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Assessment of Anti-Hyperglycemic Effects and the Probable Mode of Action of *Cynara scolymus* Leaf and Flower Head Hydroethanolic Extracts in Type 2 Diabetic Wistar Rats

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Research Article

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Abstract

Introduction: Theprevalence of type 2 diabetes mellitus has been increasing steadily all over the world. Because of the side effects of the conventional antidiabetic chemical drugs, the development of alternative medicines is needed. Thus, this study was designed to assess the effect of *Cynara scolymus* (artichoke) leaf and flower head hydroethanolic extracts on oral glucose tolerance and to suggest their probable mode of actions in experimentally-induced type 2 diabetic Wistar rats.

Materials and Methods: Type 2 diabetes mellitus was induced by a single intraperitoneal injection of streptozotocin (60 mg/kg body weight) dissolved in cold 0.01M citrate buffer (pH 4.5), 15 minutes after intraperitoneal injection of nicotinamide (60 mg/kg body weight). After induction of diabetes mellitus, the rats were allocated into four groups (each of six rats). Group 1 was kept as normal control. Group 2 was diabetic control. Groups 3 and 4were diabetic groups that were respectively treated with *Cynara scolymus* leaf and flower head extracts at dose of 100mg/kg body weight/day by oral administration for 28 days.

Results: The treatment of the diabetic rats with leaf and flower head extracts resulted in a significant improvement of the impaired oral glucose tolerance, the lowered serum insulin and C-peptide levels, andthe decreased β cell insulin content and HOMA-β cell function in association with amendment of the pancreatic islets histological architecture and integrity. The lowered visceral adipose tissue mRNA expression of adiponectin and the elevated resist in mRNA expression were significantly ameliorated in diabetic rats as a result of treatment with leaf and flower head extracts.

The lowered HOMA-IS and raised HOMA-IR in diabetic rats was significantly alleviated due to treatment with flower head extract.

Conclusion: The *Cynara scolymus* leaf and flower head hydroethanolic extracts successfully improved the oral glucose tolerance probably *via* amendment of pancreatic islets histological integrity, enhancement of the insulinogenic effects and attenuation of insulin resistance. The leaf extract had more potent antihyperglycemic effect than the flower head extract.

Keywords: Type 2 diabetes mellitus; *Cynara scolymus*; Glucose Tolerance; Insulin; Adiponectin; Resistin.

Introduction

Diabetes mellitus is a multi-factorial metabolic disorder characterized by chronic hyperglycemia with modificationsin carbohydrate, fat and proteins metabolism resulting from dysfunction of pancreatic β-cells and/or insulin resistance [1,2]. With the increasing prevalence of diabetes, development of effective pre-clinical modelshas become crucialas a very useful toolfor studying possible drug discovery approaches for both diabetic prevention and treatment. Streptozotocin (STZ) has been reported to induce both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) [3-5] depending on itsdose, single or multiple injection, the use of high-fat diet and its concomitant injection with or without nicotinamide (NA). In this regard, it was reported that the injection of NA 15 minutes before STZ in rats leads to T2DM [6,7]. The major pathophysiologic abnormalities of T2DM include insulin resistance and subsequent loss of β-cell mass and β-cell dysfunction [8-11]. Three key defects implicated in the onset of hyperglycemia in T2DM that are (1) increased hepatic glucose production, (2) diminished insulin secretion, and (3) impaired insulin action [12,13]. T2DM accounts for >90% of diabetes and is associated with metabolic disorder of carbohydrate, lipid and protein [7,14]. The sub-classes of lipids responsible for the pathogenesis of insulin resistance are intracellularly-accumulated free fatty acids (FFAs), diacylglycerol (DAG) and triacylglycerol (TG) moieties [15].

Despite the progress of modern medicine, traditional medicine remains an important pillar in the treatment of many chronic diseases including diabetes mellitus [16,17]. Medicinal plants continue to provide valuable therapeutic agents and they have always been the sources of drugs that treat many diseases including diabetes mellitus [18,19]. In this regard, the World Health Organization (WHO) has recommended the evaluation of traditional plant treatments for diabetes as they are effective, non-toxic, with less or no side effects and are considered to be excellent candidates for oral therapy [20].

The importance of the plant *C. scolymus* which is called artichoke or globe artichoke steamed from used as edible material for nutrition and from its content of phenolic acid constituent in particular cynarin and chlorogenic acid [21]. Previous studies found that after eating of an artichoke food supplement for three consecutive months reduced fasting and post-prandial glycaemia in T2DM diabetic patients [22]. Among the vegetables, the globe artichoke (*Cynara cardunculus var. scolymus* [L.] Fiori) plays an important economic role in Mediterranean agriculture [23]. Cynareae tribes present in Egypt are known for their efficacy in relieving some liver disorders [24].

The leaves of *C. scolymus* L. have been long-used in traditional medicine and now included in British and European Pharmacopeia (BP/EP), the British Herbal pharmacopeia (BHP) and the Complete German Commission E Monographs [21]. Artichokeleaf extracts decreased serum lipids, as well as hepatic and cardiac oxidative stress in rats fed on high cholesterol diet [55]. Several reports from folk medicine as well as preliminary data from scientifically designed pre-clinical and clinical studies have suggested that artichokes may have cholerectic, hypocholesterolemic and hypolipidemic properties [25-31].

The pharmacologic properties of artichoke flower heads are well documented in several *in vivo* and *in vitro* studies for the treatment of hepatobiliary dysfunction, dyspeptic syndromes, gastric diseases, as well as for inhibition of cholesterol biosynthesis and low density lipoproteins (LDL) oxidation-induced arteriosclerosis and coronary heart disease [31-33].

Little is known about the effects of *C. scolymus* leaf and flower head extracts on histopathological, ultrastructural, immunohistochemical and biochemical changes in diabetic rats. The present study aims to investigate the effects of *C. scolymus* on type 2 diabetic rats. This investigation will be monitored by histopathological, ultrastructural, immunohistochemical and biochemical parameters

including serum glucose (fasting and post-prandial), insulin and C-peptide to assess the anti-diabetic efficacy of *C. scolymus* leaf and flower head extracts and to suggest their probable mechanisms of action.

Material and Methods

Chemicals

STZ and NA were obtained from Sigma Chemical Company (USA). Proteinase K was obtained from Bioline USA Inc., Taunton, MA, USA.All other used chemicals are of analytical grade.

Preparation of Plant Extraction

C. scolymus was collected from Beheira (Egypt) and was authenticated by Dr. Mohamed A. AbdallahFadl, Associate Professor of Taxonomy, Botany Department, Faculty of Science, Beni-Suef University, Egypt. The leaves and flower heads were washed, air dried at room temperature for 3 weeks in good aerated and shaded area, and then powderedby an electrical grinder. The powder of *C. scolymus* leaves and flower heads were separately soaked in 70% ethanol for 72 hours at room temperature. The mixtures were then filtered through man filter paper and evaporated under reduced pressure using rotary evaporator to a yield of crude extracts. The hydroethanolic extracts were kept at -20˚C until used.

Experimental Animals

Healthy adult male Wistar rats weighing 120±20 g were purchased from Helwan Station of Experimental Animals, Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt). All animals were kept under observation for two weeks before the onset of the experiment to exclude any intercurrent infections. Animals were maintained under standard conditions of light, ventilation, temperature (22±2 ˚C), and humidity (55±5%).The animals were allowed to access tap water and standard pellet diet *ad libitum*. All animals' procedures were in accordance with the ethical guidelines for the use and care of animal of the Experimental Animal Ethics Committee, Faculty of Science, Beni-Suef University, Egypt (Ethical Approval Number 2015/2). All efforts were done to reduce the number and suffering of animals.

