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Antidiabetic Effect of Green Vegetable Leaf Powders in Diabetic Rats

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Abstract

This study investigated the anti-diabetic effects of purslane (PS), chard (CA), and chicory (CI) leaf powders on streptozotocin (STZ)-induced type 2 diabetic rats. The total polyphenols, flavonoids and polyphenolic profiles were determined in the leaf powders. Thirty adult male albino rats were divided equally into six groups: untreated non-diabetic control was considered negative control. Other rat groups were rendered diabetic (fasting blood glucose (FBG) level >250 mg/dL) with a twice intraperitoneal injection of STZ (35 mg/kg). Diabetic rats were fed a basal diet. They were classified into five groups: a diabetic control group (positive control), a group receiving metformin (100 mg/kg body weight) as a medication, and other groups were fed a basal diet supplemented with either PS, CA, or CI leaf powder at a 1% level. The experiment lasted for 40 days. Blood samples were analyzed for FBG, insulin, fructosamine and oral glucose tolerance test (OGTT). Feeding diabetic rats basal diet containing the investigated leaf powders decreased insulin resistance (HOMA-IR,) significantly compared to diabetic rats. The CI and PS leaf powders displayed powerful positive hypoglycemic action compared to metformin, in addition to reversing the histological abnormalities in the liver and pancreas of diabetic rats to a state close to normal. Therefore, the investigated leaves may help reduce the metabolic syndrome associated with type 2 diabetes.

Keywords: Purslane leaves ; chard leaves ; chicory leaves ; type 2 diabetes mellitus.

1. Introduction

Diabetes mellitus is recognized as a medical condition characterized by high levels of FBG in the bloodstream. It is a defect in insulin secretion or action [1]. Diabetes has long been a threat to human health [2]. Long-term therapy with chemical antidiabetic drugs has negative side effects [3]. Therefore, it is essential to find safe and natural resources against diabetes. Plant drugs are frequently considered less toxic and side effects free than synthetic ones [4]. Green leafy vegetables are widely used for nutritional and medicinal purposes [5]. *Portulaca oleracea* L. (purslane), Portulacaceae, is an annual herb. It is widely used as a green leaf in different dishes in the Mediterranean and Tropical Asian regions [6]. Treating STZ-induced C57BL/6J diabetic mice with freeze-dried juice extract of purslane at 400 mg/kg for 21 days decreased the FBG levels by 31 %, and significantly improved the OGTT and increased insulin secretion [7]. Administering diabetic mice with purslane extract intragastrically at 100 and 200 mg/kg for 28 consecutive days significantly decreased blood glucose levels [2].

Beta vulgaris L. var. *cicla* (Swiss chard), Chenopodiaceae, is used in traditional medicine as an antidiabetic agent. Oral administration of chard leaf aqueous extract at 2 g/kg/day for 45 days caused a significant reduction in the FBG level of diabetic rats compared to that obtained by subcutaneous injection of insulin (6 U/kg/day) [8]. Administration of chard leaf aqueous extract to diabetic rats at 2 g/kg daily for 42 days by gavage significantly decreased glucose level to the normal level [9]. On the other hand, the administration of chard leaf aqueous extract at 8 g/kg decreased blood glucose levels in diabetic rabbits by 15.1% [10].

Cichorium intybus L. (chicory) is a medicine and food plant in the Asteraceae family [11]. Oral

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administration of chicory leaf extract at 200 mg/kg for 21 days had a similar hypoglycemic effect on diabetic rats as that of the standard drug glibenclamide at 5 mg/kg [12].

Phytochemical screening of purslane, chard and chicory revealed the presence of bioactive compounds which are reported to have hypoglycemic activity [13, 14].

