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CUCUMIS SATIVUS AND *CUCURBITA MAXIMA* EXTRACT ATTENUATE DIABETES-INDUCED HEPATIC AND PANCREATIC INJURY IN A RAT MODEL

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Diabetes is usually associated with oxidative stress that causes hepatic and pancreatic tissue injury. This work was carried out to evaluate the effect of *Cucumis sativus* and *Cucurbita maxima* methanol extracts on the streptozotocin-induced diabetic hepatic and pancreatic injury in rats. Diabetes was induced in seven equal groups of rats by a single intraperitoneal injection of streptozotocin (40 mg/kg), in addition to the non-diabetic control group. Two diabetic groups were treated with *Cucumis sativus* methanol extract and two were treated with *Cucurbita maxima,* each at 200 and 400 mg/kg for 21 days after streptozotocin-induced diabetes. Another diabetic group was treated with both *Cucumis sativus* and *Cucurbita maxima* at 200 mg/kg of each. Another group was treated with metformin (200 mg/kg orally). The plant extracts normalized serum liver enzymes activities, oxidative stress markers, and restored serum proteins and lipid profile. They also significantly reduced blood sugar to values comparable to non-diabetic rats. The hypoglycemic effect is also confirmed by the improvement of the immunohistochemical expression of insulin in β-cells of islets of Langerhans. Hepatic and pancreatic protection was also confirmed by the improvement of the histopathological picture as compared to STZ-diabetic rats. The GC-MS analysis revealed the presence of 35 and 34 compounds in the methanol extract of cucumber and pumpkin, respectively. Finally, the methanol extract of cucumber and pumpkin could be beneficial acting synergistically in the protection of the liver and pancreas against diabetes-induced tissue damage.

Key words: *diabetes, streptozotocin-induced diabetes, hepatic injury, pancreatic injury, islets of Langerhans, Cucumis sativus, Cucurbita maxima, phytochemical analysis, hepatoprotection*

INTRODUCTION

Diabetes mellitus is a complex syndrome characterized by hyperglycemia, abnormalities in lipid profile (1), microvascular and macrovascular complications (2-4) which can lead to loss of visual, renal, and neurologic functions (5), impaired mobility and cognition, poor quality of life, and productivity (1, 2). The number of people with diabetes is expected to go up to 642 million by 2040 (6). The liver is the most susceptible organ to the oxidative stress induced by hyperglycemia leading to liver tissue injury. This is followed by impairment of carbohydrate, protein, and lipid metabolism, thereby leading to an exaggeration of the oxidative stress and further intensity of the inflammatory process (7). Both oxidative stress and inflammatory responses act as damaging agents aggravating the pathogenesis of hyperglycemia (8). The mechanism of diabetes-induced liver damage as a contribution to the combination of increased oxidative stress and exaggerated inflammatory response may be due to the accumulation of oxidative damage products in the liver, such as malondialdehyde, fluorescent pigments, and conjugated dienes (9). Despite the progress in the management of diabetes and its pathogenesis, there are still several challenges, among which are the side effects and cost of synthetic antidiabetic drugs. These challenges necessitate a change in lifestyle and the search for more potent, safer and cheaper alternatives (10). Medicinal and edible plants are promising natural sources of therapeutic agents such as hepatoprotectives, antidiabetics, and antioxidants (11). Members of the family *Cucurbitaceae* being rich in proteins, unsaturated fatty acids, phenolic compounds, carotenoids, tocopherols, phytosterol, and squalene, have proven to exert several pharmacological activities such as antioxidative (12), anticancer (13), antihypertensive, cardioprotective, and antilipemic (14). The hypolipidemic and hypoglycemic activities of cucumber, white pumpkin and ridge gourd were evaluated in alloxan-induced diabetic rats (15). The antidiabetic potential of ethanol extracts *of Cucumis sativus* in control of blood glucose levels and effectiveness on various biochemical parameters were tested (16). Pumpkin polysaccharides have been reported to have hypolipidemic and hypoglycemic properties (17). However, information on the hepatoprotective and antihyperglycemic effect of *Cucumis sativus* and *Cucurbita maxima* fruit extract and the potential synergistic effect between them are incomplete. This work aims to explore the protective effect of *Cucumis sativus* and *Cucurbita maxima* methanol extracts against diabetic-induced hepatic and pancreatic injury in streptozotocin-

induced diabetes in the rat model. The potential synergistic effect between them and the phytochemical constituents of both extracts by the GC/MS analysis were also studied.

MATERIALS AND METHODS

Ethics statement

This experiment was conducted according to the guidelines of the Institutional Animal Care and Use Committee, Veterinary Medicine, Cairo University (Vet. CU. IACUC) Approval Protocol No.: Vet CU 20022020147.

Plants used and preparation of extracts

Cucumis sativus (cucumber, CUC) and *Cucurbita maxima* (pumpkin, PUM) were obtained from the local market and identified by the Flora and Phyto-Taxonomy Researches, Horticultural Research Institute, Agricultural Research Centre, Dokki, Giza, Egypt. The fruits were air-dried, powdered and kept in dark bottles (voucher no.: CUC10 and PUM 10). Four hundred grams of each plant were extracted by percolation several times with methanol 70% until complete exhaustion. The methanol 70% was used because it has been reported to give the highest yield as compared to other solvents (18). The solvent was then evaporated by rotary evaporator at 40°C until a semisolid extract was obtained. The extract was divided into small portions and kept refrigerated (4°C) until used. Known grams (10 g) from the extract were freshly suspended in distilled water using few drops of tween 80 as a suspending agent to prepare a suspension with a concentration adjusted to 400 mg/ml.

Animals

Forty-eight Sprague Dawley rats of both sexes with average body weight (170 – 200 g) were obtained from the experimental animal colony unit, Vacsera, Helwan Egypt. The rats were kept for two weeks for acclimatization under strict hygienic measures at room temperature (25 ± 5°C) and exposed to daily natural 12-hour light-dark cycles. The rats were fed pelleted balanced diet consisting of the following ingredients purchased from Agricultural Development Company, 6-October, Giza, Egypt: sunflower oil (15%), concentrate mixture 45% (10%), yellow corn (49%), soybean meal 44% (11%), wheat bran (10%), molasses (3%), common salt (0.5%), ground limestone (0.2%), dicalcium phosphate (0.1%), lysine (0.2%), dl-methionine (0.7%) and mineral-vitamin premix (0.3%). Water and feed were offered *ad libitum*.

Induction of diabetes

The rats were supplied with fructose for one week before the induction of diabetes to prevent severe hypoglycemia (19). Streptozotocin (STZ, Aldrich Company, USA), was prepared in 0.1 M citrate buffer (pH 4.5). Diabetes was induced in the rats by intraperitoneal injection of STZ at a dose of 40 mg/kg body weight (19). Diabetes was confirmed in fasted rats by determining the blood glucose levels after 72 h post diabetes induction using Lifescan One Touch II® Glucometer (20). If the value obtained was more than 150 mg/dl, then diabetes has been successfully induced.