4.4. Induction of T2DM

T2DM was experimentally induced in rats deprived of food and water for 16 hours by intraperitoneal (IP) injection of 120 mg/kg b.wt dissolved in 0.9% NaCl solution 15 minutes before IP injection of STZ-NA at a dose of 60mg/kg/b.wt dissolved in citrate buffer at pH 4.5 [34,35]. Ten days after STZ injection, rats were screened for blood glucose levels. Animals, deprived from food and water overnight (10-12 hours), were given glucose (3 g/kg b. wt.) by oral gavage. After 2 hours of oral administration, blood samples were taken from lateral tail vein and blood glucose concentration was measured by glucometerobtained from Life Scan Corporation, Canada. Rats having serum glucose ≥ 200 mg/dl, after 2 hours of glucose intake, were considered diabetic and selected for further biochemical, physiological and histological studies.

Experimental Design

After acclimation period and induction of T2DM, rats were randomly divided into 4 groups (6 rats for each). Group 1 was considered as normalcontrol group and was administered the equivalent volume of a vehicle, 1% CMC, daily by oral gavage for 28 days. Group 2 was diabeticcontrol group that consisted of diabetic rats administered the equivalent volume of 1% CMC (as vehicle) daily by oral gavage for 28 days. Group 3 was composed of diabetic rats treated with *C. scolymus* leaf hydroethanolic extract at a dose of 100mg/kg/b.wt, dissolved in 1% CMC, by oral gavage daily for 28 days. Group 4 consisted of diabetic rats treated with *C. scolymus* flower head hydroethanolic extract at a dose of 100mg/kg/b.wt, dissolved in 1% CMC, by oral gavage daily for 28 day.

Oral Glucose Tolerance Test (OGTT)

OGTT was performed tonormal control, diabetic control and diabetic treated rats at the day before sacrifice by oral administration of glucose solution (3 g/kg b.wt) to overnight (10-12 h) fasted animals then successive blood samples were then taken at 0,30, 60, 90 and 120 minutes. Blood samples were obtained from lateral tail vein and blood glucose concentration was measured by glucometer obtained from Life Scan Corporation, Canada.

Blood and Tissue Sampling

At the end of the experiment, animalswere overnight fasted, then blood was collected from jugular vein underdiethyl etheranesthesia and left to coagulate at room temperature. Serum was separated by centrifugation at 3000 rpm for 15 min and stored at -20°C pending its use for the detection of insulin and C-peptide levels. The rats were rapidly dissected and visceral adipose tissue was excised and kept at -70°C until its use for RNA extraction and detection of mRNA of adiponectin and resistin by reverse transcriptase (RT)-PCR reaction. Pancreas was excised and fixed in 10% formalin pending histological and Immunohistochemical investigations. In addition, pieces of pancreas measuring 1 mm^3 were immediately fixed in fresh 3% glutaraldehyde-formaldehyde for electron microscopic examination.

Detection of Serum Insulin and C-Peptide Levels

Serum was used for the determination ofinsulin and C-peptide levelsusing ELISA kit purchased from Linco Research, USA, according manufacturer's instruction.

Calculation of Homeostatic Model Assessment (HOMA) Indices

HOMA-Insulin resistance (IR), HOMA-insulin sensitivity (IS), and HOMA-β cell function were calculated according to Hsing*et al*. [36], Park *et al*. [37]and Aref*et al*. [38] from the following formulas.

HOMA-β cell function = (20 × fasting insulin [μIU/ml])/(fasting glucose [mg/dl]- 3.5)

HOMA-IS =10000/(fasting insulin[μIU/ml] × fasting glucose[mg/dl])

HOMA-IR = (fasting insulin [μIU/ml] × fasting glucose [mg/dl])/405

Detection of mRNA Expression of Adiponectinand Resistin

Total RNA was isolated from visceral adipose tissue according to the method of Chomzynski and Sacchi [39], using Thermo Scientific Gene JET RNA purification kit obtained from Thermo Fisher Scientific Inc., Rochester, New York, USA. Reverse transcription (RT) of RNA into cDNA and the PCR amplification in the presence of specific primers of adiponectin and resistin was performed using Thermo Scientific Verso 1-Step RT-PCR Reddy Mix kit (Thermo Fisher Scientific Inc., Rochester, New York, USA) and Thermal cycler Techne 312(Fisher Scientific, Leicestershire, LE11 5RG). The sense and anti-sense specific primers of adiponectin and resistin were obtained from Bioresearch Technologies, South McDowell Blud, Petaluma, CA, USA. The RT-PCR productswere loaded and electrophoresedat 90 volts on 1.5% agarose gel stained with ethidium bromide in 1X Tris Borate EDTA buffer (TBE) (pH 8.3-8.5). The bands on the agarose gel were viewed by UV transilluminator in a dark chamber and photographed by a camera using Gel Documentation System obtained from Raya for the Scientific Services, Giza, Egypt. The bands were analyzed by Raya Gel Docu Advanced Program accessed from Raya for the Scientific Services, Giza, Egypt. The mRNA levels of adiponectin and resistin were normalized to β-actin.

Histological Investigation

The pancreasfrom each rat wasrapidly excised after dissection andthen fixed in 10% neutral buffered formalin for 24 hours; the organs were routinely processed and sectioned at 4–5 μm thickness. Sections of pancreas were stained with haematoxylin and eosin [40,41].

Immunohistochemistry of Pancreatic Islets

Immunolocalization technique for anti-insulin was performed on 5–6 mμ thickness sections and stained with the streptavidin–biotin–peroxidase staining method [42]. Paraffin sections were deparaffinized in xylene, rehydrated in descending grades of alcohol. Endogenous peroxidase and non-specific binding sites for antibodies were suppressed by treating the sections with 0.3% hydrogen peroxide for 20 min and 5% normal bovine serum (1:5 diluted TRIS) for 20 min at room temperature, respectively. The sections were washed in phosphate buffered saline and 10% normal goat serum was applied for 30 min to reduce non-specific binding. The sections were incubated for 1 h with anti-sera containing primary antibodies for rat insulin (polyclonal antibody) supplied by Bio Genex Cat. No. AR. 295-R and anti-TNF-α (Santa Cruz, CA, USA). The sections were incubated with biotinylated secondary antibody (Dako-K0690; Dako Universal LSAB Kit) and streptavidin horseradish peroxidase (Dako-K0690) for 30 min, and then 3,30-diam-inobenzidinetetrahydrochloride (Sigma-D5905; Sigma–Aldrich Company Ltd., Gillingham, UK) substrate kit for 10 min to obtain immunolabelling. Finally, (i) dehydrated in graded alcohol, (ii) cleared in xylene, and then (iii) mounted in DPX. The binding of antibodies was evaluated by examination under high-power light microscopy. All sections were incubated under the same conditions with the same concentration of antibodies and at the same time, so the immunostaining was comparable among the different experimental groups.

Ultra Structural Preparations

The specimens of pancreas at day 30 were cut into small pieces measuring about 1 mm^3 and immediately fixed in fresh 3% glutaraldehyde-formaldehyde at 4ºC for 18-24 hours. The specimens were then washed in phosphate buffer (pH 7.4) and post-fixed in isotonic 1% osmium tetroxide for 1 h at 4ºC and then processed. Semithin sections (1 μm) were stained with toluidine blue. Ultrathin sections (70–80 nm) were stained with uranyl acetate and lead citrate [43] and examined on a Joel CX 100 transmission electron microscope operated at an accelerating voltage of 60 kV.