Currently, STZ is utilized frequently to cause β-cell damage to reproducibly create both insulin-dependent and noninsulin-dependent diabetes mellitus. Multiple low-dose STZ has been reported to cause a modest impairment of insulin secretion, which is comparable to the feature of the latter stage of type 2 diabetes, according to Zhang et al. [15]. Shehata et al. [16] induced diabetes in rats using STZ (in three divided i.p doses with 5-day interval). High doses of STZ have been shown to severely impair insulin secretion, simulating type 1 diabetes. Tehseen et al. [17] (2024) induced diabetes type 1 in rats by a single intraperitoneal injection of STZ at a dose of 75 mg/kg b.w.

Although some investigations have been carried out to evaluate the anti-diabetic properties of the leaf extracts of the studied plants [18, 19, 20], only a few research papers used purslane and chicory leaf powders. Still, they did so at high levels (5-20%). To the best of our knowledge, no *in vivo* study regarding the anti-diabetic activity of chard leaf powder was carried out. The research aimed to evaluate the anti-diabetic effects of the examined leaf powders at a low level (1% of the diet) in STZ-induced hyperglycemic rats compared with a reference drug, metformin [21].

2. Materials and methods

Plant materials and Chemicals

Fresh PS, CA, and CI leaves weighing ten kilograms each were procured from a private farm in Giza Governorate, Egypt. The purslane (*Portulaca oleracea* L.), chard (*Beta vulgaris* L.), and chicory (*Cichorium intybus* L.) voucher specimens were 136, FUPD-6, and M38, respectively, and were deposited in the herbariums of CAIM, the Department of Pharmacognosy, Faculty of Pharmacy, Fayoum University, Egypt, and the National Research Centre (NRC), Cairo, Egypt. Leaves were cleaned with tap water and dried at 40 °C. A fine powder was made from the dried leaves. The STZ, polyphenol and flavonoid standards were obtained from Sigma Chemical (St Louis, MO, USA). All other chemicals were of analytical grade.

Leaf extract preparation

An ethanolic extract of the leaf powder was obtained by extraction 10 g of powder with 200 mL of 80 % ethanol for 30 min using a high-speed homogenizer. After filtration, the filtrate was concentrated under a vacuum and stored at -20 °C for further analysis.

Determination of total polyphenols content

Folin-Ciocalteu reagent was used to analyze total polyphenols content [22]. The absorbance was measured at 750 nm. Gallic acid was used as a standard. Total polyphenols content was expressed as mg gallic acid equivalents/g dried leaf.

Determination of total flavonoids content

The total flavonoid content was measured according to the method of Zhishen et al. [23]. The absorbance was determined at 510 nm. Quercetin was used as a standard. The total flavonoids content of the extract was expressed as mg quercetin equivalents/g dried leaf.

HPLC analysis for identifying and quantifying phenolic compounds

HPLC (Agilent, USA) was used to separate, identify and quantify the polyphenols of the extracts. According to Schneider [24] chromatographic separation was carried out on a Kinetex 5 µm EVO C_{18} 100 x 4.6 mm column (Phenomenex, USA) with a linear gradient mobile phase. Analyses were performed at a flow rate of 0.7 mL/min with a variable wavelength detector set at 284 nm and a sample injection volume of 20 µL. The phenolic compounds were identified by comparing their UV spectra and retention times with authentic standards. Quantification was carried out with calibration curves.

Experimental animals

Thirty adult male albino rats aged 13-14 weeks (261 g ± 11.2) were housed individually in cages at 22±1 °C, 85±2 % relative humidity, and 12 h light/dark cycle. The Institutional Animal Care and Use Committee, CU-IACUC, Cairo University approved the protocol with approval number (CUIIF120).

Experimental design

After one week of adaptive feeding on a basal diet [25], five rats were selected and allocated to a negative control group, Group 1 (G1). Diabetes was induced in the rest of the rats by a twice injection with a freshly prepared STZ solution (35 mg/kg, i.p) after fasting for 12 h, on alternate days 1 and 3 according to Luo et al.

