

Full Length Research Paper

Chemical screening and antihyperglycemic property of *Cleome droserifolia* ethanol extract

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The present study aimed to assess the antihyperglycemic effect of *Cleome droserifolia* (samwa) ethanol extract in alloxan-induced diabetic rats and to shed more light on the phytochemical screening of this plant and the chemical analysis of its ethanol extract. Alloxan-induced diabetic male albino rats were treated with ethanol extract at intragastric dose level of 200 mg/kg body weight for 4 weeks and were compared with the diabetic control rats given the equivalent amount of the vehicle (dimethylsulfoxide, DMSO). Oral glucose tolerance test, liver glycogen and serum insulin were determined. The chemical screening of *C. droserifolia* (samwa) indicated the presence of glycosides and/or carbohydrates, cardenolides, saponins, sterols and/or triterpenes, tannins, catechins and flavonoids. The treatment of the diabetic rats with samwa ethanol extract exerted marked amelioration of the deteriorated glucose tolerance and the impoverished liver glycogen content without concomitant increase in serum insulin concentration. Moreover, the administration of the extract caused a noticeable increase in the islets cells number with marked amelioration in the islet architecture.

Key words: Samwa ethanol extract, phytochemical screening, diabetic rats.

INTRODUCTION

With the increasing incidence of diabetes mellitus in rural populations throughout the world and the inability of current therapies to control all metabolic defects of the disease and their pathological consequences in addition to the great expense of modern therapy, there is a clear need for the development of alternative strategies for diabetes therapy. Moreover, the World Health Organization expert methods of treatment for diabetes should be further investigated (WHO Report, 1980; Comfort, 1988). Thus, renewed attention to alternative medicines and natural therapies has stimulated a new wave of research interest in traditional practices (Day and Bailey, 1988; Day, 1990; Ahmed et al., 2010; Abdel-Moneim et al., 2000; Ahmad, 2003). Traditional antidiabetic plants might provide a useful source of new oral hypoglycemic compounds for development as pharmaceutical entities, or as simple dietary adjuncts to

the existing therapies (Ahmad, 2003). Those that have been evaluated may be grouped into three categories: (1) plants from which a reputedly hypoglycemic compound or partially characterized hypoglycemic fraction has been prepared, (2) plants reported to exert a hypoglycemic effect, but the nature of the active principle is unestablished, and (3) plants that reputedly exert an hypoglycemic effect, but the scientific evidence is equivocal. A survey of the literature has shown that a large variety of compounds obtained from several plants was found to be responsible for the hypoglycemic action. Of these are glycosides, glycans, sulfide molecules, polysaccharides, oils, vitamins, alkaloids, saponin, glycoproteins, peptides and amino acids from different plant families (Chen et al., 2009; Atta-Ur-Rahman and Zaman, 1989; Marles and Farnsworth, 1995).

Cleome droserifolia (Samwa) is used as a decoction in

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Sinai by Bedouins for the treatment of diabetes mellitus. It belongs to family Cleomaceae or Capparaceae (Genus: Cleome), which may be considered as very important herb and highly used in folk treatments in Sinai.

El-Shenawy and Abdel-Nabi (2006) reported that *C. droserifolia* ethanolic extract exhibited antihyperglycemic properties and enhanced insulin release in alloxan-induced diabetic. El-Seifi et al. (1993a,b) demonstrated that *C. droserifolia* decoction fluid produced marked amelioration of glucose tolerance and serum insulin concentration of the diabetic rats *in vivo* and increased insulin release from the islets of Langerhans isolated from normal rats which suppressed the rise of blood glucose concentration, both in the fasting state and after glucose intake (Nicola et al., 1996)

The purpose of the present work was to evaluate the hypoglycemic activity of *C. droserifolia* ethanol extract in alloxan diabetic male albino rats and to shed light on the preliminary phytochemical screening of this plant and the chemical analysis of its ethanol extract.