Statistical Analysis

Results were expressed as mean ± standard error (SE). The data are analyzed by one-way analysis of variance (ANOVA) using PC-STAT, University of Georgia, followed by LSD analysis to compare various groups with each other [44]. Values of $P>0.05$ were considered non-significantly different, while values of $P<0.05$ and $P<0.01$ were significant and highly significant different respectively.

Results

Effect on Oral Glucose Tolerance

Oral glucose tolerance curves of normal, diabetic control and diabetic treated rats are illustrated in Figure 1. The serum glucose levels at all intervals (0, 30, 60, 90, 120 minutes) of glucose tolerance test were highly significantly ($p<0.01$; LSD) elevated in diabetic rats as compared to normal ones. The treatment of diabetic rats for 28 day with *C. scolymus* leaf and flower head extract successfully improved ($p<0.01$; LSD) the impaired glucose tolerance of diabetic rats. The leaf extract seemed to be more effective in improving the glucose tolerance than the flower head extract. F-probability of one-way ANOVA revealed that the effect on OGTT between groups was very highly significant throughout the experiment.

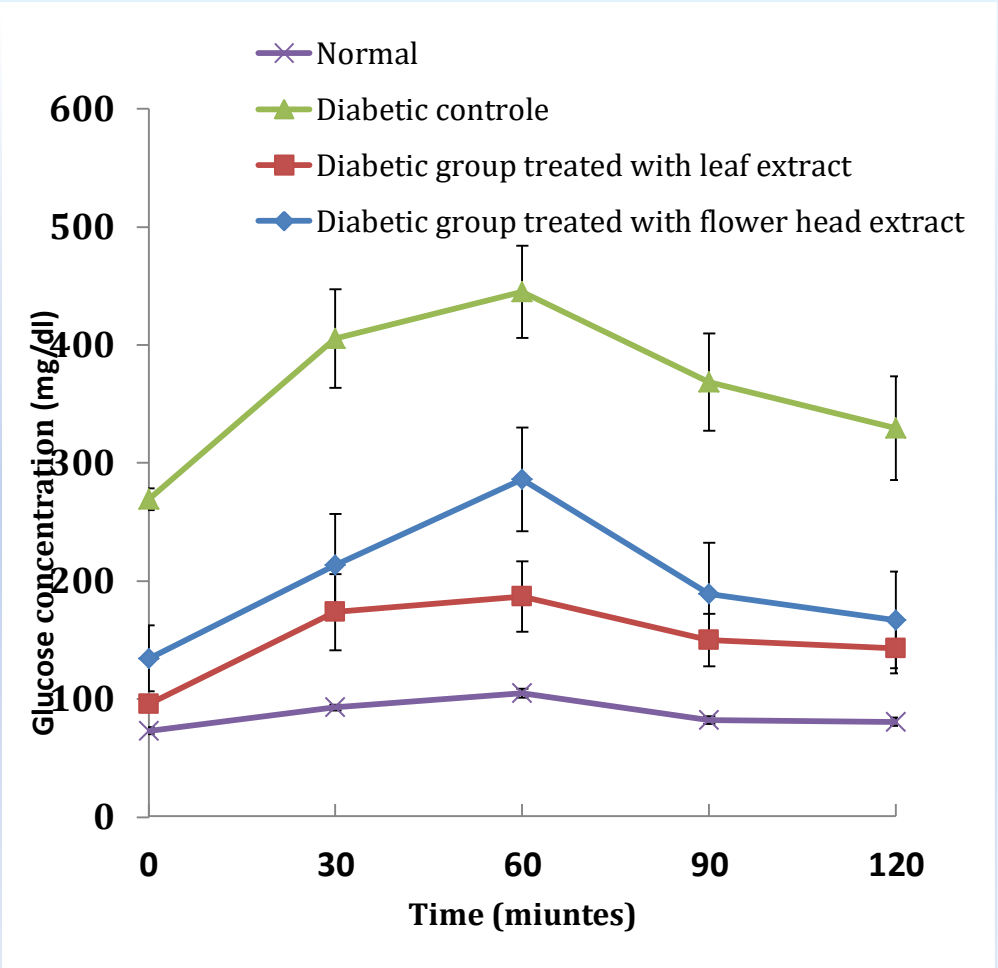

Figure 1: Oral glucose tolerance curves of normal, diabetic control and diabetic groups treated with *C. Scolymus* leaf and flower head extracts for 28 days. F probability: $p<0.001$; LSD at the 5% level: 86.7; LSD at the 1%level: 116.76.

Effect on Serum Insulin and C-Peptide Concentrations

The serum insulin and C-peptide concentrations at fasting statewere highly significantly ($p<0.01$; LSD) decreased in diabetic rats in comparison with those of normal control rats; the recorded percentage changes were -60.03and -74.03% respectively. The treatment of diabetic rats with C.scolymus leaf and flower head extracts induced a highlysignificant increase ($p<0.01$; LSD) of serum insulin and C-peptide concentration as compared to diabetic control (Table 1).The treatment with C. scolymus leaf extract appeared to be more effective in improving the serum insulin (152.00%) and C-peptide (229.78%) levels than the treatment with the C. scolymus flower head extract.

Group	Insulin (µIU / ml)	Percentage change	C-peptide (pg/ml)	Percentage change
Normal	9.45±0.55[a]		3.62±0.31[a]	
Diabetic control	3.75±0.0.46[c]	-60.03	0.94±0.15[d]	-74.03
Diabetic group treated with leaf extract	9.45±0.72[a]	152.00	3.10±0.13[ab]	229.78
Diabetic group treated with fruit extract	6.21 ±0.74[b]	65.60	2.92±0.10[bc]	210.64
F-probability	P<0.001		P<0.001	
LSD at the 5% level	1.86		0.56	
LSD at the 1% level	2.53		0.76	

Table 1: Effect of *C. scolymus* leaf and fruit extracts on insulin and C-peptide levels in NA/STZ-induced diabetic rats.

-Data are expressed as mean ±SE. Number of animals in each group is six.
-Means, which share the same superscript symbol(s), are not significantly different
-Percentage changes were calculated by comparing diabetic treated groups with diabetic control.

Effect on mRNA Expression of Adiponectin and Resistin

Data describing the effect of treatment of diabetic rats with *C. scolymus* leaf and flower head extract on adipose tissue adiponectin and resistinmRNA expression normalized to β-actin were recorded in Figures 2 and Figure 3.