[26]. The STZ solution was prepared according to Altındağ et al. [27]. Rats in the STZ-injected groups were allowed to drink glucose solution (5%) to prevent hypoglycemia. The animals with FBG levels >250 mg/dL, after three days of the second STZ injection, were diagnosed as diabetic rats [28]. The animals were grouped (n=5) as follows:

G1: Normal rats fed on the basal diet (negative control).

G2: Diabetic rats fed on the basal diet (positive diabetic control).

G3: Diabetic rats fed on the basal diet and treated with the drug metformin at 100 mg/Kg b.w [29].

G4: Diabetic rats fed on the basal diet supplemented with 1% PS powder.

G5: Diabetic rats fed on the basal diet supplemented with 1% CA powder.

G6: Diabetic rats fed on the basal diet supplemented with 1% CI powder.

The experimental basal diet consisted of: casein 12%, sunflower oil 8%, fiber 1%, salt mixture 5%, vitamin mixture 1%, corn starch 36%, sucrose 36% and the investigated leaf powder 1% [25]. The FBG was measured every ten days. At the end of the experiment (40 days), all the rats were fasted for 12 h and weighed. The blood was collected from the retro-orbital vein and the serum was separated for analysis. The animals were anesthetized with diethyl ether, and sacrificed by cervical dislocation. The liver, kidney, spleen, and pancreas were removed and weighed immediately.

Biochemical analyses

The FBG was estimated using a glucometer (OneTouch Select, LifeScan, Inc., China). Serum insulin and serum fructosamine ELISA kits (SinoGeneclon Co., Ltd., China) were determined according to the instructions.

The fructosamine test evaluates all blood proteins that glucose binds to. In this test the nitroblue tetrazolium dye is reduced to formazan (a colored end-product) which is directly proportional to the fructosamine concentration [30]. Determination of serum insulin is based on two monoclonal antibodies. During incubation, insulin in the sample reacts with anti-insulin antibodies attached to micro-titration wells and anti-insulin antibodies conjugated to horseradish peroxidase enzyme (HRP). The enzyme-labeled antibody that is not bound is removed with a quick wash. The reaction with TMB substrate is used to identify the bound HRP complex [31].

Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula according to Albareda et al. [32].

$$HOMA - IR = [fasting\ insulin\ level\ in\ mU/L\ x\ fasting\ glucose\ level\ in\ mmol/L]/22.5$$

Oral glucose tolerance test (OGTT)

At the end of the experiment, and after a 12 h fast, the rats were administered orally with glucose solution (2 g glucose/kg b.w). Blood glucose was measured at 0, 30, 60, 90, and 120 min after administration of glucose. Blood glucose was measured using the glucometer. The OGTT was calculated by the area under the curve (AUC) according to Zhao et al. [33] as follows:

$$AUC = (G0 + G0.5) \times 0.25 + (G0.5 + G1) \times 0.25 + (G1 + G2) \times 0.5$$

Where G0, G0.5, G1 and G2= blood glucose levels at zero time, and after 0.5, 1 and 2 h of glucose administration, respectively.

Organs relative weight

The relative weight of the liver, kidney, pancreas and spleen was calculated according to the following formula:

$$\text{Organ relative weight } \% = \frac{\text{Organ weight}}{\text{Final body weight}}\ x\ 100$$

Histopathological assays

The histopathological assays were performed according to Mbara et al. [34]. The pancreas and liver were washed with an ice-cold 0.9% saline solution and fixed in 10% formalin before dehydrating and embedding in paraffin. Sections cut into five μm were deparaffinized and hematoxylin and eosin (H & E) were stained before examining under a light microscope at 200 × magnification.

Statistical analyses

Total polyphenols, total flavonoids and biochemical determinations were carried out in triplicate. The data are reported as mean ± SD. Statistical analysis was performed by ANOVA and Tukey's test at $p<0.05$ using XLSTAT 2014.5.03 Software (Addinsoft, New York, USA).

