups) after 30 days of treatment.

Groups	Control (C)	Diabetic (STZ)	Diabetic + CU (STZ + CU)	Control + CU (C + CU)
TG [a]	103.71 ± 3.04	234.42 ± 7.94 ***	108.75 ± 3.64 +++	101.17 ± 2.56
TC [b]	110.54 ± 1.81	193.75 ± 2.45 ***	118.36 ± 1.79 +++	105.83 ± 3.58
HDL-C [c]	37.41 ± 3.18	13.25 ± 2.10 ***	24.53 ± 3.24 **++	36.78 ± 5.32
LDL-C [d]	120.34 ± 6.23	182.65 ± 3.19 ***	128.32 ± 6.01 +++	122.68 ± 4.20

Data are expressed as mean ± S.D. (n = 7); Treated groups (STZ); (STZ + CU); (C + CU) *vs* control group (C): $^{**}p < 0.01$; $^{***}p < 0.001$; (STZ + CU) group *vs* (STZ) group: $^{++}p < 0.01$; $^{+++}p < 0.001$; [a, b, c,d]: (mg/dl).

Glucose tolerance test

The effects of *Centaurium umbellatum* aqueous extracts on glucose tolerance test (GTT) were presented in TABLE 4. Blood glucose levels in diabetic rats reached a peak 1 h after glucose (2 g/kg bw) administration. Although the glucose levels started to decline, they continued to be high after 2 h. In diabetic rats treated

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with *C. umbellatum*, a significant decrease by 59% in glucose levels was observed at 120 min compared to the values obtained at 30 and 60 min.

TABLE 4 : Glucose tolerance test (GTT) in adult rats (control and experimental groups) after 30 days of treatment.

Groups	Blood glucose level [a]			
	0 min	30 min	60 min	120 min
Control (C)	92 ± 1	196 ± 11	123 ± 3	106 ± 2
Diabetic (STZ)	258±15 ***	358±14 ***	375±13 ***	335±7 ***
Diabetic + CU (STZ + CU)	130±4 ** +++	200±3 ** +++	163±8 ** +++	138±2 * +++
Control + CU (C + CU)	86 ± 2	177 ± 12	122 ± 2	87±1 ***

Data are expressed as mean ± S.D. (n = 7); Treated groups (STZ); (STZ + CU); (C + CU) *vs* control group (C): $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; (STZ + CU) group *vs* (STZ) group: $^{+++}p < 0.001$; [a]: (mg/dl).

DISCUSSION

This study was undertaken to determine if a daily intraperitoneal administration of *Centaurium umbellatum* leaf extract could attenuate diabetic disruptions in adult rats. To our knowledge, this is the first study showing the hypoglycemic and the hypolipidemic effects of *C. umbellatum* on streptozotocin-induced diabetic rats.

In our experiment, we observed a significant increase in the levels of blood glucose and water consumption in streptozotocin-induced diabetic rats. Previous findings have reported that STZ induced *diabetes mellitus* (DM) and insulin deficiency lead to increase blood glucose[23], by inducing necrosis of pancreatic beta cells, thus causing hypo-insulinemia and hyperglycemia[24]. The treatment of diabetic rats with the aqueous leaf extracts of *Centaurium umbellatum* significantly decreased blood glucose and water consumption levels. In consistence with the present data, a number of other plants have been observed to have similar pattern of hypoglycemic effects[25]. *C. umbellatum* extracts could exert its hypoglycemic activity by stimulating the few surviving beta cells to release more insulin rather than by aiding the regeneration of pancreas necrotic beta cells. These abilities might be accomplished by the presence of phenolic acids in CU extracts, as previously reported by Hatjimanoli and Debelmas[16]. Thus could likely explain the significant increase in the serum insulin levels, observed in the current study, when streptozocin-induced diabetic rats were treated with *C. umbellatum* for 30 days.

In addition, glycogen is the primary intracellular storable form of glucose. Its level in various tissues especially in liver indicates a direct reflection of insulin activity, since it regulates glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Since, STZ caused a selective destruction of pancreatic beta cells resulting in a marked decrease of insulin levels, it could be predicted that glycogen levels in tissues like muscle and liver decreased, as the influx of glucose in the liver was inhibited in the absence of insulin and recovered on insulin treatment[26-28]. Our results showed that, upon treatment of diabetic rats with *C. umbellatum* leaf extracts, there was a significant elevation in both liver weight and hepatic glycogen content. These effects could be attributed to the presence of phenolic acids in CU extracts. In fact, according to Jung *et al.*[29], the phenolic acids were found to elevate a glucokinase activity and a production of glycogen in the liver in type 2 diabetes. Our results corroborated with previous studies[30,31] which have found an improvement of diabetic status after herbal plant extract treatment.

Another important aspect to be discussed in our study was the altered lipid metabolism, which was often linked with DM. It is well known that insulin activates enzyme lipoprotein lipase, which hydrolyzes triglyceride under normal conditions[32]. The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma[33]. Moreover, it has been demonstrated that insulin deficiency in diabetes leads to a variety of disruptions in metabolic and regulatory processes, which in turn lead to accumulation of lipids[34]. In our study, we observed a significant increase in TC, TG, LDL-C and a decrease in HDL-C levels in the serum of streptozotocin-induced diabetic rats. On the other hand *Centaurium umbellatum*, produced a favorable effect on these parameters, indicating its lipid lowering activities. Thus might partly be due to the insulin stimulatory effect of this plant and to the low secretion of cholesterol biosynthesis enzymes.

Additionally, the *C. umbellatum* extract also significantly decreased the blood glucose level in glucose loaded rats (GTT) and this fact could be attributed to the potentiation of serum insulin effect by increasing the

pancreatic secretion of insulin from surviving beta cells or its release from bound insulin. In this context, a number of other plants have been observed to have similar pattern of hypoglycemic effects[35]. This spoke in favor of the view that *C. umbellatum* could play an important role for the treatment of diabetic and hyperlipidemic patients.

In conclusion, our study demonstrates that *Centaurium umbellatum* leaves can be used to treat hyperglycemia and hyperlipidemia of *diabetes mellitus*. This plant may be developed as an oral hypoglycemic agent or as a functional food for diabetic patients with hyperlipidemia and for persons with high risk of diabetes. Consequently, consumption of *C. umbellatum* leaves may prevent the complication of hyperglycemia associated with diabetes. Finally, the precise mechanism (s) and site (s) of its activity and the active constituent (s) of *C. umbellatum* leaves still need to be determined in addition to toxicological studies in further experiments.

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