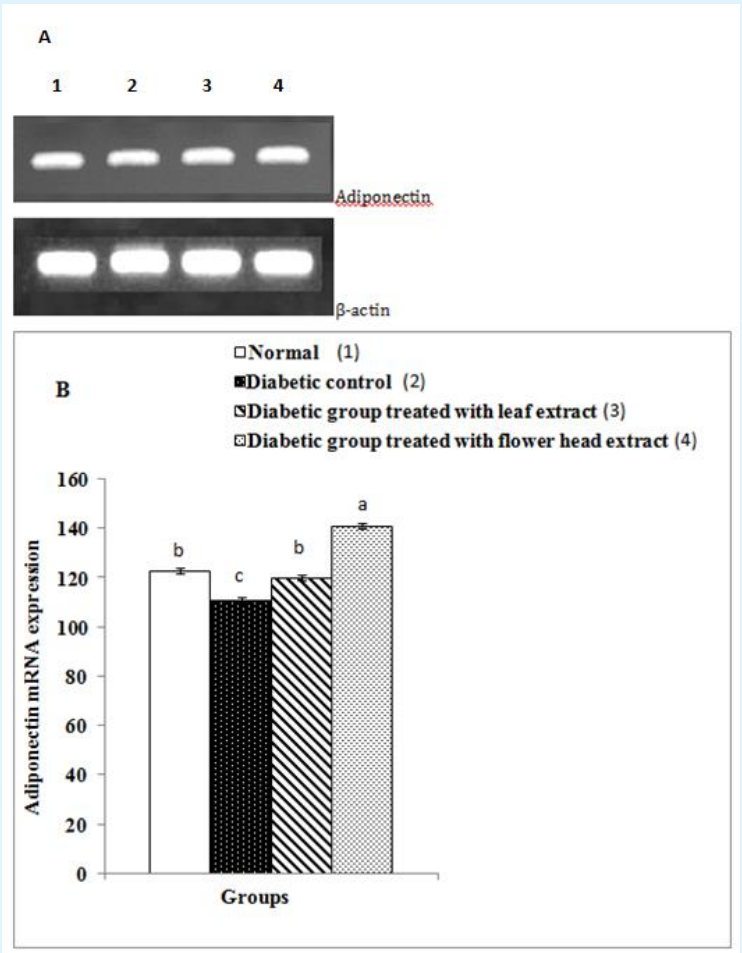

Figure 2: Effect of *C. scolymus* leaf and flower head extracts on adipose tissue adiponectin relative to β-actin mRNA expression in NA/STZ diabetic rats.
A represents gel electrophoretogram depicting PCR products.
B corresponds to the densitometric analysis of PCR products. Means, which share the same superscript symbol(s), are not signficantly different. Number of detected samples in each group is three.

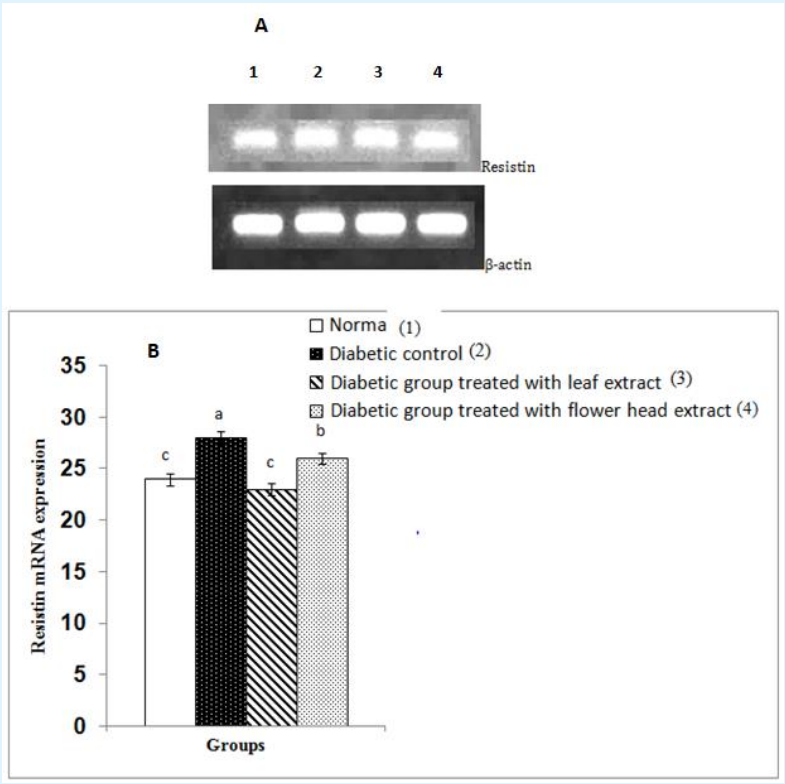

Figure 3: Effect of *C. scolymus* leaf and flower head extracts on adipose tissue resistin relative to β-actin mRNA expression in NA/STZ diabetic rats.
A represents gel electrophoretogram depicting PCR products.
B corresponds to the densitometric analysis of PCR products. Means, which share the same superscript symbol(s), are not signficantly different. Number of detected samples in each group is three.

Densitometric analysis revealed a highly significant ($p<0.01$; LSD) decreases of adipose tissue adiponectin and increase in resistin mRNA expression in NA/STZ diabetic rats as compared to the normal rats. The treatment of diabetic rats with *C. Scolymus* leaf and flower head extracts produced a significant increase of the lowered adiponectin mRNA expression and a significant decrease of the elevated resistin mRNA expression. The flower head extract seemed to be more effective in increasing the adiponectin mRNA expression while the leaf extract appeared to be more potent in decreasing the elevated resistin mRNA expression.

Effect on Homeostatic Assessment (HOMA) Indices

HOMA-β cell function, HOMA-IS and HOMA-IR of normal, diabetic control and diabetic rats treated with *C. scolymus* leaf and flower head extracts are represented in Table 2.

Group	HOMA-β cell function	Percentage change	HOMA-IS	Percentage change	HOMA-IR	Percentage change
Normal	2.76 ± 0.10^{a}		14.67 ± 0.97^{a}		1.72 ± 0.12^{b}	
Diabetic control	0.27 ± 0.04^{c}	-90.22	10.58 ± 1.84^{b}	-27.88	2.66 ± 0.25^{a}	54.65
Diabetic group treated with leaf extract	2.08 ± 0.12^{a}	670.37	11.56 ± 1.20^{ab}	9.26	2.23 ± 0.19^{ab}	-16.17
Diabetic group treated with flower head extract	1.26 ± 0.22^{b}	366.66	17.48 ± 2.04^{a}	65.22	2.03 ± 0.23^{b}	-23.68
F-probability	P<0.001		P<0.05		P<0.05	
LSD at the 5% level	0.53		4.65		0.61	
LSD at the 1% level	0.72		6.34		0.83	

Table 2: Effect of *C. scolymus* leaf and flower head extracts on HOMA-β cell function, HOMA-IS and HOMA-IR in NA/STZ-induced diabetic rats.

HOMA-β cell function and HOMA-IS were highly significantly ($p<0.01$; -90.22%)and significantly ($p<0.05$; -27.88%) decreased respectively in diabetic rats while HOMA-IR was highly significantly ($p<0.01$; 54.65%) increased. The treatment with *C. scolymus* leaf and flower head extracts induced a highly significant increase in HOMA-β cell function; the leaf extract seemed to be more effective in improving the impaired HOMA-β cell function than the flower head extract. The HOMA-IS was also detectably increased as a result of treatments with *C. scolymus* leaf and flower head extracts. While the effect of leaf extract was non-significant ($p>0.05$), the effect of flower head extract highly significant ($p<0.01$). In contrast, HOMA-IR was remarkably decreased after the treatments with *C. scolymus* leaf and flower head extracts; the decrease was significant only as a result of flower head extract.

HistopathologicalResults

The histopathological examination of normal control pancreas sections showed closely packed lobules of pancreatic acini. Islets of Langerhans were embedded within the exocrine portions and alpha cells (Figure 4a). Diabetic control revealed histopathological changes of endocrine portions represented by marked decrease of β-cells and dissolution of some islets in other parts(Figures 4b and c). Congested stromal blood vessels was also seen(Figure 4b). Pancreas of rats treated with *C. scolymus* leaf (Figure 4d) and flower (Fig. 4e) showed nearly normal structure of islets of Langerhans.