3. Results and Discussion

Total polyphenol and flavonoid contents

The highest TPC content was recorded for the CA leaf powder (14.7±0.7 mg GAE/g DM) followed by that of CI (12.63±0.4 mg GAE/g DM) and PS (9.06±0.68 mg GAE/g DM). On the other hand, the TFC content of the CI, PS, and CA leaves was found to be 22.97±0.89, 17.09±0.98, and 10.25±1.34 mg QE/g DM, respectively. The TPC and TFC of purslane

leaves were 10.06± 0.26 mg GAE/g DM and 8.97± 0.18 mg QE/g DM, respectively [35]. Meanwhile, the TPC and TFC of chard leaves were 11.12± 0.56 mg/g DM and 7.92± 0.39 mg/g DM, respectively [36]. These values were lower than the levels obtained in the current study. The TPC of chicory leaves was 16.7± 1.8 mg/g DM [37]. Furthermore, the TPC and TFC of chicory leaves were 8.659 mg GAE/g DM and 1.123 mg QE/g DM, respectively [38]. These values were much lower than the ones recorded in this investigation. These variations could be due to cultivar differences and climatic conditions.

HPLC of polyphenols

HPLC was used to find the concentration of polyphenolic components in the extracts under investigation (Figure 1).

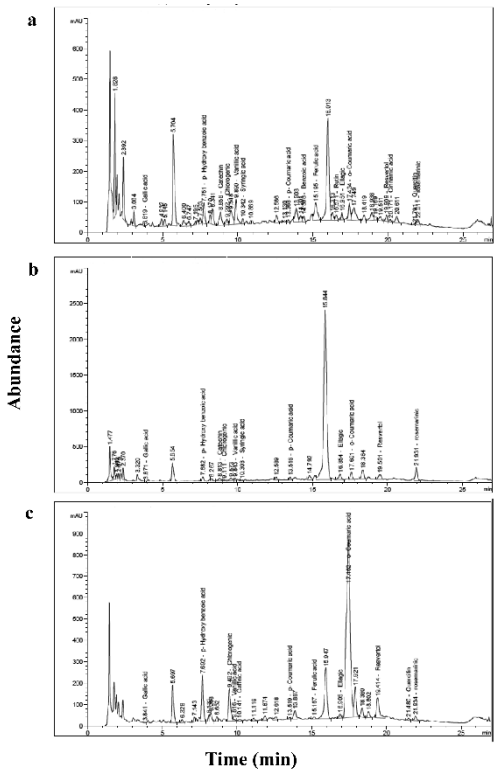

Fig. 1. HPLC chromatograms of purslane (a), chard (b) and chicory (c) leaf ethanolic extracts

The major phenolic components in the investigated CI leaf extract were found to be o-coumaric acid, ρ-hydroxybenzoic acid and chlorogenic acid. Their levels in CI extract exceeded their counterparts in PS and CA extracts. Chlorogenic acid, ρ-hydroxy benzoic acid, and vanillic acid were identified in chicory leaf extract obtained by 50% aqueous ethanol [38]. Although benzoic acid was the primary polyphenol in the PS extract, it was not identified in the other investigated extracts. Vanillic acid was found in the PS extract at a higher level than in different extracts. Meanwhile, CA extract was characterized by a high level of chlorogenic acid.

Rosmarinic acid and resveratrol were the two main flavonoids found in the extracts under investigation. Quercetin was absent in the CA extract, whereas rutin was not found in the other extracts. Flavonoids possess antidiabetic properties [39].

Effect of the investigated leaf powders on blood glucose level

In the normal control group (G1), FBG maintained normal during the study (Figure 2).