Study design

The rats were assigned randomly into eight groups of six rats each. Rats of group I received an equal volume (1 ml) of distilled water and were kept as a negative control. Diabetes was induced in the rats of groups II – VI as described in the induction of diabetes. The rats of group II were given 1 ml of distilled water orally and kept as a non-treated diabetic group (positive control). The rats of group III and IV were treated orally with CUC methanol extract daily at a dose of 200 and 400 mg/kg, respectively. The rats of group V and VI were treated orally with PUM methanol extract daily at a dose of 200 and 400 mg/kg. The rats of group VII were treated orally with both CUC and PUM methanol extract at a dose of 200 mg/kg of each. The rats of group VIII were treated orally with metformin, a reference hypoglycemic drug, at a dose of 200 mg/kg. The treatment lasted for 21 days after the establishment of STZ-induced diabetes. The used doses were selected since previous studies (16) reported that there were no signs of discomfort or toxicity in doses up to 1000 mg/kg in rats.

Samples

One drop of blood was taken every week (on days 7, 14, and 21 of the experimental periods) in the morning from the tail vein of each rat for estimation of blood glucose levels directly. At the end of the experiment, a blood sample (1.5 ml) was taken from the medial canthus of the eye under pentobarbital anesthesia. The blood samples were allowed to clot and clear serum was obtained by centrifugation at 3000 r.p.m for 15 minutes. The clear non-hemolyzed supernatant sera were quickly removed for analysis of various biochemical parameters. The sera were kept at –20°C until biochemical analysis. The animals were then euthanized by an overdose of the anesthetic solution (thiopental sodium 100 mg/kg intraperitoneal) and tissue specimens were collected immediately from the liver and pancreas of each animal, rinsed with isotonic saline and fixed in 10% buffered formalin. Paraffin-embedded tissue sections were performed for histopathological examination. Paraffin-embedded tissue sections from the pancreas were also used to determine insulin expression in islets of Langerhans. Another liver specimen was minced and homogenized with 10% (w/v) phosphate-buffered saline (0.1 M, pH 7.4). After centrifugation, the supernatant of the homogenate was collected and used to estimate the antioxidant parameters.

Hepatoprotective assessments

1. Serum biochemical parameters

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities, and total proteins, albumin, total bilirubin, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) levels were determined spectrophotometrically using commercial test kits (Biodiagnostic Co, Egypt) according to the manufactures' instructions. Low-density lipoprotein-cholesterol (LDL-C) was calculated according to Friedewald's equation (21). The total globulins were calculated by subtracting the obtained value of albumin from the total proteins.

2. Determination of oxidant/antioxidant markers

Glutathione-s-transferase (GST) activity, reduced glutathione (GSH) content, lipid peroxidase (MDA, malondialdehyde); CAT, catalase activity, and nitric oxide (NO) level were measured in the liver homogenate spectrophotometrically using commercial test kits (Biodiagnostic Co, Egypt) according to the manufactures' instructions. All parameters were analyzed using Spectrophotometer (T80 UV/VIS PG instrument Ltd, UK).

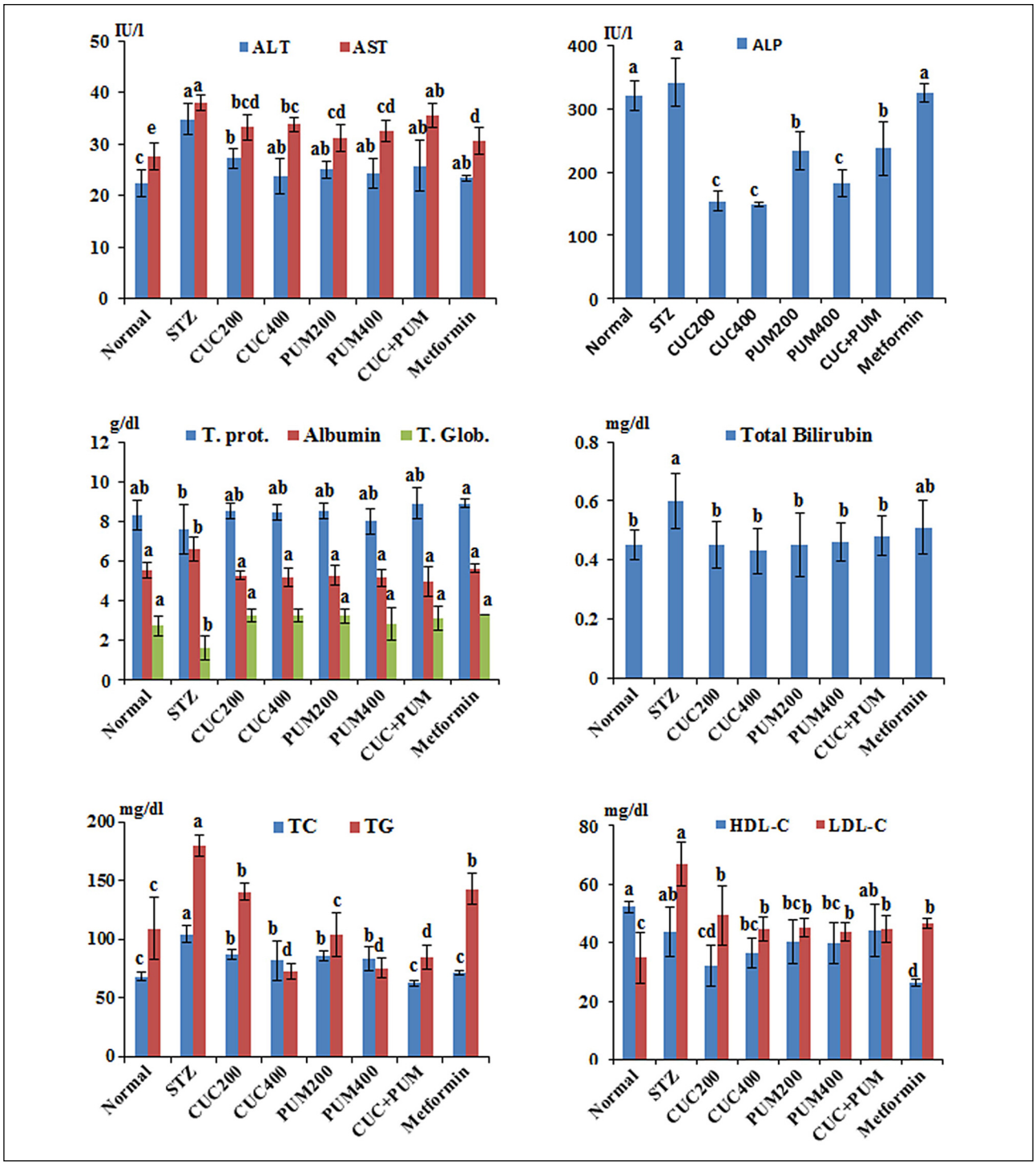

Fig. 1. Effect of cucumber (CUC) and pumpkin (PUM) methanol extract (200 and 400 mg/kg) each alone or in combination (200 mg/kg of each) on the serum liver enzyme (ALT, AST and ALP) activities, protein profile (total proteins (T. prot.), albumin and total globulins (T. Glob.) and lipid profile (total cholesterol (TC), triglycerides (TG), high- and low-density lipoprotein cholesterol (HDL-C; and LDL-C), in STZ-diabetic rats. Results are mean ± S.D., $p<0.05$, n=6.