MATERIALS AND METHODS

Plant

C. droserifolia, belonging to the family Cleomaceae, is a perennial and wild shrublet that grows on rocky fragments in Sinai, Red Sea and Gebel Elba. Its local name is samwa and it was purchased from spice shops in Cairo (Egypt).

Preparation of plant extract

The aerial parts of herb were used; the ethanol extracts were prepared by soaking 100 g of the dry powdered plant materials in 1 L of ethanol (95%) at room temperature for 48 h. The extracts were filtered after 48 h, first through a Whatmann filter paper No. 42 (125 mm) and then through cotton wool. The extracts were concentrated using a rotary evaporator with the water bath set at 40°C. The remained residual part of the herb was further extracted with ethanol and the filtrate was concentrated under vacuum using rotary evaporator. The resultant extract was stored in a glass container at -20°C pending bioassay.

Phytochemical screening of the tested plant "*C. droserifolia*"

Phytochemical screening was performed using standard procedures (Sofowora, 1993; Trease and Evans, 1989) glycosides and/or carbohydrates, cardenolides, saponins, sterols and/or triterpenes, tannins flavonoids and amino acids.

Test for carbohydrates (Molisch's test)

To 0.5 g of extract diluted to 5 ml in water was added, 2 drops of alcoholic α -naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

Test for cardenolides (Keller-Killiani test)

To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. To

this solution 1 ml of concentrated sulphuric acid was added slowly, while the test tube is tilted. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may be formed just above the brown ring and gradually spread throughout this layer.

Test for saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil then mixed vigorously. Formation of an emulsion was observed.

Test of phytosterols

Salkowski's test

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid then mixed and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes.

Liebermann Burchard's test

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added. Formation of brown ring at the junction indicated the presence of phytosterols.

Test of tannis

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added. A brownish green or a blue-black coloration occurred.

Test for flavonoids

Three methods were used to test for flavonoids. First, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow coloration that disappeared on standing indicated the presence of flavonoids. Second, a few drops of 1% aluminium solution (aluminum acetate was used to prepare this solution) were added to a portion of the filtrate. A yellow coloration indicated the presence of flavonoids. Third, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was mixed with 1 ml of dilute ammonia solution. A yellow colouration indicated the presence of flavonoids. L of ethanol (95%) at room temperature for 48 h. The extracts were filtered after 48 h, first through a Whatmann filter paper No. 42 (125

Test of amino acids

To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicated the presence of amino acid.

Experimental animals

White albino rats (*Rattus rattus*) weighing 180 to 200 g each were

used as experimental animals in the present investigation. They were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. The chosen animals were housed in stainless steel cages. All animals were kept at room temperature ($25 \pm 5^\circ\text{C}$), with a natural 12 h lighting cycle, and were allowed access of water *ad libitum* and fed a standard diet of known weight and composition (16% protein, 3.2% fats, 13.2% fibers and 67.6% carbohydrates, vitamins and minerals).

Induction of diabetes

Diabetes mellitus was induced in the adult healthy rats by a single intraperitoneal injection of alloxan monohydrate (2,4, 5, 6-tetraoxohexa- hydroypyrimidine) (Sigma Company) at a dose level of 150 mg/kg body weight dissolved in phosphate citrate buffer (pH 4) (Gold, 1970; Klebanoff and Greenbaum, 1954). Rats were deprived of food for 16 h before alloxan injection.

After seven days of alloxan injection, rats were deprived of food overnight (12 to 14 h) and they were then given glucose (3 g/kg body weight) by gastric intubation. After 2 h of glucose administration, blood samples were taken from the retro-orbital venous plexus according to the method of Sorg and Buckner (1964) centrifuged and serum glucose level for each rat was measured. Rats with serum glucose concentrations after 2 h of oral glucose administration ranging from 180 to 300 mg/dl were considered as mild diabetic animals and included in the experiment.