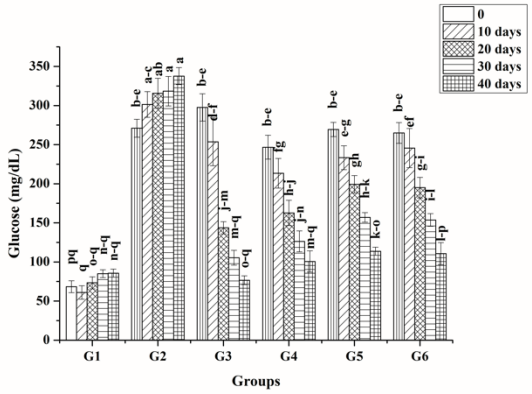

Fig. 2. Blood glucose level of diabetic rats fed, for 40 days, a basal diet supplemented with 1% leaf powder of purslane (G4), chard (G5) and chicory (G6) compared with the non-diabetic control group (G1), diabetic non-treated group (G2), and diabetic rats treated with 100 mg drug (metformin)/Kg b.w (G3). Groups (1, 2 and 3) were fed a basal diet. Data are presented as the mean ±standard deviation of the mean (SD). Bars with different letters indicate significant difference ($p < 0.05$, n = 3) by Tukey's test.

Since STZ is toxic to pancreatic islet cells, it has been used to induce hyperglycemia in lab animals [40]. The glucose level in diabetic rats (G2) increased significantly ($p<0.05$) to >300 mg/dL after ten days of feeding a basal diet, after which no significant ($p<0.05$) increase in this level was noted. Feeding diabetic rats a basal diet containing PS powder (G4) for ten days significantly ($p<0.05$) decreased glucose level to <220 mg/dL. Feeding diabetic rats diets supplemented with the investigated leaf powders (G4, G5, and G6) or treated with drug (G3) for 40 days decreased glucose levels not significantly ($p<0.05$) different from that of the control group (G1). PS polysaccharides showed anti-diabetic effects by reducing FBG in diabetic mice [41]. Feeding diabetic rats on a diet containing 20% chicory leaf powder for one month decreased glucose levels significantly to the normal state [42]. Previous studies indicated that the identified polyphenols and flavonoids in the

current study have remarkable anti-diabetic activity [43, 44, 45].

Oral glucose tolerance test (OGTT)

Figure 3a shows OGTT results. At zero time of the experiment, the blood glucose level in the control and diabetic treated groups ranged from 98.5 to 122.25 mg/dL, while it was 308 mg/dL in the diabetic untreated group.