Hypoglycemic assessments

1. Determination of blood glucose

Blood sugar (mg/dl) was estimated in blood samples with Lifescan One Touch II® Glucometer which has been tested for accuracy and precision against a Beckman Synchron CX7 analyzer that depends on the glucose oxidase colorimetric method (20).

2. Immunohistochemical expression of insulin in β-cells of the islets of Langerhans

The avidin-biotin complex peroxidase (ABC) technique was applied for immunostaining of insulin expression (β cells of Langerhans) in formalin-fixed, paraffin-embedded tissue sections of the pancreas. Sections of 5 µm thick were deparaffinized in xylene and rehydrated through graded alcohols followed by microwaved (heated) in 0.01 M citrate buffer solution for 20 min for antigen retrieval. Endogenous peroxidase

Table 1. Effect of cucumber (CUC) and pumpkin (PUM) methanol extracts either alone (200 and 400 mg/kg) or in combination (200 mg/kg of each) on the oxidant/antioxidant parameters on the liver homogenates of STZ- diabetic rats (mean ± SD, n = 6).

Groups	**GST** (U/g tissue)	**GSH** (mM/g tissue)	**MDA** (nmol/g tissue)	**Catalase** (µmol/g tissue)	**Nitric oxide** (µmol/100g tissue)
Normal control	660.0 ± 31.4^{bc}	49.4 ± 7.1^{b}	6.11 ± 0.5^{b}	802.4 ± 49.8^{c}	1.9 ± 0.2^{b}
STZ	560.5 ± 33.0^{a}	27.8 ± 2.8^{a}	8.82 ± 0.1^{d}	645.0 ± 49.0^{a}	2.0 ± 0.2^{c}
CUC200 + STZ	638.0 ± 13.5^{b}	29.6 ± 3.9^{a}	6.8 ± 1.3^{ab}	728.2 ± 59.1^{b}	2.0 ± 0.2^{b}
CUC400 + STZ	560.0 ± 10.0^{a}	31.0 ± 4.4^{a}	6.64 ± 2.9^{ab}	729.4 ± 56.1^{b}	1.3 ± 0.1^{a}
PUM200 + STZ	656.0 ± 15.2^{bc}	26.8 ± 2.2^{a}	7.3 ± 0.5^{bcd}	767.6 ± 49.1^{bc}	1.4 ± 0.0^{a}
PUM400 + STZ	650.0 ± 12.2^{bc}	28.0 ± 1.2^{a}	7.0 ± 0.5^{bcd}	766.4 ± 48.3^{bc}	1.4 ± 0.0^{a}
CUC + PUM + STZ	533.0 ± 27.8^{b}	28.2 ± 5.4^{a}	4.1 ± 0.5^{a}	788.0 ± 7.6^{bc}	1.5 ± 0.1^{a}
Metformin	673.0 ± 12.0^{c}	30.4 ± 4.4^{a}	8.1 ± 1.0^{cd}	775.8 ± 14.6^{bc}	1.5 ± 0.1^{a}

Means of different letters in the same column are significantly different at $P < 0.05$;
GST, glutathione s-transferase; GSH, reduced glutathione; MDA, malondialdehyde.

activity was blocked by incubation with 3% hydrogen peroxide (H_2O_2) for 15 min. After washing the slides with phosphate-buffered saline (PBS), all sections were incubated with 5% normal goat serum (Dako, USA) for 30 min at room temperature to block non-specific binding. The slides were then incubated overnight at 4°C with rabbit anti-insulin primary polyclonal antibody (Difco Lab, USA). After washing 3 times with PBS, the sections were incubated for 30 mins at room temperature with goat anti-rabbit immunoglobulin G biotinylated secondary antibody. The sections were then incubated with horseradish peroxidase-labeled streptavidin (Dako, USA). The site of antibody binding was visualized using DAB (3,3-diaminobenzidine tetrahydrochloride) as chromogen which gave dark brown precipitate. Hematoxylin was used as the counterstain. All sections were examined under an optical microscope (Model CX41, Olympus, Japan).

Histopathological examination

The formalin-fixed tissue samples from the liver and pancreas were embedded in paraffin, processed routinely, stained with hematoxylin and eosin (H & E) and subjected to histopathological examination microscopically (22).

Phytochemical analysis of methanol extracts of the tested plants

Phytochemical analysis was performed to explore the active constituents of the tested plants. GC-MS analysis of methanol extracts of cucumber and pumpkin were performed at Micro Analytical Center- Cairo University using a Perkin Elmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS) equipped with an Elite-1 fused silica capillary column (30 mm × 0.25 mm ID × 1 iMdf, composed of 100% dimethyl poly siloxane). An electron ionization system with an ionization energy of 70 eV was used for detection. The flow rate of helium (99.999%) and the carrier gas was 1 ml/min. The injected volume (2 µl) was employed (split ratio of 10:1). The Injector temperature was 250°C and the Ion-source temperature was 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 100°C to 200°C/min, then 5°C/min to 280°C, ending with a 10 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scanning interval of 0.5 seconds and fragments from 50 to 1000 Da. Total GC running time was 10 minutes at the scanning speed of 2000. The software adopted to handle mass spectra and chromatograms was Turbo Mass Ver5.2.0.

Statistical analysis

Data were presented as mean ± SD. Differences between means were tested for significance by ANOVA followed by the Duncan test using SPSS version 16 computer program (SPSS Inc., Chicago, USA). The difference of means at $P < 0.05$ is considered significant.

RESULTS

Hepatoprotective effect

STZ-induced diabetes significantly ($P < 0.05$) increased serum ALT, AST and ALP activities as compared to normal non-diabetic rat. Oral administration of methanol extract of CUC and PUM alone or in combination decreased the ALT, AST and ALP activities as compared to STZ-diabetic rats or normal non-diabetic ones (*Fig. 1*).

STZ significantly ($P < 0.05$) decreased the total protein and total globulin levels as compared to the negative control non-diabetic rats. Little effect was observed on the levels of albumin and total bilirubin in the serum of diabetic rats. Oral administration of methanol extract of CUC and PUM alone or in combination maintained the concentration of total proteins at levels comparable to normal non-diabetic ones (*Fig. 1*).

STZ markedly ($P < 0.05$) increased the TC and TG concentrations in the serum of diabetic rats compared to the negative control rats. Oral administration of methanol extract of CUC and PUM alone or in combination normalized serum TC and TG levels. STZ significantly ($P < 0.05$) increased LDL-C but decreased HDL-C in the serum of diabetic rats as compared to the negative control rats. Oral administration of methanol extract of CUC and PUM alone or in combination normalized serum HDL-C and LDL-C (*Fig. 1*). STZ significantly ($P < 0.05$) decreased the GST, GSH and CAT activities (*Table 1*). On the other hand, the MDA and NO concentration were significantly increased in diabetic rats.

Oral administration of methanol extract of CUC and PUM methanol extract into STZ-diabetic rats significantly attenuated these changes (*Table 1*). CUC was more effective. In comparison to the positive control, the CUC and PUM methanol extract-treated rats exhibited values of oxidative stress markers more or less similar to the negative control. CUC appeared to be more effective. Co-administration of both CUC and PUM into diabetic rats significantly alleviated the STZ-induced changes in MDA and NO levels and CAT activity, similar to metformin treatment (*Table 1*).