Animal grouping

The selected animals were divided into three main groups including normal, diabetic control and diabetic treated animals. The diabetic treated group was daily administered samwa ethanol extract, dissolved in dimethylsulfoxide (DMSO) as a vehicle, at a dose level of 200 mg/kg body weight by intragastric intubation for 4 weeks. The first two groups were given the same equivalent amount of a vehicle for the same period and by the same route as the diabetic treated animals.

Blood and tissue sampling

At the end of the tested periods, normal, diabetic control and diabetic-treated rats were sacrificed under diethyl ether anaesthesia. Blood samples were taken and centrifuged at 3000 rpm for 15 min. The clear non-haemolysed supernatant sera were quickly removed and kept at -20°C till used. Oral glucose tolerance test was also performed on the day before sacrifice by oral administration of 3 g glucose/kg body weight, then blood samples were taken from retro-orbital venous plexus after 0, 30, 60, 90 and 120 min of glucose intake.

The pancreas was rapidly excised from each animal, fixed in 10% neutral buffered formalin and transferred to National Cancer Institute, Cairo University, Egypt for preparation and staining with trichrome Periodic acid-Schiff (PAS) method.

The liver was also immediately removed from each rat. A part of the tissue was used for glycogen estimation.

Biochemical analyses

Glucose was determined in serum according to the enzymatic method described by Siest and Schielef (1981) using kits supplied by BioMerieux Chemicals Company (France). Serum insulin was assayed in the Radioactive Isotopes Unit, National Research Centre (Dokki, Giza) by coat-A-count radioimmunoassay kits supplied by Diagnostic Products Corporation, Los Angeles, USA, according to the method of

Marschner et al. (1974). Hepatic glycogen content was estimated according to the method described by Kemp and Adrienne (1954) using reagents prepared in the laboratory.

Statistical analysis

Student "*t*" test (Snedecor and Cochran, 1980) was performed to evaluate the effect of treatment with each of the tested agents. For each parameter, the diabetic control group was compared with the normal one while the diabetic treated group was compared with the diabetic control one. Values with $P > 0.05$ were considered non-significant but values with $P < 0.05$, $P < 0.01$ and $P < 0.001$ were considered statistically significant, highly significant and very highly significant, respectively.

RESULTS

Chemical screening of samwa ethanol extract

The preliminary phytochemical analysis of *C. droserifolia* (samwa) herb revealed the presence of glycoisdes and/or carbohydrates, cardenolides, saponins, sterols and/or triterpenes, tannins flavonoids, and amino acids.

Biochemical effects

Oral glucose tolerance curves of normal, diabetic control and diabetic treated rats are as shown in Figure 1. The serum glucose concentration at all points (0, 30, 60, 90 and 120 min) of glucose tolerance test was very highly significantly ($P < 0.001$) elevated in the diabetic control rats as compared to the corresponding values of normal ones. The administration of extract for 4 weeks induced a marked amelioration of the glucose tolerance of diabetic rats. The effect was significant at all times of glucose tolerance test due to samwa ethanol extract.

Serum insulin concentration and liver glycogen content variations as a result of diabetes and its treatment with the tested agents are shown in Table 1. Both variables were dramatically ($P < 0.001$) depleted in the diabetic non-treated group after the 4th week of experimental period. However, while serum insulin concentration exhibited a non-significant change in the entire diabetic treated groups as compared to the diabetic control one, the liver glycogen content depicted a significant increase. The effect of the extract on liver glycogen content was very highly significant ($P < 0.001$) and the percentage change was +936.269%. Both ratios of serum insulin-increasing to glucose-decreasing activity and serum insulin-increasing to liver glycogen-increasing activity exhibited positive values for the diabetic control rats and negative values for the diabetic treated ones (Table 1).

