

HYPOGLYCEMIC EFFECT OF *CLEOME DROSERIFOLIA* ETHANOLIC LEAF EXTRACT IN EXPERIMENTAL DIABETES, AND ON NON-ENZYMATIC ANTIOXIDANT, GLYCOGEN, THYROID HORMONE AND INSULIN LEVELS

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Key words: diabetes mellitus, *Cleome droserifolia*, antioxidant, biochemical aspects

The major conclusions to be drawn from the present study are that the insulin level increase by C. droserifolia extract could be secondary to its antioxidant properties.

SUMMARY

Cleome (C.) droserifolia has been mentioned in Egypt as a traditional medicine to be of value in the treatment of diabetes mellitus. The study was designed to examine the hypoglycemic effect C. droserifolia leaf ethanolic extract in normal (normoglycemic) and in alloxan-treated diabetic mice. At a dose of 0.31 g kg⁻¹ i.p., C. droserifolia reduced the mean basal blood glucose concentrations of fasted normal mice by 6.8% on day 30 of the experiment. C. droserifolia significantly increased glycogen content by 48.2% as compared to diabetic group. Serum albumin alpha globulin and beta globulin concentrations were significantly decreased (by 30.7%, 30.9% and 70.3%, respectively) in diabetic group (p<0.001) as compared to control mice. Hepatic glutathione levels were significantly elevated in the treated group as compared to diabetic mice. Significant increases in the levels of T₃, T₄, and T₃/T₄ ratio were observed following treatment with C. droserifolia (p<0.001). Insulin was significantly decreased (p<0.0001) in diabetic mice and increased upon treatment with C. droserifolia (p<0.01). Ethanolic extract not only exhibited antihyperglycemic properties but also reduced oxidative stress in alloxan-induced diabetic mice while enhancing insulin release.

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INTRODUCTION

Diabetes is a serious metabolic disorder with micro- and macrovascular complications that result in a significant morbidity and mortality. The increasing proportion of the aging population, consumption of calorie rich diet, obesity and sedentary lifestyle have led to a tremendous increase in the number of diabetics worldwide (1). Although providing good glycemic control, current therapies do little in preventing complications. Besides this, these drugs are associated with side effects. Thus, it is necessary to continue looking for new and if possible more efficacious drugs. Diabetes mellitus (DM) is a chronic metabolic condition characterized by disorder of glucose homeostasis. Numerous experimental and clinical observations have indicated that hyperglycemia may directly or indirectly contribute to excess formation of free radicals.

Cleome (C.) droserifolia, family Capparaceae, grows in different regions of North Sinai, Egypt (2). It also has a long history of medicinal use, especially in Sinai for the treatment of DM in individuals with non-insulin dependent diabetes (3). It has hypoglycemic properties as it significantly suppressed the rise in

peripheral blood glucose concentrations in albino rats (4). The methanol extract of *C. droserifolia* has two flavonoids as active components (5). The administration of crude plant extract of *C. droserifolia* significantly decreases glucose levels and proves some biochemical parameters in alloxanized rats (110 mg kg^{-1}). The efficacy of the extract was compared with melatonin treatment in rat (6). However, the duration of this study was only 21 days and the degree of hyperglycemia was only mild (200 mg dL^{-1}). Since diabetes is a chronic disorder requiring long-term therapy, there was the need to assess the effect of *C. droserifolia* over a longer period of time and in a more severe form of diabetes (plasma glucose $>300 \text{ mg dL}^{-1}$). Therefore, this study was undertaken to assess the effect of *C. droserifolia* on thyroid hormones, insulin, hepatic glycogen content, non-enzymatic antioxidants (hepatic glutathione and uric acid in serum) and total protein in alloxanized mice (165 mg kg^{-1}) over a 30-day period.