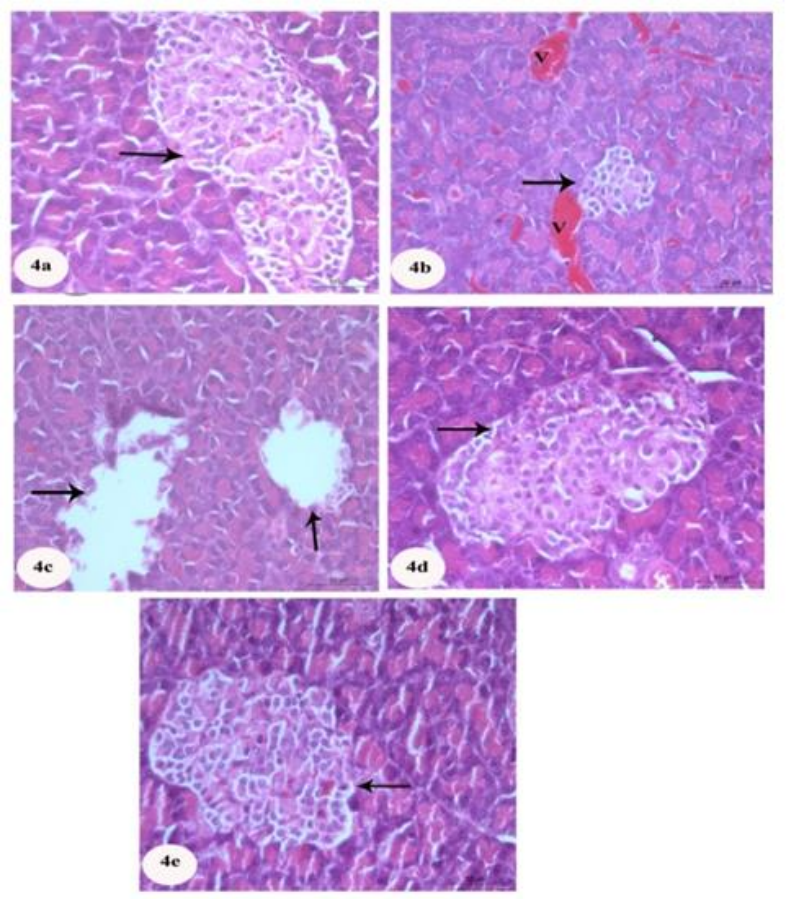

Figure 4: Photomicrographs of sections of pancreata of rats showing their histological structure and integrity in the tested groups. (a) normal group with closely packed lobules of pancreatic acini. Islets of Langerhans were embedded within the exocrine portions. (b&c) diabetic control revealed histopathological changes of endocrine part of the pancreas represented by marked decrease of β-cells and dissolution of some islets (arrows) in other parts. Congested stromal blood vessels (V) were also seen. Pancreas of rats treated with *C. scolymus* leaf (d) and flower (e) showing nearly normal structure of Islets of Langerhans (arrows). (H&E stain; Scale bar =50μm).

ImmunohistochemicalStaining of Insulin

Immunohistochemically, β-cells of the normal group stained with strong positive reaction for anti-insulin antibodies as brown granules in the cytoplasm of great number of the β-cells (Figure 5a). In diabetic group, the immune reactivity for anti-insulin antibodies was obviously decreased (Figure 5b). After treatment with *C.*

scolymus leaf extract, positive immunoreactions of β-cells for anti-insulin antibodies were obviously increased (Figure 5c). Treatment with *C. scolymus* flower head extract revealedmarkedly increased positive immunoreactions of β-cells for anti-insulin antibodies (Figure 5d).

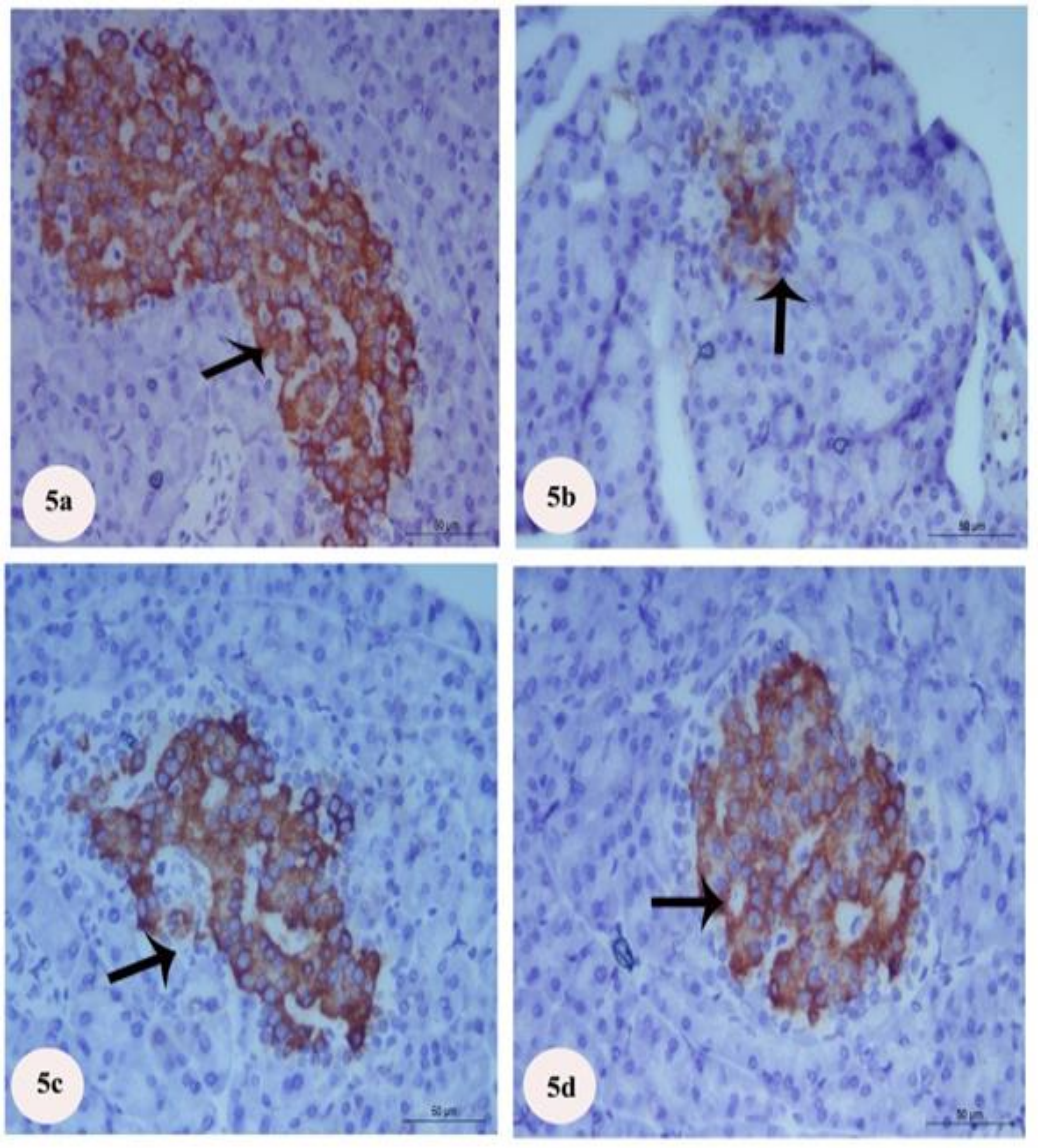

Figure 5: Photomicrographs of immunohistochemistical staining of insulin in islets of Langerhans. a: Normal group showing pancreatic β-cells with positive immunostaining (arrow) which are distributed over the center of pancreatic islets and stained with deep brown color. b: Diabetic control showing significant decrease of the immunostaining particles for insulin (arrow). c&d: Diabetic rats treated with *C. scolymus* leaf and flower respectively showing an increase in the staining particles (arrow). (Immunohistochemical staining of insulin; Scale bar =50µm).