Table 2. Effect of cucumber (CUC) and pumpkin (PUM) methanol extracts either alone (200 or 400 mg/kg) or in combination (200 mg/kg of each) on the blood sugar level of rats (mg/dl) (mean ± SD, n = 6).

Groups	Weeks post treatment		
	1	2	3
Normal control	121.0 ± 7.0^{a}	136.6 ± 17.6^{bc}	110.3 ± 10.8^{a}
STZ	244.7 ± 8.0^{c}	199.0 ± 18.8^{d}	157.3 ± 5.9^{c}
CUC200 + STZ	124.2 ± 17.0^{a}	143.4 ± 10.5^{c}	126.8 ± 10.9^{abc}
CUC400 + STZ	118.0 ± 7.4^{a}	142.8 ± 5.4^{c}	113.6 ± 9.4^{ab}
PUM200 + STZ	117.6 ±11.5^{a}	122.2 ± 11.8^{ab}	130.0 ± 18.2^{abc}
PUM400 + STZ	159.3 ± 22.3^{b}	131.8 ± 16.1a^{bc}	124.4 ± 17.0^{abc}
CUC + PUM + STZ	138.5 ± 12.4^{a}	144.5 ± 4.9^{c}	121.4 ± 12.6a^{bc}
Metformin	131.3 ± 17.2^{a}	112.0 ± 9.0^{a}	130.3 ± 6.1^{abc}

Means of different letters in the same column are significantly different at $P < 0.05$.

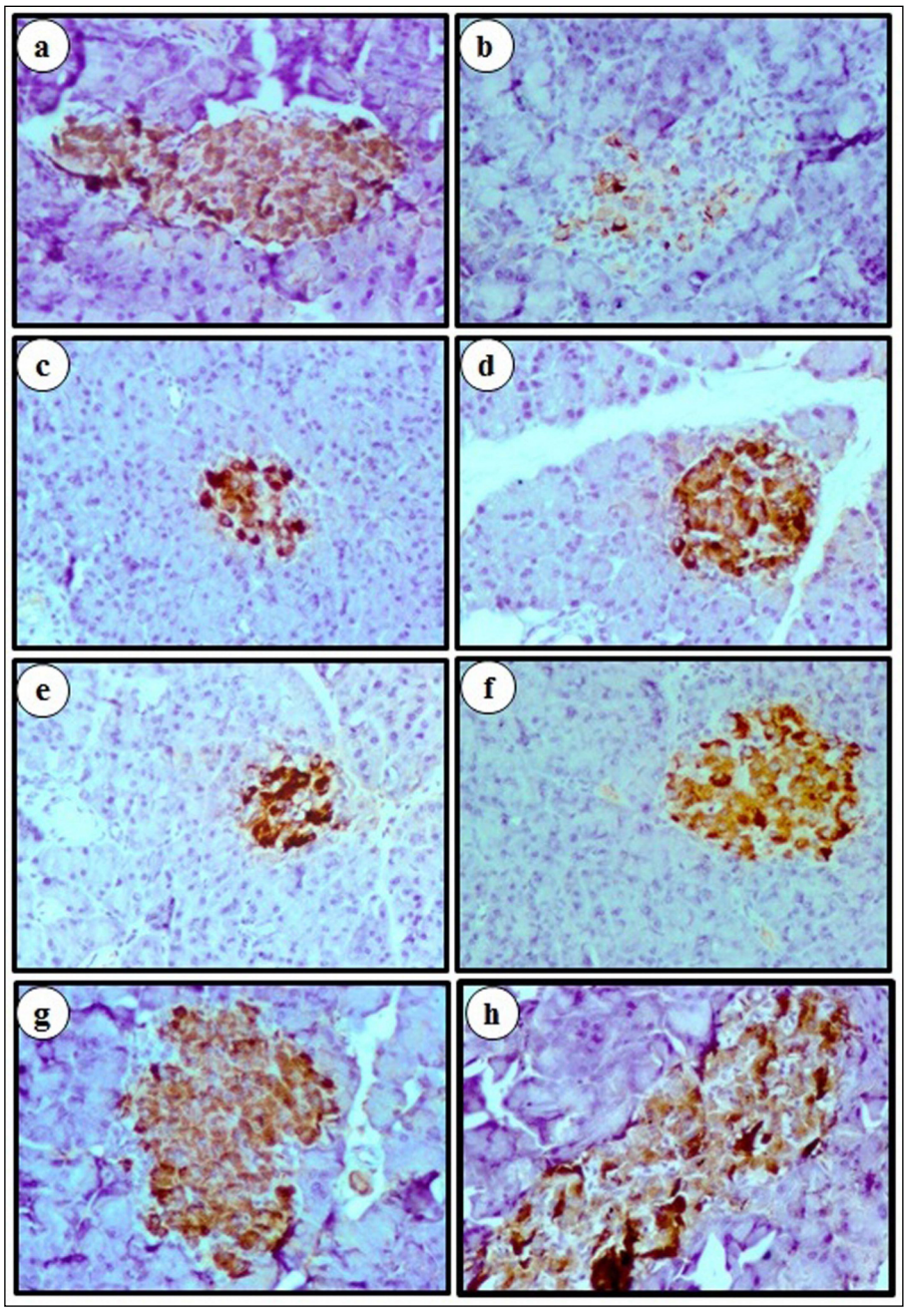

Fig. 2. Immunoreactivity of insulin in β-cells of Langerhans islets of rats: (a): normal control showing strong immunoreactivity of insulin in β-cells of Langerhans islets (dark brown granules in the cytoplasm of β-cells); (b): diabetic showing marked reduction in number and area of insulin-positive β-cells; (c): diabetic rats treated with cucumber (200 mg/kg) showing a marked increase in the number and area of insulin-positive β-cells; (d): diabetic rats treated with cucumber (400 mg/kg) showing strong positive immunostaining in most β-cells; (e): diabetic rats treated with pumpkin (200 mg/kg) showing improvement in the number and area of positive immunoreactive β-cells; (f): diabetic rats treated with pumpkin (400 mg/kg) showing strong positive immunoreaction in the most of β-cells; (g): diabetic rats treated with cucumber and pumpkin extracts (200 mg/kg of each), showing strong positive insulin expression in pancreatic β-cells more or less similar to negative control and (h): diabetic rats treated with metformin (200 mg/kg) showing strong positive immunoreaction in most of β-cells. DAB, diamino benzidine tetra-hydrochloride and hematoxylin counterstain, magnification × 200.