MATERIAL AND METHODS

Preparation and dose of extracts

The raw material was collected from Arish, North Sinai, Egypt, and authenticated to *C. droserifolia* (Forssk.) by Dr. W. Kamel. Voucher specimen was deposited at Department of Botany, Faculty of Science, Suez Canal University. The leaves and small branches of the plant were thoroughly washed with distilled water to remove dirt and soil. They were shade dried. The materials were coarsely powdered and extracted by cold percolation using 95% ethanol till exhaustion (7). These extracts were concentrated for further studies at reduced temperature and pressure in a rotary evaporator. The extract was evaporated to dryness to give crude ethanolic extract. Then, extracts were suspended in 1% Tween-80 for intraperitoneal (i.p.) administration. It was daily administered to mice at a dose of 0.31 g kg^{-1} body weight. This dose was determined according to preliminary studies that proved its effectiveness.

Chemicals and instruments

Alloxan induced diabetic animals: Aldrich Chemical Co.; Tween-80 synthesis grades: Stharlau Chemie S. A.; glutathione: Aldrich Chemical Co. Ltd., Germany; kit for total protein: Bio-Rad Laboratories, Hercules,

CA. USA; enzyme-linked immunosorbent assay kit: Merckodia AB, Uppsala, Sweden; electrolyte buffer (Tris-barbital-sodium barbital): Electra HR buffer Helena, Laboratories, U.K., Cat # 5805; Ponceau's fixative dye solution: Helena Biosciences, Sunderland, Tyne and Wear, SR 53XB; all other chemicals: E. Merck, Germany; spectrophotometer: UV-visible, Unicom, Helios Alpha-9423 UVA 1000K, England; homogenizer: Potter-Elvehjem; electric centrifuge: Remi Udyog, New Delhi; densitometer scanning: Tumor Marker Center, Faculty of Medicine, Suez Canal University.

Animals and experimental design

Thirty male Swiss albino mice, weighing 20-22 g, were used in the study. They were obtained from Experimental Research Center of Theodor Bilharz Institute, Cairo, Egypt. Before initiation and during the experiment, mice were fed standard chow and had free access to tap water. After randomization into various groups, the mice were acclimatized for two weeks in the new environment before initiation of the experiment.

All animals were randomly divided into three groups of ten animals each. Group I were given 0.31 g kg^{-1} of crude ethanolic extract and used as normal control mice (CNT). Group II and group III were made diabetic by a single intraperitoneal (i.p.) injection of alloxan and served as diabetic control (DCNT) and treatment group (CD), respectively. Daily treatment (crude ethanolic extract 0.31 g kg^{-1} , i.p.) was started from day 4 of alloxan administration for 30 days.

Diabetes developed 3 days of the intraperitoneal administration of a single dose of 165 mg kg^{-1} body weight of alloxan, freshly prepared in distilled water, to 16-hour fasted rats (8). Three days after alloxan administration, blood glucose levels were measured in all animals. The mice exhibiting plasma glucose levels over 300 mg dL^{-1} 3 days after alloxan administration were included in the study.

Sample collection

Blood samples of fasted rats were collected using capillary tubes (Micro Hematocrit Capillaries, Mucaps) introduced into the medial retro-orbital venous plexus under light ether anesthesia and with 0.1 M EDTA as

anticoagulant (9). Serum was separated in an electric centrifuge at 3000 xg for 10 min. In addition, liver was excised from each animal after sacrifice by decapitation, dissected into ice-cold saline and then thoroughly rinsed. The tissue was cut into fragments and homogenized to a known volume of certain buffer. Aliquots from the homogenate were used for further studies.

Glucose and protein determination

Blood glucose concentrations were measured using Glucometer Elite (10). Hepatic glycogen was measured according to the anthrone-H₂SO₄ method, with glucose as the standard (11). Total protein content of blood serum was determined using the Bio-Rad protein assay reagent.