Immunohistochemical Staining of TNF-alpha

Immunohistochemical sections of control group showed no reaction for TNF-alpha (Figure 6a), while diabetic group revealed marked increase in intensity of TNF-alpha of pancreatic islets compared to normal group (Figure 6b). Treatment with *C. scolymus* leaf and flower head extract showed an obvious reduction in intensity of TNF-alpha of pancreatic islets (Figures6c and d) reflecting decrease in the expression of this cytokine.

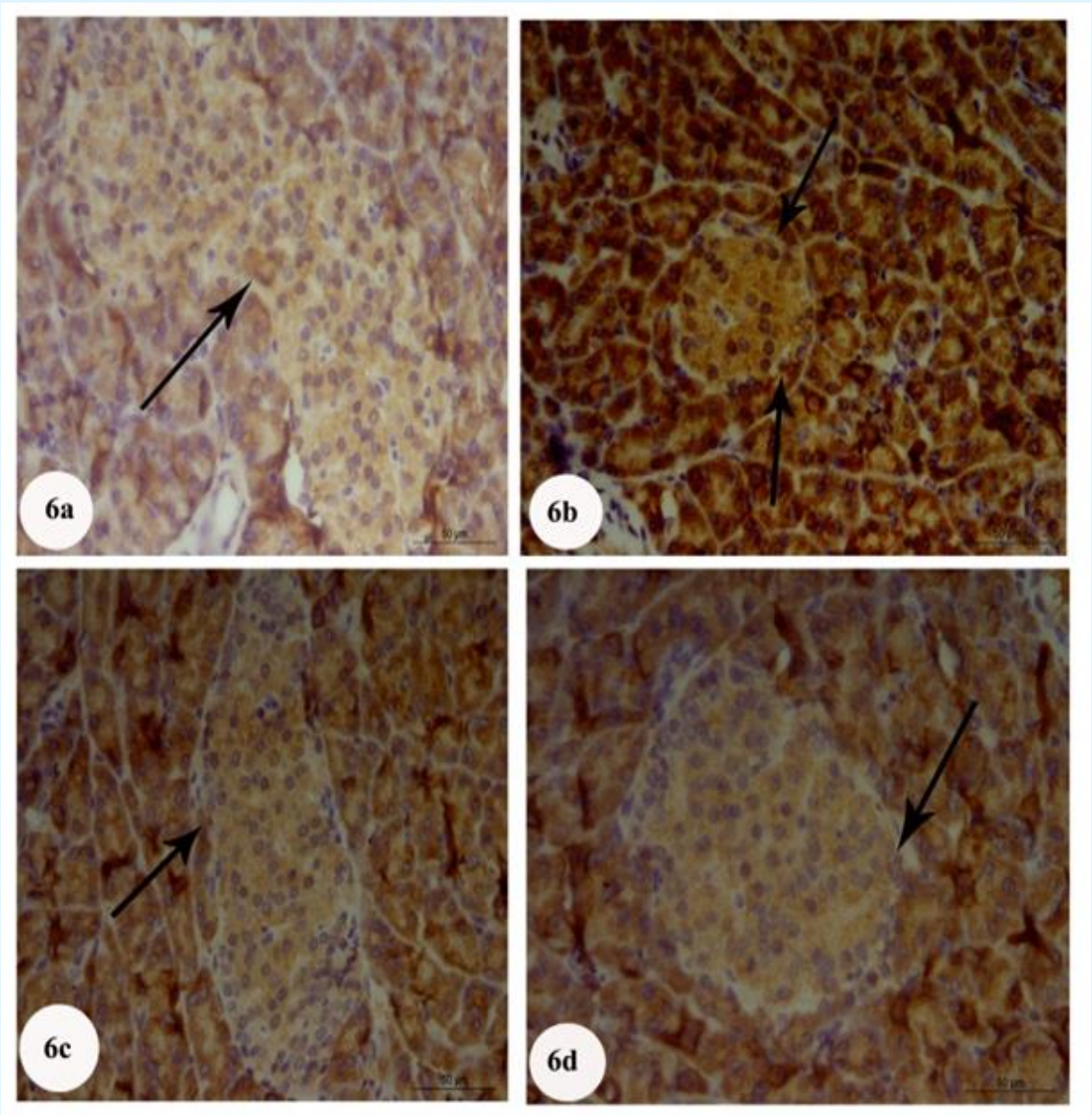

Figure 6: Photomicrographs of pancreas sections immunohistochemically stained for TNF-alpha. (a) normal islets of Langerhans (arrow). (b) diabetic group showing marked increase in intensity of TNF-alpha of islets (arrows). Diabetic rats of pancreas islets treated with *C. scolymus* leaf (c) and flower respectively (arrow) (d) showing reduced intensity of TNF-alpha. (Immunohistochemical staining of TNF-alpha; Scale bar = 50µm).

Ultra Structure Results

Acinar Cells: Ultra structure of control pancreas showed acinar cells with euchromatic nuclei, well-developed cisternae of rough endoplasmic reticulum, mitochondria and numerous electron dense secretory granules of variable sizes in the apical part (Figure 7a). Diabetic control showed marked changes in pancreatic acini represented by damaged swelling mitochondria, cytoplasmic vacuolation and an obvious decrease of zymogen granules. Nucleus with fragmented chromatin was also observed (Figures 7b and c).

Diabetic rat pancreas treated with *C. scolymus* leaf and flower head extracts showed marked improvement represented by increase in zymogen granules, regular contours of nuclei and well-flattened rough endoplasmic except few vacuoles (Figures 7d-e).