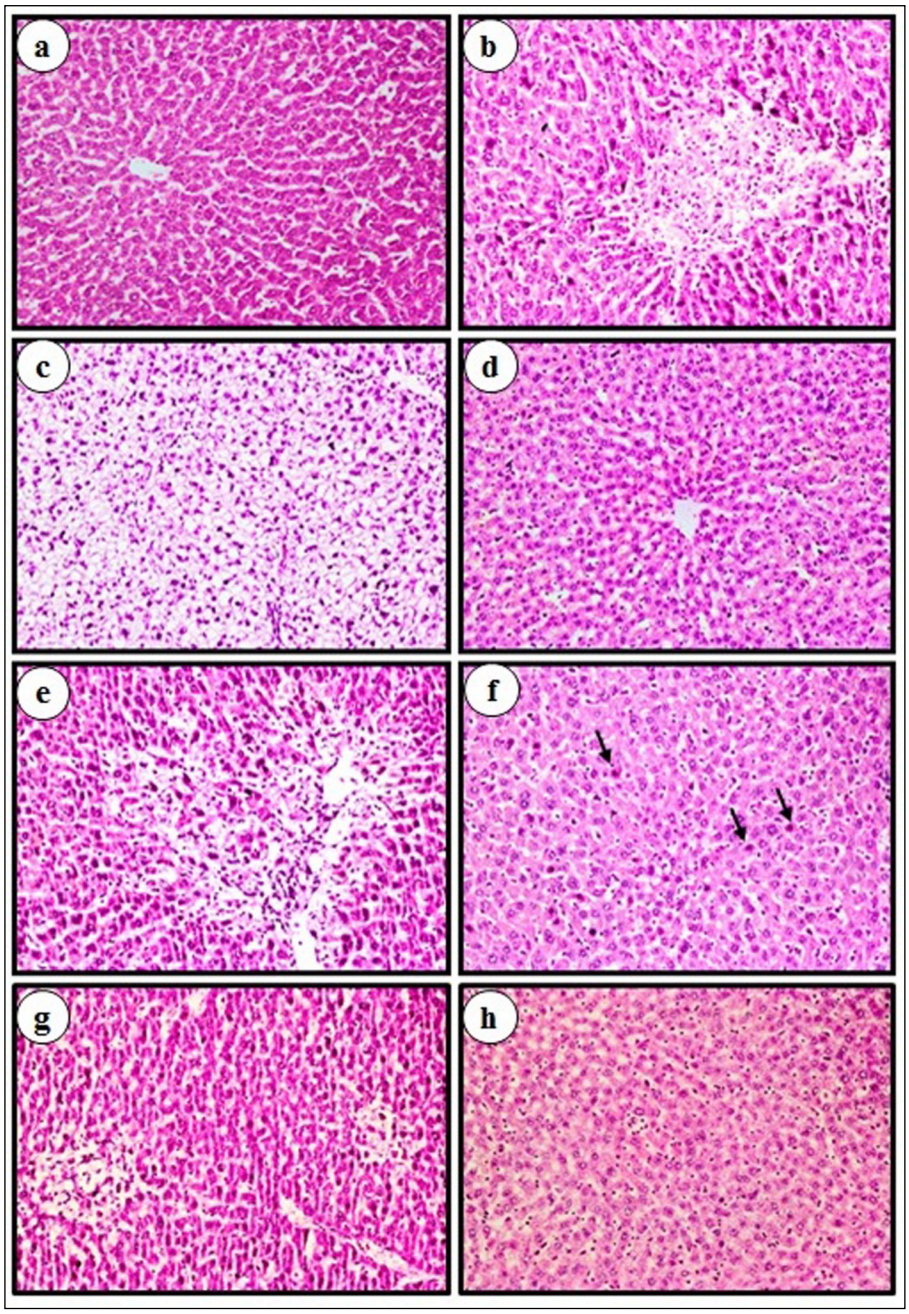

Fig. 3. Histopathological examination of the liver sections of rats: (a): normal control rat, showing normal hepatic architecture; (b): STZ-induced diabetic rat, showing severe necrotic area, mononuclear cell infiltration as well as disorganized cords with individual cell necrosis; (c): diabetic rat treated with cucumber (200 mg/kg), showing diffuse vacuolar degeneration with activation of Kupffer cells; (d): diabetic rat treated with cucumber (400 mg/kg), showing normal hepatic parenchyma associated with mild infiltration of mononuclear cells; (e): diabetic rats received pumpkin (200 mg/kg), showing the focal area of extensive centrilobular coagulative necrosis associated with infiltration of mononuclear cells; (f): diabetic rat received pumpkin (400 mg/kg), showing normal hepatocytes and coagulative necrosis of single cells (arrows); (g): diabetic rat received pumpkin and cucumber (200 mg/kg, each), revealed focal areas of hepatic cell necrosis associated with infiltration of mononuclear cells, and (h): diabetic rat treated with metformin (standard antidiabetic drug, 200 mg/kg), exhibited normal architecture of hepatic parenchyma, in addition to mild mononuclear inflammatory cells infiltration. H & E, magnification × 100.

Hypoglycemic effect

STZ significantly ($P < 0.05$) increased the blood glucose level for at least 17 days. Blood sugar was then declined to levels comparable to normal levels. Oral administration of methanol extract of either CUC or PUM significantly decreased the blood sugar level in a dose-dependent manner through the whole experimental period when compared to STZ-diabetic rats. Co-administration of both CUC and PUM into diabetic rats showed a strong hypoglycemic effect when compared to STZ-diabetic rats. The blood sugar level in this group was comparable to the normal non-diabetic and metformin-treated rats (*Table 2*).

Immunohistochemistry of pancreatic islets

The pancreas of normal rats showed a strong immunopositive reaction for anti-insulin antibodies that appeared as dark brown granules occupying the cytoplasm of β-cells of the islets of Langerhans. The exocrine portion of the pancreas was completely negative for insulin (*Fig. 2a*). The pancreas of STZ-induced diabetic rats showed a marked reduction in immunohistochemical expression of insulin in β-cells whereas scattered cells were faint to moderate immunopositive for insulin (*Fig. 2b*). The pancreas of cucumber- or pumpkin-treated diabetic rats showed a dose-dependent increase in the number and percentage area of immunoreactive β-cells of the islets. The pancreatic tissue revealed the maintenance and restoration of normal immunochemical expression of insulin in the pancreatic β-cells with strong positive immunostaining (*Fig. 2c-2f*). The pancreas of diabetic rats received an extract of both pumpkin (200 mg/kg) and cucumber revealed strong positive immunoreactivity in pancreatic β cells nearly similar to the control group (*Fig. 2g*) and metformin-treated rats (*Fig. 2h*).