Blood electrophoretic pattern

The technique of cellulose acetate zone electrophoresis was used to analyze albumin and globulin in serum (12). Sample electrophoresis in Tris-barbital-sodium barbital, pH 8.8, was performed for 25 minutes, with the current adjusted to 250 volts. Separated protein bands were visualized after being stained (Ponceau's fixative dye solution) for 5 minutes and rinsed in 95% glacial acetic acid:ethanol (3:7; V/V). Bands corresponding to albumin, α_1 -, α_2 -, β - and γ -globulins were quantified by densitometer detection.

Glutathione assay and uric acid determination

Hepatic levels of reduced glutathione (GSH) were determined (13). GSH in protein-free supernatant was determined at 412 nm and expressed in mg g⁻¹ tissue. The GSH concentrations of the samples were derived from the standard curve prepared using known amounts of GSH.

Serum uric acid was determined at 546 nm using the uricase-PAP enzymatic colorimetric method (14) and expressed in mg dL⁻¹.

Hormone determination

Plasma T₃ and T₄ were measured using the respective enzyme-linked immunosorbent assay (ELISA) kits (15,16). Plasma insulin concentrations were also estimated using an ELISA kit with rat insulin as standard (17).

Statistical analysis

All data were processed using the Microsoft SPSS version 11.0 software packages for statistical evaluation. Results were expressed as mean \pm SE. Results were analyzed for statistical significance by one way ANOVA followed by Tukey-Kramer multiple comparisons test.

RESULTS

Glucose levels on day zero showed no significant intra-group variation. Seventy-two hours after administration of alloxan, they increased approximately fourfold (p<0.0001) while remaining unchanged in non-diabetic controls (Table 1). The extract administration decreased plasma glucose levels by 41.1% on day 30 of the experiment (p<0.001).

Hepatic glycogen content decreased significantly by 56.6% in diabetic controls as compared to non-diabetic animals. *C. droserifolia* significantly increased glycogen content by 48.2% as compared to diabetic group (Table 1).

Serum total protein increased significantly upon *C. droserifolia* extract treatment as compared to diabetic group (Table 2). Serum albumin and globulin (alpha

Table 1. Effect of 30-day administration of *Cleome droserifolia* extract on glucose levels (mg dL⁻¹) in alloxan (165 mg/kg) diabetic mice

| Group | Glucose content (mg dL ⁻¹) | | | glycogen content (mg g ⁻¹) | |
|-------|--|------------------------|--------------------------|--|------------------------|
| | Basal | Day 0 | Day 30 | Day 0 | Day 30 |
| CNT | 77.4 \pm 6.8 (n=10) | 75.3 \pm 3.1 (n=10) | 72.1 \pm 1.4 (n=10) | 4.32 \pm 0.8 (n=10) | 4.40 \pm 0.6 (n=10) |
| DCNT | 76.5 \pm 8.2 (n=10) | 314.8 \pm 11.6 (n=8) | 321.6 \pm 16.8** (n=8) | 3.37 \pm 1.1 (n=10) | 1.91 \pm 0.4** (n=7) |
| CD | 82.2 \pm 5.4 (n=10) | 319.1 \pm 13.6 (n=8) | 189.4 \pm 10.1* (n=8) | 3.76 \pm 1.3 (n=10) | 2.83 \pm 1.3* (n=8) |

CNT, normal control; DCNT, diabetic control; CD, treatment with *Cleome droserifolia*; values are given as mean \pm SD for the number of animals indicated. Diabetic control was compared with normal control at the respective time point and treated group was compared diabetic control. Values were considered statistically significant at *p<0.001 and **p<0.0001.

and beta) concentrations decreased significantly (by 30.7%, 30.9% and 70.3%, respectively) in diabetic group ($p < 0.001$) as compared to controls. Gamma globulin increased in diabetic mice as compared to controls, while these fractions of *C. droserifolia* induced a highly significant increase in all these parameters except for gamma globulin concentration, which was found to have greatly decreased. The A/G ratio was higher in diabetic mice than in the control and CD groups (Table 2).