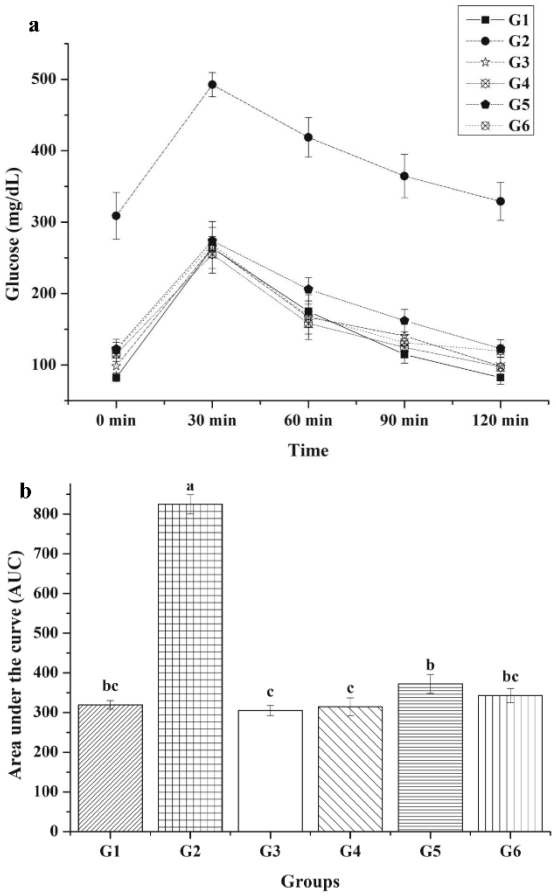

Fig. 3. Oral glucose tolerance tests (OGTT) (a) and AUC (b) of diabetic rats fed, for 40 days, a basal diet supplemented with 1% leaf powder of purslane (G4), chard (G5) and chicory (G6) compared with the non-diabetic control group (G1), diabetic non-treated group (G2), and diabetic rats treated with 100 mg drug (metformin)/Kg b.w (G3). Groups (1, 2 and 3) were fed a basal diet. Data are presented as the mean± standard deviation of the mean. Bars with different letters indicate significant difference (p<0.05, n = 5) by Tukey's test.

The blood glucose level of all groups increased sharply to a maximum at 30 min after glucose loading. Then the blood glucose level decreased gradually. The glucose levels of the normal control group and diabetic treated groups decreased to normal levels at 120 min after glucose loading, whereas the diabetic untreated group remained hyperglycaemic (300 mg/dL). As shown in Figure 3b, the AUC value of the diabetic untreated group was significantly (p<0.05) higher than those of the other investigated groups. The AUC value of the chard group (G5) was significantly (p<0.05) higher than those of the purslane group (G4) and drug group (G3). There is no significant (p>0.05) difference in AUC values between the treated diabetic groups and the normal control group (G1). In addition, the efficacy of the investigated leaf powders was comparable to that of the drug.

Insulin

In response to elevated blood glucose levels, pancreatic islet cells release insulin. Due to insulin insufficiency, diabetes induces serious malfunctions in the target tissues [46]. STZ stimulation increased blood glucose, decreased serum insulin levels, and caused selective destruction of pancreatic β-cells [2]. In the current study, the lowest level of insulin was noticed in non-treated diabetic rats (G2) that were fed a basal diet for 40 days, as shown in Figure 4a.

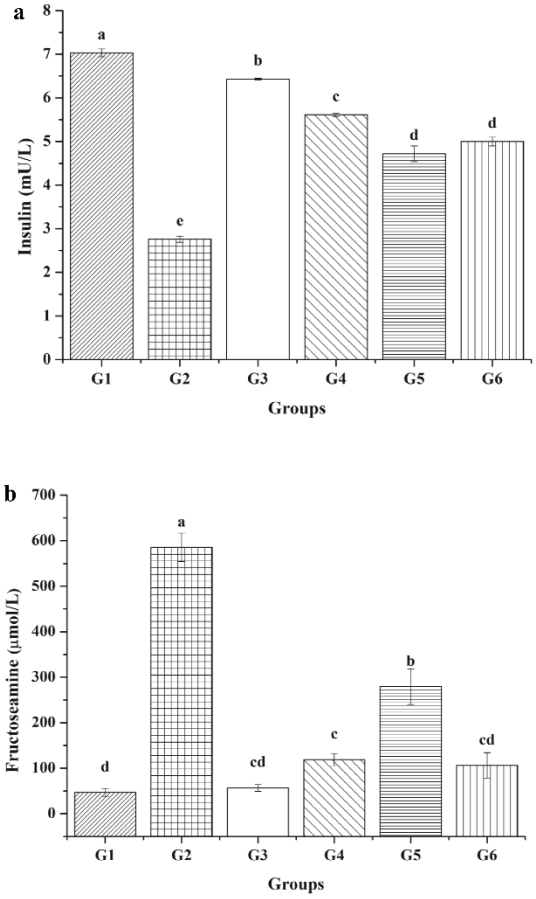

Fig. 4. Insulin (a) and fructoseamine (b) levels of diabetic rats fed, for 40 days, a basal diet supplemented with 1% leaf powder of purslane (G4), chard (G5) and chicory (G6) compared with a non-diabetic control group (G1), diabetic non-treated group (G2), and diabetic rats treated with 100 mg drug (metformin)/Kg b.w (G3). Groups (1, 2 and