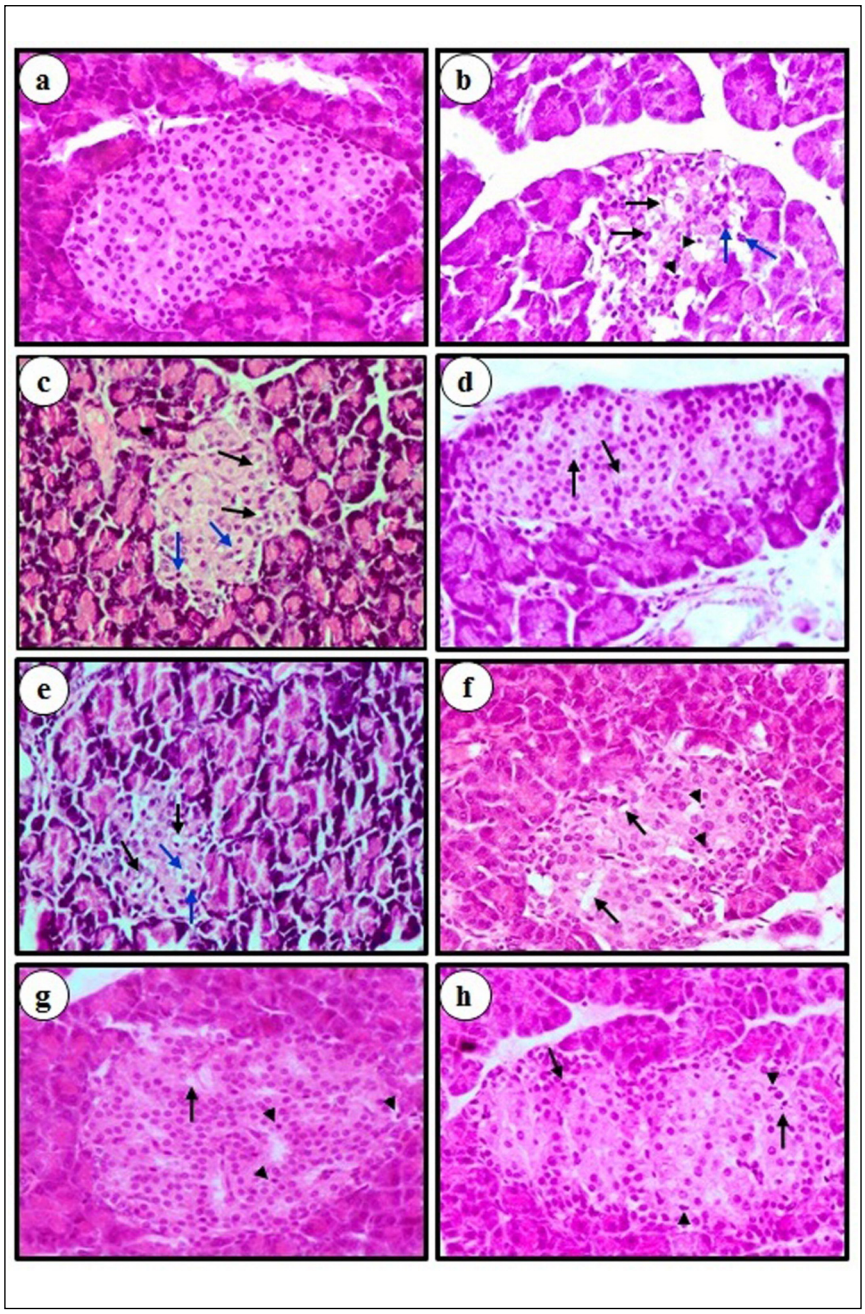

Fig. 4. Histopathological examination of pancreas of (a): normal control rat, showing intact architecture and normal cellular population of the islet of Langerhans; (b): diabetic rat, showing degenerative and necrotic changes of most of the endocrine cells of islet of Langerhans with vacuolation of some cells (black arrows), and necrosis of others with either pyknotic (arrow heads) or karyorrhexic nuclei (blue arrows); (c): diabetic rat treated with cucumber (200 mg/kg), showing vacuolar degeneration (black arrows) and necrosis (blue arrows) of some cells of islets of Langerhans; (d): diabetic rat treated with cucumber (400 mg/kg), showing normal polygonal appearance with relatively large, hyperchromatic rounded nuclei and finely granular abundant cytoplasm in most of the cells of islets of Langerhans, a few cells were vacuolated (arrows); (e): diabetic rat received pumpkin (200 mg/kg), showing islet of Langerhans with ill-defined boundary, vacuolated cells (blue arrow) and necrotic cells with pyknotic nuclei and vacuolated (degranulated) cytoplasm (black arrows); (f): diabetic rat received pumpkin (400 mg/kg), showing normal appearance of the cells of islets of Langerhans. A few cells appeared vacuolated (arrows) while others were necrotic (head arrows) with pyknotic nuclei (arrows); (g): diabetic rat received pumpkin and cucumber (200 mg/kg, each), showing the cells of islet of Langerhans with normal morphology and population. A few scattered vacuolated (arrows) and necrotic cells with pyknotic nuclei are present (arrowheads), and (h): diabetic rat treated with metformin (standard antidiabetic drug, 200 mg/kg), showing a normal architecture of the pancreas with the cells of normal morphology and population, in addition to a few scattered vacuolated and necrotic cells with either pyknotic (arrows) or karyrrhexic nuclei (arrowheads). H & E, nagnification × 200.

Histopathological examination

1. Histopathological examination of liver

The liver of streptozotocin-diabetic rats showed multiple areas of extensive coagulative necrosis in the hepatic parenchyma with distortion of the hepatic cords associated with hepatic cell necrosis with massive focal aggregations of mononuclear inflammatory cells in some portal areas associated with partial hyperplasia of the epithelium lining of the bile duct (*Fig. 3b*) compared to the liver of normal rats (*Fig. 3a*). The liver of diabetic rats treated with cucumber and pumpkin extract in their smaller dose shows mild to moderate vacuolar and coagulative necrosis with infiltration of mononuclear inflammatory cells, activation of Kupffer cells (increased in number and size). Meanwhile, the liver of diabetic rats treated with cucumber or pumpkin extract at their higher dose shows more or less hepatic parenchyma similar to that of the normal control group with granular cytoplasm of the hepatocytes. The liver of diabetic rats received an extract of both pumpkin (200 mg/kg) and cucumber (200 mg/kg) or those treated with metformin showed more or less normal hepatic architecture of hepatic parenchyma with granular cytoplasm and vesicular nuclei of hepatocytes and marked activation of Kupffer cells (*Fig. 3c-3h*).

2. Histopathological examination of the pancreas

The pancreas of streptozotocin-diabetic rats shows degenerative and necrotic changes of most of the endocrine cells of the islet of Langerhans. Some cells were vacuolated and others were with either pyknotic or karyorrhexic nuclei (nuclear fragmentation) (*Fig. 4b*) as compared to the pancreas of normal rat (*Fig. 4a*). The pancreas of diabetic rats treated with cucumber or

Table 3. Phyto-components of methanol extracts of cucumber by GC-MS analysis

No	RT (min)	Name	Area sum %
1	5.12	5-aminolevulinic acid (for photodynamic therapy)	2.13
2	5.4	L-histidine, 3-methyl-(α-amino acid)	4.61
3	5.58	L-arginine (α-amino acid)	1.71
4	6.024	alpinetin (vasorelaxant)	1.07
5	6.22	L-lysine (α-amino acid)	1.59
6	6.39	melibiose (disaccharide)	2.09
7	6.74	hexadecanedioic acid (palmetic acid)	0.52
8	7.1	stevioside	0.87
9	8.33	hexamethylinositol	0.90
10	8.72	dodecanoic acid (saturated fatty acid)	9.95
11	9.12	3,5-dihydroxyphenol	2.11
12	9.7	vincadifformine (cytotoxic drugs)	43.05
13	9.83	3'-benzyloxy-5,6,7,4'-tetramethoxyflavone	0.64
14	10.045	mangiferin	0.51
15	10.25	5,7-dimethoxylflavone (anti-inflammatory)	1.31
16	10.799	methyl 6,7-dimethoxycoumarin-4-acetate	0.52
17	11.44	isomyristic acid	1.12
18	11.59	5-hydroxyisovanillic acid	1.15
19	11.85	arachidic acid (saturated fatty acid)	1.42
20	12.08	6-octadecenoic acid , (Z)-	0.46
21	13.28	strychane,1-acetyl-20α-hydroxy-16-methylene	0.79
22	13.5	gitoxigenin	1.17
23	13.57	hydroquinine (a cinchona alkaloid)	0.70
24	14.05	phytol	0.91
25	14.49	Cis-vacenic acid (monosaturated omega-7 fatty acid)	0.85
26	14.6	4',6-dimethoxyisoflavone-7-O-β-D-glucopyranoside	0.57
27	14.8	zearalenone estrogenic metabolite	0.72
28	15.78	isolongifolol	5.75
29	15.8	linoleic acid	0.45
30	16.04	genkwanin (O-methylated flavone)	5.93
31	16.6	vitexin	0.97
32	17.6	β-sitosterol	0.59
33	18.17	inosine, 1-methyl	0.68
34	20.24	hexa-hydro-farnesol	0.56
35	21.3	quercetin 3,5,7,3',4'-pentamethyl ether	0.47