A significant decline in hepatic GSH was observed in diabetic mice as compared with normal control mice ($p < 0.001$). By the end of the study, the hepatic GSH levels were significantly elevated in the treated group as compared with diabetic mice (Table 3). The results revealed a significant increase in serum uric acid level (24.2%) in diabetic mice. *C. droserifolia* significantly decreased ($p < 0.03$) uric acid level by day 30 of its administration (Table 3).

Serum T_3 decrease ($p < 0.001$) and slightly altered serum T_4 ($p < 0.03$) levels were observed in diabetic mice as compared with control group. Consequently, the T_3/T_4 ratio was significantly decreased in diabetic group. Significant increases in the levels of T_3 , T_4 , and

T_3/T_4 ratio were observed following treatment with *C. droserifolia* ($p < 0.001$). Insulin was significantly decreased ($p < 0.0001$) in diabetic mice and increased upon treatment with *C. droserifolia* (Table 3).

DISCUSSION

The main goals of the present study were (a) to determine the antihyperglycemic effect of ethanolic *C. droserifolia* extract in normal (normoglycemic) and in alloxan-treated diabetic mice in a moderate form of diabetes (plasma glucose > 300 mg/dL) over a longer period of time (4 weeks); (b) to quantify the non-enzymatic antioxidant status, hepatic glycogen content and protein content; and (c) to assess the effect of *C. droserifolia* on serum insulin, triiodothyronine (T_3) and thyroxin (T_4) levels in induced diabetic mice.

The increased serum glucose recorded in diabetic mice as compared to control mice (Table 1) may have derived from glycogenolysis and/or gluconeogenesis in the former. These mechanisms have been extensively reported to be the causative reasons eventually leading to hyperglycemia in different diabetic states (18). The administration of *C. droserifolia* extract (0.31 g kg^{-1}) for

Table 2. Effect of 30-day administration of *Cleome droserifolia* extract on serum total protein and its fractions in alloxan (165 mg kg^{-1}) diabetic mice

| Parameter | CNT | DCNT | CD |
|-----------------------|----------|-----------|------------|
| Total protein | 8.8±0.2 | 3.1±0.9** | 7.3±0.2* |
| Albumin (%) | 53.7±8.6 | 37.2±4.6* | 48.3±6.1 |
| Globulin α (%) | 17.7±2.3 | 11.7±1.6* | 12.6±3.2 |
| Globulin β (%) | 29.6±1.1 | 8.8±5.7** | 19.3±6.1** |
| Globulin γ (%) | 19.0±4.4 | 24.8±0.8* | 14.2±5.6** |
| A/G ratio | 1.1±0.2 | 1.7±0.4 | 1.4±0.2 |

CNT, normal control; DCNT, diabetic control; CD, treatment with *Cleome droserifolia*; values are given as mean \pm SD. Values were considered statistically significant at * $p < 0.01$ and ** $p < 0.001$. Diabetic control was compared with normal control, and treated group was compared with diabetic controls.

Table 3. Effect of 30-day administration of *Cleome droserifolia* extract on serum total protein and its fractions in alloxan (165 mg kg^{-1}) diabetic mice

| Parameter | CNT | DCNT | CD |
|---------------------------------|-------------------------|--------------------------------|--------------------------------------|
| Serum uric acid (mg dL^{-1}) | 3.3±0.1 (3.1-3.8) | 4.1±0.1* (3.9-4.4) | 3.2±0.1* (3.1-3.5) |
| Hepatic GSH (mg g^{-1}) | 4.7±0.1 (4.4-5.0) | 1.9±0.1 ^a (1.6-2.4) | 3.3±0.1 ^b (2.9-3.8) |
| T_3 (ng dL^{-1}) | 270.8±7.4 (254.8-288.6) | 168.2±11.1** (142.8-196.8) | 230.9±6.8 ^b (216.9-248.4) |
| T_4 (nmol dL^{-1}) | 43.4±0.7 (41.8-45.0) | 39.2±2.1 (33.7-43.4) | 44.2±0.8 (43.0-46.4) |
| T_3/T_4 ratio | 20.1±0.4 (18.7-20.6) | 13.9±1.1* (11.0-16.1) | 16.8±0.3 (18.3-22.4) |
| Insulin (μU mL^{-1}) | 27.7±0.6 (25.8-28.8) | 14.9±0.6* (13.8-16.0) | 22.5±1.3 ^b (19.5-25.2) |