Histological effects

In normal animals (Figure 2A), the islets of Langerhans,

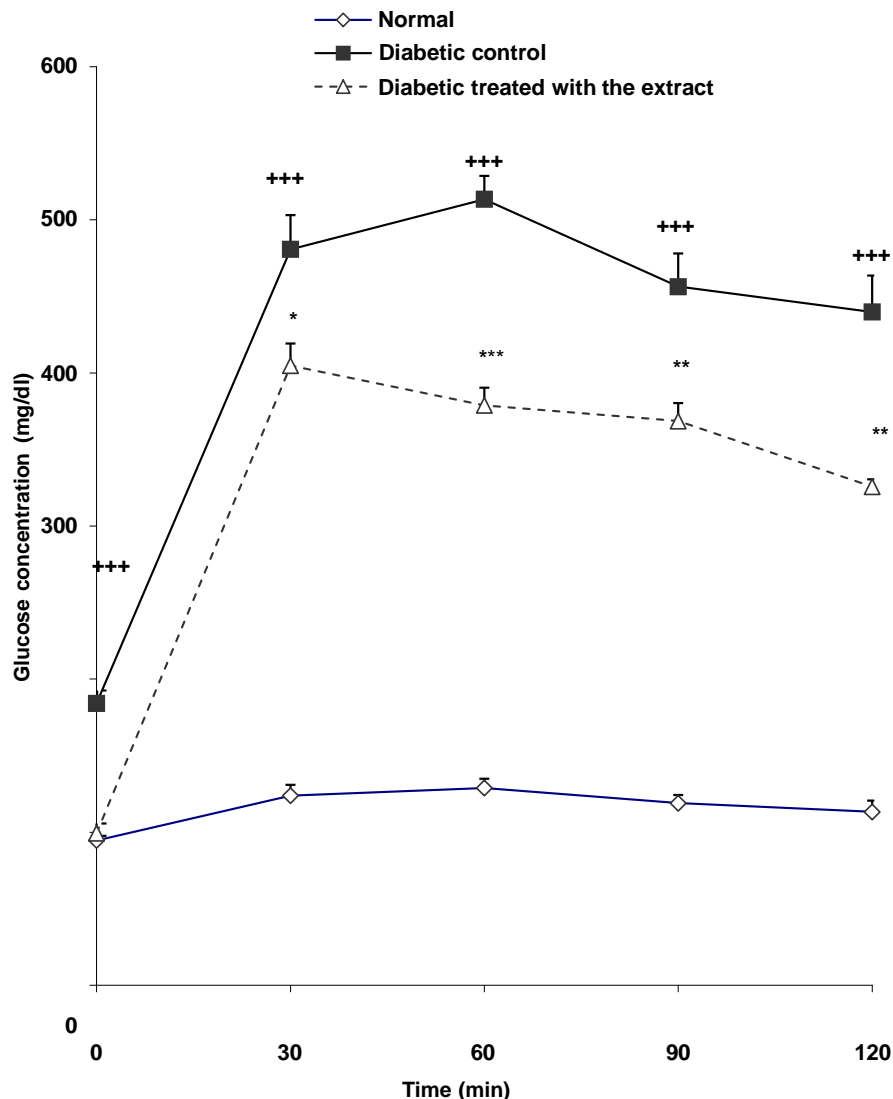


Figure 1. Effect oral administration of samwa-chloroform extract on glucose tolerance test (OGTT) of alloxan diabetic male albino rats. +++Very highly significant as compared to normal. *, **, *** Significant, highly significant and very highly significant.

Table 1. Fasting serum glucose and insulin concentration and liver glycogen content of normal, diabetic non-treated and diabetic treated rats.

Parameter	Normal	Normal-treated	Diabetic	Diabetic-treated
Liver glycogen (mg/g tissue)	7.66±0.67	11.500±1.80	1.16±0.06	12.00±1.78*
Fasting glucose (mg/dl)	94.60±2.98	102.63±1.02 ⁺	184.45±8.44	99.32±6.33*
Insulin (µIU/ml)	24.54±1.24	31.67±4.60	13.39±1.56	10.50±1.23

Data are expressed as mean ± standard error (SE). Number of animals in each group is five. Values significant compared to those of normal rats: *P < 0.0.05. Values significant compared to those of diabetic non-treated rats: * P < 0.0.05

which constitute the endocrine portion of the pancreas are diffused throughout the pancreas as irregular masses

surrounded by the exocrine part consisting of pancreatic acini. The cells of the islets are arranged in irregular