pumpkin extract shows the endocrine cells of the pancreatic islets with either mild to moderate degenerative changes or appeared normal with relatively large, hyperchromatic rounded nuclei and finely granular abundant cytoplasm and mild infiltration of mononuclear cells around the blood capillaries more or less similar to the control or metformin-treated group (*Fig. 4c-4h*).

Phytochemical screening

Gas chromatography-mass spectrometry (GC/MS) analysis of cucumber and pumpkin is recorded in (*Tables 3* and *5*) and illustrated in *Fig. 5a* and *5b*. GC/MS analysis of the tested plants revealed the presence of 34 and 35 compounds in CUC and PUM methanol extract, respectively. The major components are vincadifformine cytotoxic drugs (43.05%), dodecanoic acid (saturated fatty acid, 9.95%), genkwanin (O-methylated flavone, 5.93%), isolongifolol (5.75%) and L-histidine, 3-methyl-(α-amino acid (4.61%), 5-aminolevulinic acid (for photodynamic therapy) (2.13%) and 3,5-dihydroxyphenol (2.11%) in the PUM extract. On the other hand, PUM contains 3,5-dihydroxyphenol (26.65%), genkwanin (O-methylated flavone, 20%), zearalenone estrogenic metabolite (12.44%), hexamethylinoic acid (6.83%), hydroquinine (a cinchona alkaloid (4.72%), L-histidin, 3-methyl-(3.26), alpinetin (2.25) and La-arginine (2%) as major components.

DISCUSSION

Streptozotocin is a glucosamine-nitrosourea compound that, as other alkylating agents in this class, is toxic to cells by causing damage to the DNA, though other mechanisms may also contribute (19, 23). DNA damage induces activation of poly ADP-ribosylation, which is likely more important for diabetes induction than DNA damage itself (23). Streptozotocin is similar

Table 4. Phyto-components of pumpkin methanol extract by GC-MS analysis.

No	**RT** (min)	Name	**Area sum** %
1	5.12	5-aminolevulinic acid	1.90
2	5.4	L-histidin, 3-methyl-	3.26
3	5.58	L-arginine	2.00
4	6.024	alpinetin	2.25
5	6.22	L-lysine	1.53
6	6.39	melibiose	1.28
7	6.74	hexadecanedioic acid	0.41
8	7.1	stevioside	0.63
9	7.4	azithromycin	1.27
10	8.33	hexamethylinoic acid	6.83
11	8.72	dodecanoic acid	2.10
12	9.12	3,5-dihydroxyphenol	26.65
13	9.7	vincadifformine (cytotoxic drugs)	0.35
14	9.83	3'-benzyloxy-5,6,7,4'-tetramethoxyflavone	0.27
15	10.045	mangiferin	1.10
16	10.25	5,d7-dimethoxylflavone	0.43
17	10.799	methyl 6,7-dimethoxycoumarin-4-acetate	0.46
18	11.44	isomyristic acid	0.5
19	11.59	5-hydroxyisovanillic acid	0.42
20	11.85	arachidic acid (saturated fatty acid)	0.50
21	12.08	6-octadecenoic acid , (Z)-	0.56
22	13.28	strychane, 1-acetyl-20-α-hydroxy-16-methylene-	0.89
23	13.57	hydroquinine (a cinchona alkaloid)	4.72
24	14.05	phytol	0.86
25	14.49	Cis-vacenic acid	0.28
26	14.6	4',6-dimethoxyisoflavone-7-O-β-D-glucopyranoside	0.58
27	14.8	zearalenone estrogenic metabolite	12.44
28	15.8	linoleic acid	0.70
29	16.04	genkwanin (O-methylated flavone)	20.00
30	16.6	vitexin	1.64
31	17.6	β-sitosterol	1.25
32	17.88	beta-carotene	0.40
33	18.17	inosine, 1-methyl	0.54
34	20.24	hexa-hydro-farnesol	0.57

to glucose to be transported into the cell by the glucose transport protein (2), but is not recognized by the other glucose transporters. This explains its relative toxicity to beta cells of the pancreatic islets, since these cells have relatively high levels of glucose transporter 2GLUT2 (24). This suggested the use of the drug as an animal model of diabetes (25). Streptozotocin decreases nicotinamide-adenine dinucleotide (NAD) in pancreas islet beta cells, which are more sensitive than other cells to STZ challenge, and causes degeneration of the beta cells in the islet of Langerhans and intermediates induction of diabetes within three days (26). STZ also causes renal, hepatic, cardiac and adipose tissue damage and increases oxidative stress, inflammation and endothelial dysfunction (4, 27). However, the most important deleterious effects of STZ are the hepatic changes, including lipid peroxidation, mitochondrial swelling, peroxisome proliferation and inhibition of hepatocyte proliferation which are suggested to be due to the direct effects of STZ on hepatocytes rather than the secondary effects of diabetes (28). These changes are usually associated with hydropic degeneration, dilation and congestion of the central veins that are triggered by ROS (29). These mechanisms could explain the significant alterations in blood glucose, GST, CAT, GSH, MDA and NO values, decreased total proteins and globulins levels and increased TC and TG levels in the serum of diabetic rats (30). STZ increased LDL-C but decreased HDL-C in the serum of diabetic rats as compared to the normal control rats. Hypercholesterolemia was also reported to be a major disorder of streptozotocin-induced diabetes mellitus in rats (1). All these deleterious effects are confirmed by severe degenerative and necrotic changes of most islets of Langerhans cells, nuclear fragmentation, decrease in the size (atrophy) and the number of islets, and vacuolar degeneration of the epithelial cells in some exocrine acini. The massive destruction of β-cells of Langerhans by STZ is also confirmed by the marked