CNT, normal control; DCNT, diabetic control; CD, treatment with *Cleome droserifolia*; values are given as mean \pm SD; the values in parentheses indicate minimum and maximum value. Diabetic control was compared with normal control and treated group was compared with diabetic control. Values were considered statistically significant at * $p < 0.01$ and ** $p < 0.001$.

30 days caused a reduction in glucose levels by 6.8% in comparison with the basal levels in control mice. It has been shown that the destruction of β -cells of the pancreas is directly proportional to the dose of the diabetogenic agent (19). Since the antihyperglycemic response of *C. droserifolia* was different in two models (alloxan 110 mg kg⁻¹ and 165 mg kg⁻¹) of a varying intensity of hyperglycemia (plasma glucose >200 and >300 mg dL⁻¹, respectively), it implies that the antihyperglycemic effect of *C. droserifolia* is at least partially dependent on insulin release from the pancreas, as the extract showed a greater antihyperglycemic effect in the milder form of diabetes but a lower response in the moderate form of diabetes (6). Such a response has also been seen previously with the extracts of *Eugenia jambolana* (20) and *Momordica charantia* (21). However, *C. droserifolia* significantly suppressed the rise of peripheral blood glucose concentrations as compared to diabetic mice. Decrease of blood glucose in diabetic rat due to *C. droserifolia* has been previously reported (6), giving an additional support to our findings.

Glycogen is the primary intracellular storable form of glucose and its levels in various tissues are a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthetase and inhibiting glycogen phosphorylase. The observed depletion of liver glycogen stores in diabetic mice (Table 1) was consistent with the results of other authors (22), indicating that it could be due to the loss of glycogen synthetase-activating system (23) and/or increased activity of glycogen phosphorylase (22).

Since alloxan causes selective destruction of β -cells of the islets of Langerhans resulting in a marked decrease of insulin levels (24), it is rational to believe that glycogen levels in liver tissues decrease, as they depend on insulin for the influx of glucose. Moreover, this alteration in hepatic glycogen content is normalized by insulin treatment. *C. droserifolia* showed a trend towards a significant increase in glycogen content as compared to diabetic mice.

The greater decrease of serum total protein (hypoproteinemia) in diabetic mice (Table 2) might be ascribed to liver damage. In fact, the decreased amino acid uptake or hepatic protein synthesis has been reported to be depressed due to liver disease. *C. droserifolia* significantly increased serum total protein

and albumin concentration as compared to diabetic control group, which may have been due to the increasing serum insulin level (Table 3). In this regard, the decrease of serum total protein and albumin in diabetic animals was restored to control rate by insulin treatment, which accelerates amino acid transport through cells and stimulates the protein manufacturing machinery of the cell (18). The present study showed a decrease in alpha and beta globulin needed for the transport of T₃ and T₄ as described (25). Therefore, serum depletion of T₃ and T₄ might be due to the lack of thyroid binding proteins needed for their transport. On the other hand, the observed increase in gamma globulin fraction in diabetic mice may represent a responsive mechanism enhancing the immunity of the mice.

The possible mechanism for β -cell destruction by alloxan has been reported to include generation of some types of oxygen free radicals and alternation of endogenous scavengers of these reactive species (26). It has been suggested that reactive oxygen species are a contributory factor in the development of diabetes complications (27). There are many reports indicating changes in the parameters of oxidative stress in diabetes mellitus (28). Among the antioxidant defense mechanisms are GSH and uric acid that remove reactive oxygen species once formed (29). In the present study, hepatic GSH decreased significantly by 59.6% in diabetic mice as compared to control group. These observations are in accordance with the findings that alloxan results in hepatic GSH content depletion in rat due to the higher level of free radical generation that convert more reduced GSH to its oxidized form (28,30). In contrast, *C. droserifolia* raised hepatic GSH as compared to diabetic group (Table 3). This could indicate that the *C. droserifolia* extract can either increase the biosynthesis of GSH and/or reduce the oxidative stress leading to less degradation of GSH. The increased uric acid content in diabetic groups suggests that it acts together with GSH to offer significant protection against oxygen free radical-induced liver injury (31). It acts directly with various reactive oxygen species and possesses antioxidant activity in diabetic rats.