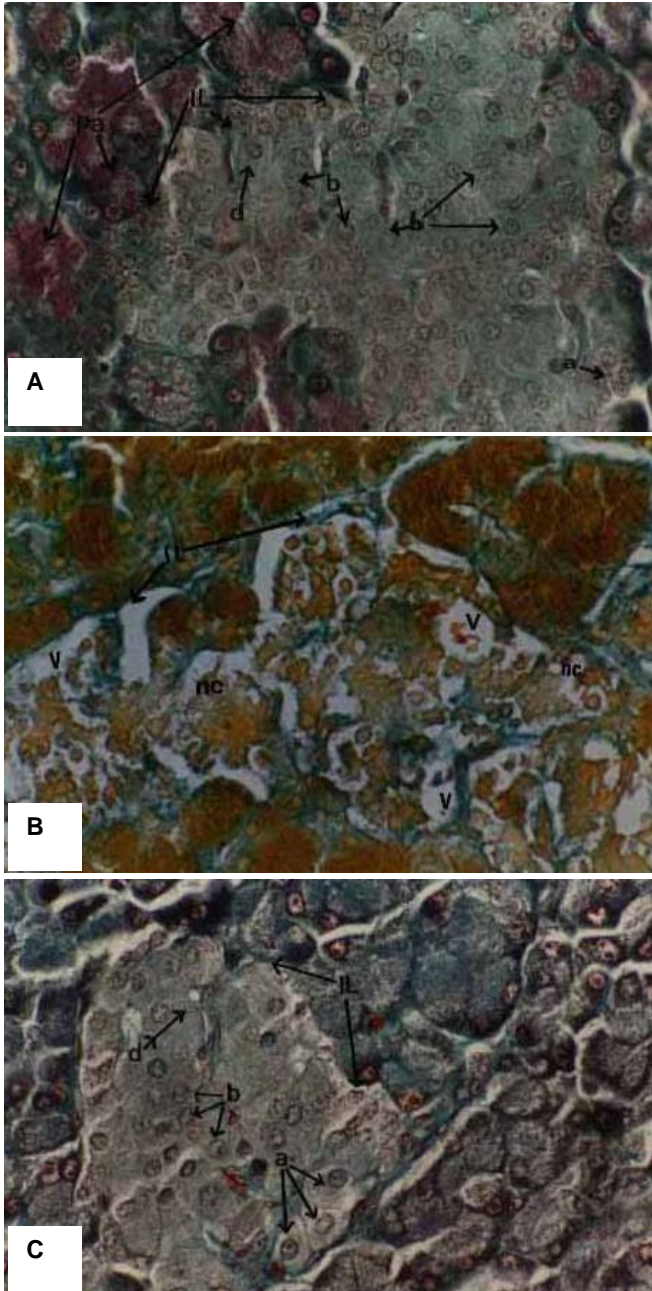


Figure 2. Photomicrographs of a section of the pancreas of rats showing normal rats (A), alloxan- induced diabetic non-treated rats (B) and diabetic treated rats with samwa ethanol extract (C) showing increased alpha (a) and beta (b) cells as compared with the diabetic non-treated rats. The vacuolation and necrotic foci disappeared. Delta cells (d) are also visible

cells and include three main types, alpha (a), beta (b) and delta (d) cells. All these cells are irregular, granular and polygonal with central and spheroid nuclei. Beta cells are the most abundant cells found, occupying the core of the islets and contain numerous granules. Alpha and delta cells form the periphery of the islets.

Delta cells are usually located adjacent to the alpha cells and somewhat larger in size.

Following alloxan injection, at the dose level of 150 mg/kg body weight profound alterations in the pancreatic islets cells were observed and the normal architecture of the islets were disrupted. These alterations include great reduction of islets cells, vacuolation, and necrotic foci (Figure 2B).