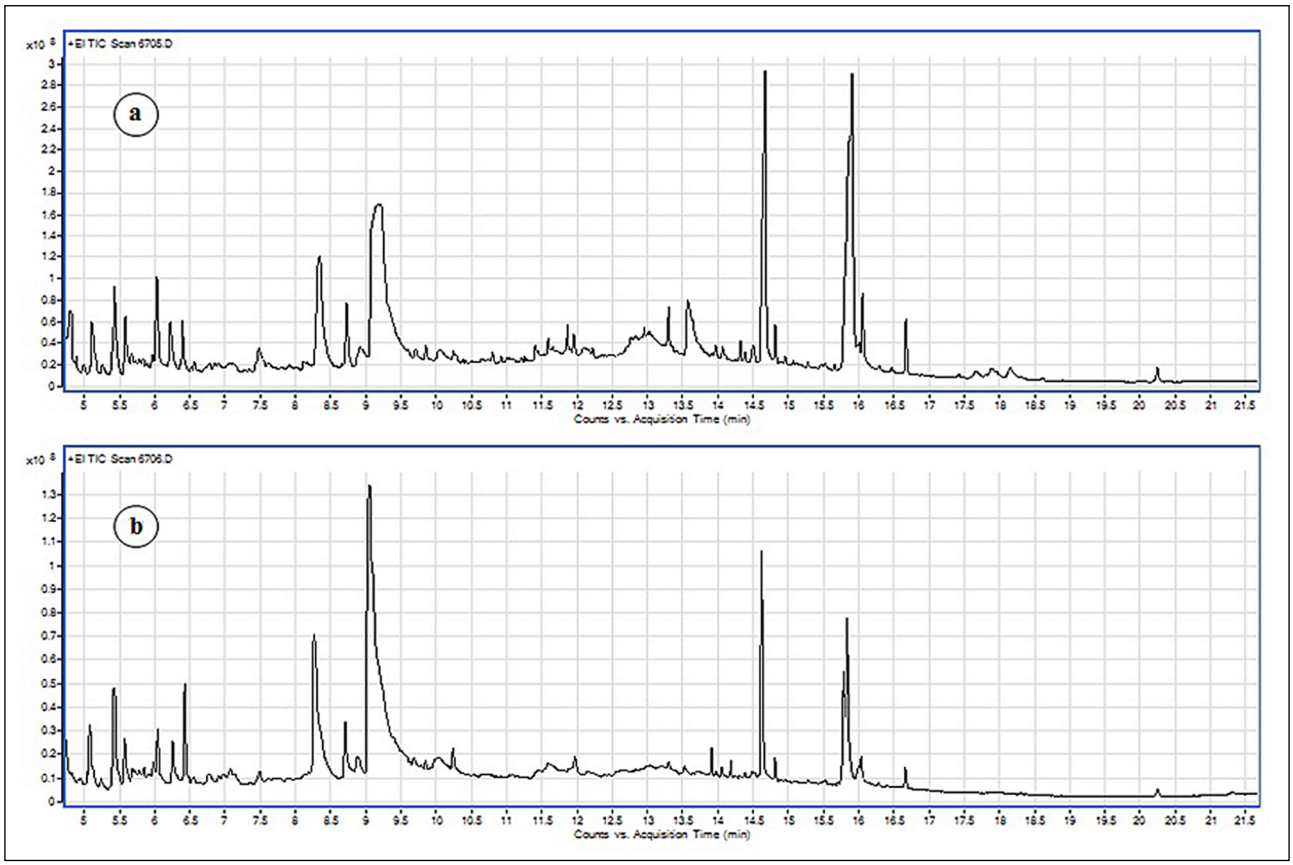

Fig. 5. GC-MS peaks of (a): cucumber, and (b): pumpkin methanol extracts.

reduction in immunohistochemical expression of insulin in β-cells (31).

The marked hepatoprotective by the CUC and PUM methanol extract was indicated by the marked reduction in serum liver enzymes, improvement of protein and lipid profile and confirmed by the improvement of the histopathological picture. The improvement of hepatic function may be correlated to the effect of these extracts against oxidative stress and cytotoxicity as due to their content of carotenoids, polyphenols (flavonols and phenolic acids), tocopherols, minerals (K, Ca, Mg, Na, Fe, Zn, Cu, Mn), vitamins (C, B1, folates) and heavy metal ions (32, 33). In addition, carotenoid content may be responsible for hepatocytes membrane-stabilizing effect, thus preventing the leakage of liver enzymes (34). Moreover, the antioxidant effect could be attributed to the phytocomponent vitexin which is a flavone glucoside that was identified in both the CUC and PUM methanol extract. Vitexin and other flavone C-glycosides all have notable concentration-dependent antioxidant activities (35). Moreover, the antioxidant activity of the fruits of *Cucumis sativus* was also previously reported for its seeds (36).

The antioxidant effect of the CUC extract could also be attributed to its contents of quercetin which was identified by GC-Ms analysis. Quercetin has been reported to inhibit the oxidation of other molecules and is classified as an antioxidant (37). Quercetin has potent antioxidant effects by several mechanisms, including its activity on glutathione (GSH), enzymatic activity, signal transduction pathways, and reactive oxygen species (ROS) as well as the formation of metal ion complexes (38). Moreover, treatment with CUC effectively counteracted diabetes-induced oxidative stress-mediated hepatic damage and could be beneficial in lessening liver dysfunction in diabetic rats. Oral administration of methanol extract of CUC and PUM in combination maintained the concentration of total protein at levels comparable to the normal non-diabetic ones, normalized serum triglycerides and cholesterol, as well as serum HDL and LDL indicating a synergistic effect. Cucumber and white pumpkin extracts reduced the total TC and LDL-C levels in diabetic rats (15, 39). The ethanol extracts of the powdered fruit of *Cucumis sativus* significantly lowered the elevated TC as well as LDL-C levels (40) and could be used for the treatment of dyslipidemia (39).

The mechanism of the hepatoprotective effect of cucumber could be due to the decreased production of reactive oxygen species and antioxidant properties (41). This is confirmed by the marked decrease in oxidative products such as MDA and NO level. The significantly decreased blood sugar after the oral administration of methanol extract of either CUC or PUM methanol extract could be attributed to its phytoconstituents such as mangiferin which has been reported to possess an antidiabetic effect in mice (42). The hypolipidemic and hypoglycemic effect of the methanol extract of pumpkin was attributed to its polysaccharides contents, flavonoids, alkaloids, and/or polyphenolic components (17, 43). The antidiabetic effect of CUC and PUM is confirmed by the disappearance of severe degenerative and necrotic changes of endocrine cells of the pancreatic islets as compared to non-treated diabetic rats. The improvement of the histopathological and immunohistochemical picture could be explained by a membrane-stabilizing effect of the carotenoids of the tested extracts (41). The revealed positively strong immunoreactivity in pancreatic β-cells in diabetic rats that received the extract of

both pumpkin and cucumber suggests a synergistic effect between them.

In the present study, the GC-MS analysis of the tested plants revealed the presence of 35 and 34 compounds in the methanol extract of cucumber and pumpkin, respectively. Traditional chemical methods of the tested plants revealed the presence of amino acids, monosaturated omega-7 fatty acid, saturated fatty acids, disaccharides, glycosides, phytosterols, terpenoids, alkaloids, saponins, flavonoids and tannins (44). This diversity of phytoconstituents could explain the different pharmacological actions of the tested plants (45). Moreover, the fact that most of these constituents are similar in both CUC and PUM, as reported by the GC-MS analysis, could explain the synergistic effects between them. This study demonstrates that methanol extract of CUC and PUM could be beneficial acting synergistically as protective against hepatic and pancreatic tissue damage. It is advisable to isolate the active constituent (s) for further investigation.

Conflict of interests: None declared.

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