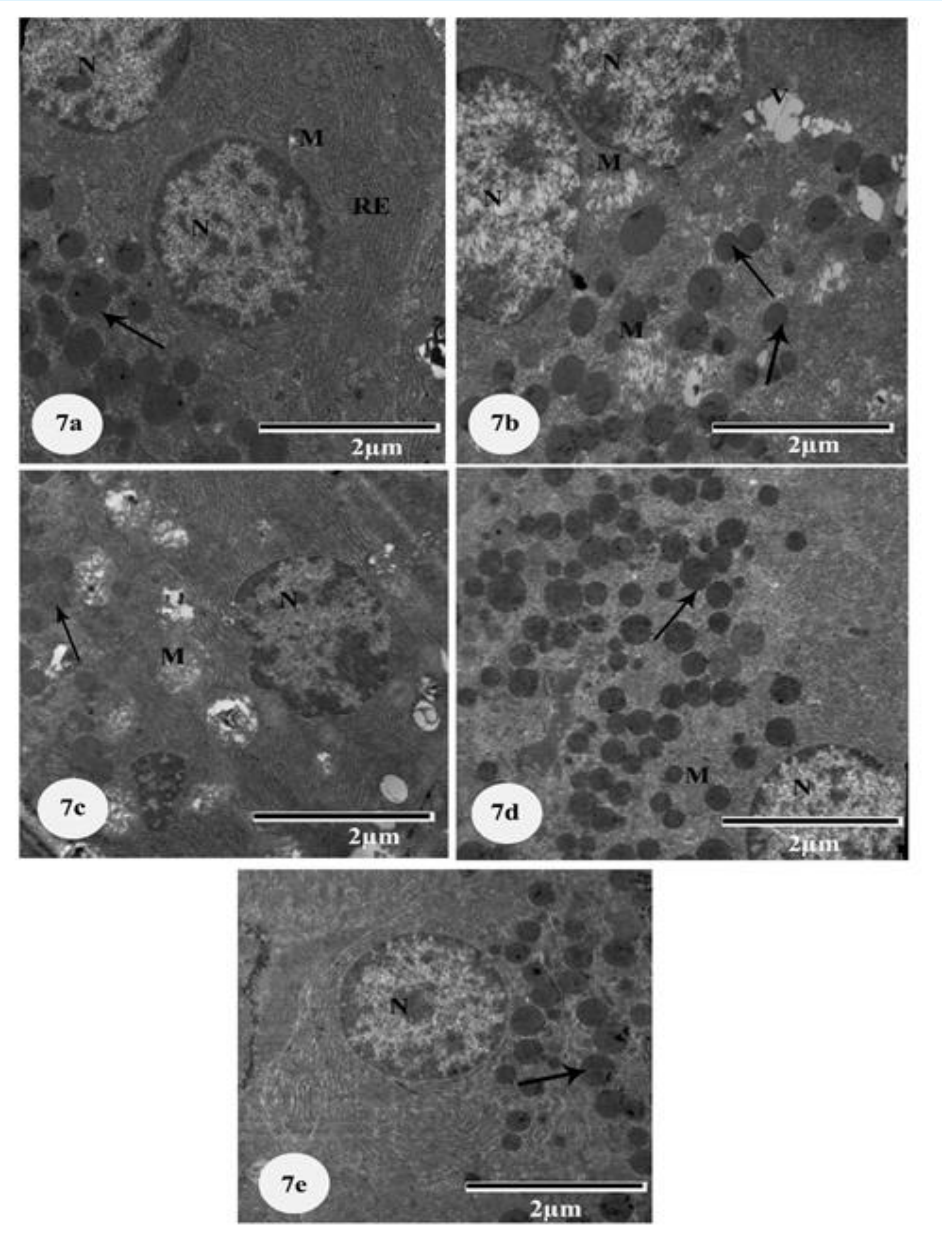

Figure 7: Electron photomicrographs of exocrine portion of pancreas showing normal acinar cells with euchromatic nuclei (N), well-developed cisternae of rough endoplasmic reticulum (RE) (a), mitochondria (M) and numerous electrons dense secretory granules (arrow) of variable sizes in the apical part. Electron photomicrographs of pancreas of diabetic control (b-c) showing marked changes in pancreatic acini represented by damaged swelling mitochondria (M), cytoplasmic vacuolation (V) and an obvious decrease of zymogen granules (arrows). Nucleus (N) with fragmented chromatin was also observed. Diabetic rat pancreas treated with C. scolymus leaf (d) and flower head (e) showing marked improvement represented by increase in zymogen granules (arrow), normal structure of mitochondria (M) and regular contours of nuclei (N). Scale bar =2 µm.

β-Cells: Islets of Langerhans of control rats were formed mainly of β-cells. Their cytoplasm contains numerous electrons dense secretory granules surrounded by wide lucent halo, mitochondria, Golgi apparatus and euchromatic nucleus (Figures 8a and b). The β-cells of diabetic rats showed vacuolation and decrease of secretory granules, fusion of some secretory granules and pyknotic nuclei (Figures 8c-d).

Treatment with *C. scolymus* leaf and flower head extract revealed euchromatic nucleus, few vacuoles in β-cells and increase of secretory granules compared to the diabetic ones (Figures 8e-f).

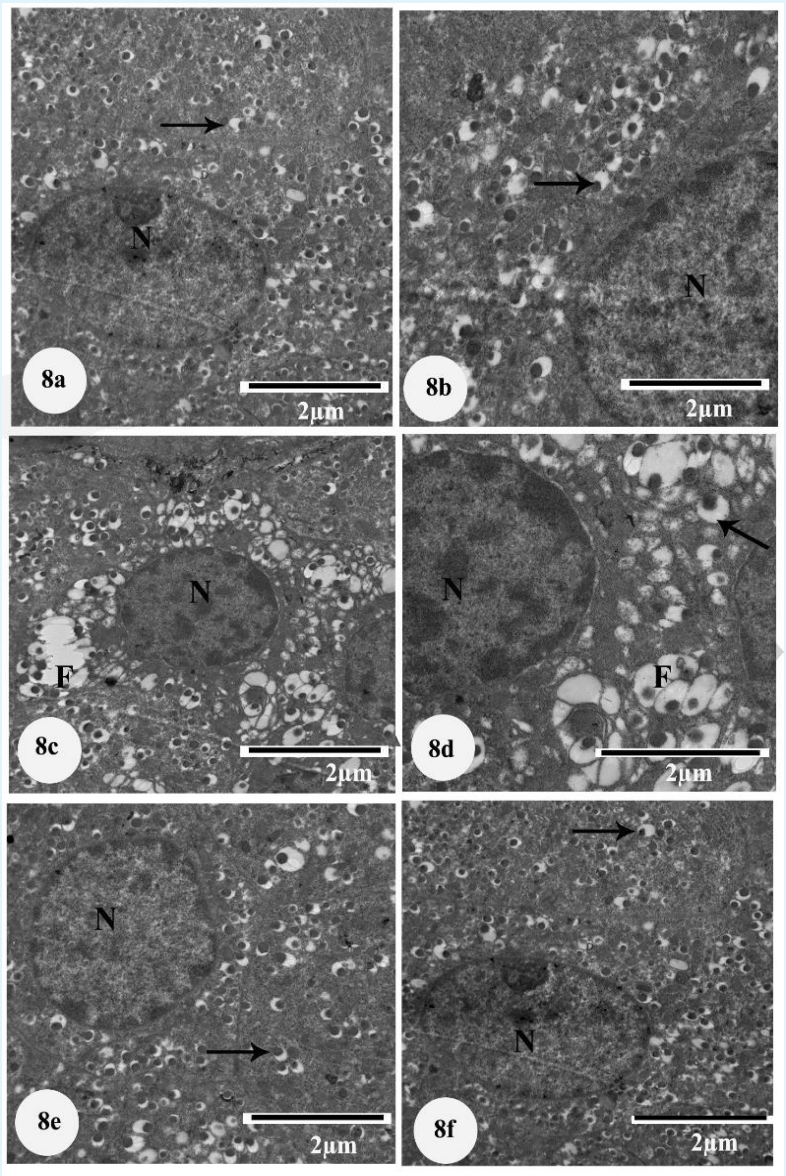

Figure 8: Electron photomicrographs of rat β-cells. a-b: Normal islets of Langerhans were formed mainly of β-cells. The cytoplasm of these β-cells contains numerous electron dense secretory granules surrounded by wide lucent halo (arrow), mitochondria (M) and euchromatic nucleus (N). 8c-d: Diabetic rat pancreas showing β-cells obvious vacuolation and decrease of secretory granules, fusion of some granules (F). Electron photomicrographs of diabetic rat pancreas treated with *C. scolymus* leaf (e) and flower head (f) hydroethanolic extract showingeuchromatic nucleus (N), few vacuoles in β-cells and increase of secretory granules (arrows) compared to the diabetic one appeared nearly similar to the control group. (Scale bar =2 µm).

Discussion

Traditional herbal medicines have been used for a long time to treat diabetes, and many controlled trials have been done to investigate their efficacy.In the present study, the hydroethanolic extracts of *C. scolymus* leaves and flower heads were chosen to assess their anti-hyperglycemic efficacies and to suggest their possible mechanism(s) of action in experimentally T2DM induced by intraperitoneal injection of STZ following NA administration [45]. To explain the mode of action of NA and STZ in this animal model of T2DM, Novelli*et al*. [46] suggested that NA injection before STZ injection resulted in a partial loss of β-cell mass by necrotic actions. In addition, STZ-NA caused a significant reduction in the number of insulin receptors on many insulin target tissues reflecting insulin resistance in such tissues [45].