As a result of treatment with the tested extract, the islets regained their normal architecture, the number of alpha and beta cells per islet was profoundly increased, vacuolation and necrotic foci disappeared, and alpha and beta cells appeared more intact and granulated (Figure 2C).

DISCUSSION

In the present study, alloxan was used to induce an experimental hyperglycemic state in albino rats. Several reports have been published on the effect of alloxan and streptozotocin on the glycaemic state of different species of animals (Ahmed, 2003; El-Shenawy and Abdel-Nabi, 2006; El-Seifi et al., 1993a,b; Gunnarson et al., 1974; Annamala and Augusti, 1980; Al-Awadi et al., 1985; Noreen, et al., 1988; Rawi et al., 1996; Abdul-Moneim et al., 1997; Ahmad, 2001). These studies indicated that both substances have cytotoxic effect on β -cells in the pancreatic islets and can induce chronic or permanent diabetes mellitus in these animals. Although alloxan and streptozotocin are structurally different, it has been reported that their immediate action converges into common mechanisms such as generation of oxygen free radicals, alteration of endogenous scavengers of these reactive species, the enhancement of DNA strand breaks, the suppression of nicotinamide adenine dinucleotide (NAD) level and ultimately inhibition of β -cell functions or their destruction (Ahmed, 2003; Marles and Farnsworth, 1995; Gaulton et al., 1985; Yoon, 1990; Pustai et al., 1996).

After several trials with various doses of alloxan, a single intraperitoneal dose of 150 mg/kg body weight in male albino rats was used in the present investigation which led to chronic hyperglycemia and marked decrease of serum insulin level (-45.436%). This result agrees well with the theoretical and practical consideration which postulated that the diabetogenic dose of alloxan could affect β -cells, insulin secretion and led to elevated blood glucose and impaired glucose tolerance (Ahmed, 2003; Begum and Bari, 1985; Rawi, 1995). The present data are also in accordance with that of Noor and Ashcroft (1989) who reported that the basal insulin level was lowered by 53.38% in adult male rats one week post-intravenous injection of 35 to 40 mg alloxan/kg body weight. Moreover, Rawi et al. (1998) found that serum insulin level of male albino rats decreased by 83.216% after

the 4th week of diabetes induction with 150 mg/kg body weight alloxan injected intraperitoneally.

The treatment of alloxan diabetic rats in this experiment with samwa ethanol extract produced marked amelioration of the impaired glucose tolerance. These results are in accordance with the previous reports, which revealed that *C. droserifolia* water extract produced an improvement in the hypoglycemic condition of diabetic rats (El-Seifi et al., 1993). Furthermore, Nicola et al. (1996) found that samwa extract significantly suppressed the rise in peripheral blood glucose concentrations, both in the fasting state and after glucose intake, which indicated the lowering effect of the plant extract on hepatic glucose output. Our result also indicated that serum insulin concentration was not significantly affected as a result of treatment of alloxan diabetic rats with the tested extract. Moreover, the diabetes induced decline in the liver glycogen content was markedly alleviated after samwa ethanol extract administration for 4 weeks. The rise in hepatic glycogen content of the diabetic treated rats may be attributed to the increase in the activity of glycogen synthetase enzyme (Rawi et al., 1996; Abdel-Moneim et al., 1997; Shanmugasundaram et al., 1983). Another possible explanation for the increase in liver glycogen is that the treatment of alloxan diabetic rats with sulfur containing ingredients of samwa extract might have caused inhibition of glucose-6-phosphatase followed by inhibition of glycogen phosphorylase activity and consequently reduced glycogen breakdown (Saleh et al., 1974; Blumenthal et al., 1979; McGuinness et al., 1987; Parto et al., 1991). The improvement of glucose tolerance and liver glycogen content as a result of treatment with the extract, in spite of the non-significant change of serum insulin level, led us to suggest that the extract may enhance insulin action and/or have insulin mimetic effects rather than being stimulants of insulin secretion. This assumption is confirmed by the ratios of insulin-increasing to glucose-decreasing activity and values of serum insulin increase percent to liver glycogen increase percentage, which exhibited positive values for the studied diabetic control animals and negative values for diabetic treated ones. This indicates that the serum glucose-lowering and liver glycogen-increasing activities of samwa ethanol extract are independent of the insulin-increasing activity. Thus, it is worth mentioning that the extract may have potent extra pancreatic effects. Consistent with this study, Nicola et al. (1996) reported that the hypoglycemic effect of *C. droserifolia* extracts was not accompanied with insulin secretion, but the effect was mediated either by the potentiation of peripheral and hepatic insulin sensitivity or decreasing the intestinal glucose absorption (Nicola et al., 1996). In contrast, other authors revealed that *C. droserifolia* decoction fluid and ethanolic extract markedly increased serum insulin level in streptozotocin and alloxan-induced diabetic rats, which in turn had a