Serum T₃ and T₄ levels are valuable indicators of thyroid function (18). In diabetic mice, T₃ level decreased approximately by 37.9% as compared to controls (Table 3). These decreases reflected the

significant reduction of T_3/T_4 ratio in diabetic group. Several systemic non-thyroid diseases induce subnormal T_3 levels indicating impaired microsomal capacity to convert T_4 to T_3 and the mechanism behind it is the development of oxidative states (32). Therefore, it is reasonable to conclude that liver damage could be the mechanism underlying the observed decrease of T_3 in diabetic mice. Diabetes suppressed thyroid hormones (T_3 and T_4) and serum insulin levels that regulate the basal metabolic rate (33). However, *C. droserifolia* restored serum T_3 back to its normal levels (Table 3), which seems to be secondary to its effect as an antioxidant. The effect of *C. droserifolia* in increasing thyroid hormone levels may reflect its effects on insulin increase. Namely, insulin has been reported to be able to stimulate hepatic T_4/T_3 conversion and to improve the synthetic capacity of thyroid cells. Moreover, T_3/T_4 ratio increased significantly upon *C. droserifolia* administration as compared with diabetic mice.

Insulin deficiency in the diabetic state results in the impairment of glucose utilization leading to an increased generation of oxygen free radicals (34). The present study revealed a very highly significant decrease in fasting serum insulin level of diabetic rats and this finding agreed with the results recorded in streptozotocin-induced diabetic rats (33). A single daily administration of the extract for 30 days significantly reduced fasting glucose in diabetic rats, causing a significant change in insulin levels. The possible improvement in insulin action secondary to its increased levels and manifested as the hypoglycemic effect of *C. droserifolia* might be attributed to its ability to improve the physical state of plasma membrane and its related activities as glucose transport, which is basically controlled by insulin (18).

The present study provided some useful insight into the molecular effect of administration of alcoholic extract of *C. droserifolia*. Study results revealed significant enhancement of insulin levels after the administration of *C. droserifolia* extract, which could be the mechanism behind the hypoglycemic effects of *C. droserifolia*. The role of *C. droserifolia* in increasing insulin levels could be secondary to its property as an antioxidant (4). Therefore, *C. droserifolia* could have a protective effect on pancreatic cells against oxidative stress-induced cellular damage, which certainly affects the synthetic capacity of these cells.

The results of this experimental animal study indicate that the herb possesses a hypoglycemic activity, and thus lends credence to the suggested folkloric use of *C. droserifolia* leaves in the management and/or control of type 2 DM in some communities in Egypt (3). Since ethanol extracts of plants are known to usually contain many chemical compounds, each of which is capable of producing definite biological activities *via* different mechanisms, it is difficult to draw any logical conclusion on the mechanism of the hypoglycemic effect of such a diverse mixture of chemical compounds contained in the plant extract used in this study. Yet, it is possible that the hypoglycemic effect of the plant extract may be due, at least in part, to its flavonoid content (20). However, from the present data it can be suggested that the *C. droserifolia* extract may exert antioxidant activities that protect the tissues from destructive damage of lipid peroxidation (6) and is unlikely to be due to the stimulation of pancreatic β -cells and subsequent secretion of insulin. The major conclusions to be drawn from the present study are that *C. droserifolia* extract not only exhibits hypoglycemic properties but also reduces oxidative stress in alloxan-induced diabetic mice and increases insulin release.

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