In the present study, the treatment of the diabetic rats with hydroethanolic extract of *C. scolymus* leaf and flower head resulted in a significant amelioration of the

impaired glucose tolerance. The *C. scolymus* leaf extract had higher antidiabetic potency and improvement effect on OGT in NA/STZ- induced diabetic rats than *C. scolymus* flower head extract. This finding is in accordance with the data of Fantini*et al*. [47] who found that the hydroethanolic extract of *C. scolymus* flowering heads has hypoglycemic effects in both normal and obese rats and Nazni*et al*. [22] who stated that *C. scolymus* powder produced marked decrease of fasting and post-prandial blood glucose levels in diabetic subjects. In addition, Abdel Magied et al. [31] found that the treatment of diabetic rats with aqueous extracts of leaves and flower heads of two varieties of artichoke induced a significant decrease of the elevated serum glucose level. In the same way, Kuczmannová *et al*. [48] reported that daily oral administration of the *Cynara cardunculus* extract for 1 and 5 weeks significantly decreased glycemia after streptozotocin (STZ) administration. The hypoglycemic effects *C. scolymus* extract may be attributed to its effect to reduce hepatic glucose output by inhibiting hepatic glucose-6-phosphatase activity as indicated byEid and Haddad [49]. In disagreement with the present study, Fallah Huseini, *et al*. [50] found that *C. scolymus* hydroethanolic extract had no significant effects on the blood levels of fasting glucose, postprandial glucose, glycosylated hemoglobin in type 2 diabetic patients.

The improvement of OGT due to the treatment of diabetic rats with extract of *C. scolymus* leaf and flower head hydroethanolic extracts was associated with a significant increase in the lowered serum insulin and C-peptide levels as well as the calculated HOMA-β cell function and also concomitant with a remarkable amendment of pancreatic islets' histological architecture as well as the increase in the islets' size and number of β-cells within the islets.

In the present study, the adiponectin mRNA expressionin visceral adipose tissue was significantly decreased in NA/STZ-induced type 2 diabetic rats as compared with the normal, and the treatment with extract of *C. scolymus* leaf and flower head resulted in a significant amelioration. In agreement with these results, Szkudelska*et al*. [51] reported that adipocytesisolated from epididymal fat tissue of STZ-NA-induced diabetic rats exhibited impaired secretion of adiponectin. In the same way, Ahmed *et al*. [52] found that serum adiponectin level was significantly depleted in HFD/STZ-induced type 2 diabetic ratswhile it was significantly alleviated as a result of the treatment of these diabetic rats with hesperidin and naringin. Moreover, it has been shown that mice lacking adiponectin expression have reduced insulin sensitivity or are more likely to suffer from insulin resistance [53].

In contrast to adiponectin in the current study, the resistin mRNA expression exhibited a significant elevation in adipose tissue of diabetic rats as compared with the normal. The treatment of diabetic rats with *C. scolymus* leaf and flower head extracts resulted in a marked improvement.This result goes parallel with that of Ahmed, *et al*. [52]Adel Abdel-Moneim*et al*. [54] who found that serum resistin level and adipose tissue resistin mRNA expression were significantly increased in high fat diet/streptozotocin-induced type 2 diabetic rats as compared with normal group, and the treatment of HFD/STZ diabetic rats with hesperidin and naringin induced a significant amelioration. It is worth mentioning here that resistin, a low molecular weight protein secreted from adipose tissue, acutely induces insulin resistance and glucose intolerance [55,56,57]. In addition, down-regulation of resist in expression and release from adipose tissue has been implicated in the insulin-sensitizing actions of many anti-hyperglycemic agents [58].

The depletion inadipose tissue adiponectinm RNA expression and the increase in resistin mRNA expression in NA/STZ-induced type 2 diabetes mellitus in association with reduced calculated HOMA-IS and raised calculated HOMA-IR reflects the role of these two adipokines in inducing insulin resistance and thereby impaired glucose tolerance in such animal model of diabetes mellitus.The treatment of NA/STZ-induced type 2 diabetic rats with *C. scolymus* leaf and flower head extract produced a marked increase in HOMA-IS and a decrease in HOMA-IR. However, while the effect of flower head extract was significant, the effect of leaf extract was not.

In the present study, the NA/STZ-induced diabetic rats showed marked changes of the islets of Langerhans represented by few small pancreatic islets compared to the normal rats. These changes are similar to the results of Al-Hariri *et al*. [59] who observed few islets of Langerhans in diabetic group compared with normal group. The *C. scolymus* leaf and flower head extract treated rats exhibited marked increase in the number and size of islets of Langerhans as well as the number of islets cells within each islet. The improved pancreatic islet histological architecture and integrity attributed the increase in serum insulin and C-peptide levels as well as the enhanced HOMA-β cell function.

In NA/STZ-induced diabetic rats of the current study, the immune reactivity for anti-insulin antibodies was markedly decreased in number of insulin cells. The *C. scolymus* leaf and flower head extract treated diabetic groups exhibited a high immunoreactivity evidenced by increase in the density and areas stained yellowish brown colour. These results are in accordance with Coskun*et al*. [60], Mendez and Haro Hernandez [61], Ao*et al*. [62], Simsek*et al*. [63] and Abdul-Hamid and Moustafa [64].

In the present study, pancreatic islets of NA/STZ-induced diabetic rats showed a significant increase of TNF-alpha expression.The treatment of diabetic rats with *C. scolymus* leaf and flower head extract showed a remarkable reduction in the TNF-alpha. These results are consistent with Ferreira *et al*. [65] who observed an increase the intensity of TNF-alpha immune stained cell in pancreas of diabetes group, while treatment with minocyclineor glibenclamide daily for 30 days resulted in marked reduction in the intensity of TNF-alpha immune stained cell. Since plasma TNF-alpha is associated to insulin resistance, one can assume that this cytokine plays a significant role in the pathogenesis of chronic insulin resistance in humans [66]. Moreover, TNF-alpha is a proinflammatory cytokine that may havea role in the mild damage of islets cells by inflammation in NA/STZ-induced diabetes. Its decrease in the treated diabetic rats may be in turn of importance in the improvement of insulin resistance and islets histological architecture and integrity.

The present ultra-structural study of the diabetic group showed marked changes in pancreatic acini represented by decrease of secretory granules, cytoplasmic vacuolation and damaged mitochondria. Moreover, β-cells showed obvious vacuolation and decrease of secretory granules. On the other hand, the β cells of *C. scolymus* leaf and flower head extract treated diabetic groups showed an increase of secretory granules number and less vacuolated cytoplasm in comparison with the diabetic control group. These evidences were concomitant with the present immunohistochemical results which indicated a high immunoreactivity of anti-insulin antibodies reflecting increased insulin expression in the β cells of treated diabetic rats as compared with the diabetic control group. The present finding is in agreement with the result of Ahmed *et al*. [67] and Abdul-Hamid and Moustafa [68] who observed an increase of insulin secretory granules in STZ-induced diabetic rats treated with various plant constituents as compared with the corresponding diabetic control.

In conclusion, the present study suggested that the *C. scolymus* leaf and flower head hydroethanolic extract improved the oral glucose tolerance *via* enhancement of the insulinogenic effects and attenuation of insulin resistance. The leaf extract was more potent in improving the oral glucose tolerance. Moreover, while the leaf extract seemed to have more potent insulinogenic effect, the flower head extract had stronger activity in improving insulin sensitivity and in suppressing insulin resistance.

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