great role in improving the glycemic state in these animals (El-Shenawy and Abdel-Nabi, 2006; El-Seifi et al., 1993a,b; Rawi, 1995).

From the histological point of view, it was found that the diabetic rats exhibited degenerative changes in the islets of Langerhans with marked loss of their cells. The treatment of the diabetic animals with the extract produced marked amelioration of the islets architecture with an increased number of α and β -cells. The newly formed islet cells may be regenerated from the remained ones that survived alloxan intoxication or from the duct epithelium of the exocrine portion of the pancreas (Abdel-Moneim et al., 2001; Ahmed, 2003; Rerup, 1970). The non-significant decrease of fasting serum insulin level in the diabetic treated rats accompanied with improved glucose tolerance and islets architecture with increased number of β -cells was explained by the forthcoming authors. Bondansky et al. (1982), Judzewitsch et al. (1982) and Hosker et al. (1985) suggested that blood glucose control without an elevated serum insulin level may be attributed to a drug-induced postprandial kick of insulin release resulting in lower serum glucose values and therapy also reduced basal insulin levels. In our opinion, the non-significant change of fasting serum insulin concentration of the diabetic treated rats, in the present study, in spite of increased β -cells, may be due to an increase in the hepatic insulin extraction (Wajchenberg et al., 1992) as a result of treatment and/or that the newly formed β -cells were still too immature to secrete insulin into the blood.

The hypoglycemic effect of the studied plant extract may be attributed to the presence of certain constituents. Some of these compounds were reported to be active *in vitro* and *in vivo*. Previous work indicated that flavonoids (Koval'ov et al., 1985), alkaloids (De and Saha, 1975; Snell, 1979), peptides and terpenoids (Meir and Yaniv, 1985; Ng et al., 1986), sulfur-containing compounds (Rawi, 1995; Swanston-Flatt et al., 1991), inorganic ions (Mertz, 1993; White and Campbell, 1993), amino acids (Rawi, 1995; Sheela and Augusti, 1992), vitamins (Özden et al., 1989; Ceriello et al., 1991) and carbohydrates (Hanefield et al., 1991; Yang et al., 2008; Ahmed, 2010) may fit in this category. These compounds were shown to have insulin mimetic effects when administered to diabetic rodents and patients (Siest and Schielef, 1981; Chi et al., 1982; Farva et al., 1986; Sitprija et al., 1987). Although sufficient evidence is available to support the effect of sulfur-containing compounds as antidiabetic agents (Swanston-Flatt et al., 1990a,b), it is not clear whether they induce their effect through activation of insulin action at the receptor or post-receptor level or via their direct action on pathways of carbohydrate metabolism. However, some investigators suggested that the mechanisms of sulfur-containing compounds involve the reaction of SH-groups with insulin inactivating compounds

by competing with insulin on these compounds, thereby protecting insulin from degradation (Rawi et al., 1996; Howard and Frank, 1966; Augusti and Benaim, 1975).

Conclusion

Conclusively, this study revealed that samwa ethanol extract improved glucose tolerance in alloxan-induced diabetic rats. Conclusive evidence provided by the present work supports the extrapancreatic actions of the tested extract.

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