3) were fed a basal diet. Data are presented as the mean ±standard deviation of the mean (SD). Bars with different letters indicate significant difference (p<0.05, n = 5) by Tukey's test

Although none of the experimental diets (G4 – G6) increased insulin levels in the diabetic rats after 40 days to the normal level (G1), these diets and the drug treatment (G3) increased significantly (p<0.05) insulin level of the diabetic rats compared to G2. The insulin level of diabetic rats treated with drug (G3) for 40 days was significantly (p<0.05) higher than that of those fed the experimental diets (G4-G6). PS improved insulin levels and boosted insulin secretion by restoring impaired pancreatic β-cells [2, 47]. No significant difference in the insulin level of diabetic rats was noticed between G5 and G6.

Fructosamine

Fructosamine is a marker of glucose control and an indicator of early glycation end products reflecting an increase in diabetes [48]. The fructosamine level in diabetic rats fed a basal diet (G2) for 40 days was significantly (p<0.05) higher than that of the control group (G1) as shown in Figure 4b. The increment in fructosamine level is directly proportional to FBG [49]. Meanwhile, the fructosamine level of diabetic rats treated with a drug (G3) or fed a basal diet containing CI powder (G6) for 40 days was not significantly (p<0.05) different from that of the control group (G1). However, the fructosamine level of diabetic rats fed PS powder (G4) was not significantly (p<0.05) different from that of the drug group (G3). Although the fructosamine level of diabetic rats fed a basal diet containing CA powder (G5) was significantly lower than that of G2, it was significantly higher than those of the other investigated groups (G1, G3, G4, G6).

HOMA-IR level

When the regular concentration of insulin cannot connect to the insulin receptors in the liver, muscles, and fat cells, it is known as insulin resistance [50]. The effect of the investigated leaf powders on insulin resistance (HOMA-IR) was calculated and indicated in Table 1. The HOMA-IR value of the untreated diabetic group (G2) was significantly (p<0.05) higher than that of the normal control group (G1). However, feeding the diabetic rats with the basal diet containing the investigated leaf powders reversed significantly (p<0.05) the increase in HOMA-IR. No significant (p>0.05) variation in HOMA-IR level between treated diabetic groups and the normal control group (G1) was noticed suggesting an improvement in insulin sensitivity. These results are consistent with those reported by Jung et al. [51] who found that feeding mice on a high-fat diet containing *Portulaca oleracea* powder at 10% for 12 weeks kept serum insulin levels and HOMA-IR not significantly different from the normal control but significantly different from those of the high-fat diet group.

Table 1: HOMA-IR of STZ-induced diabetic rats after 40 days of feeding basal diet supplemented with either purslane, chard or chicory leaf powders

Groups	HOMA-IR
G1	$1.49^{b} \pm 0.10$
G2	$2.31^{a} \pm 0.13$
G3	$1.22^{b} \pm 0.09$
G4	$1.41^{b} \pm 0.21$
G5	$1.32^{b} \pm 0.05$
G6	$1.37^{b} \pm 0.20$

-Values are presented as the means ± SD of 5 animals
-Means with different superscript in the column differ significantly (p<0.05).
G1: normal control group; G2: diabetic untreated group
G3: diabetic rats fed a basal diet and treated with the drug metformin at 100 mg/Kg b.w
G4: diabetic rats fed a basal diet supplemented with purslane leaf powder at 1% level
G5: diabetic rats fed a basal diet supplemented with chard leaf powder at 1% level
G6: diabetic rats fed a basal diet supplemented with chicory leaf powder at 1% level

According to HPLC results resveratrol and rosmarinic acid were the main identified flavonoids in the investigated leaf powders. Both compounds have the potential to be useful adjuvants in the treatment of diabetes, since resveratrol improved peripheral insulin sensitivity [52] and rosmarinic acid preserved β-cells function against STZ-induced damage [53].

Body weight

After 40 days of the experiment, there was a significant (p<0.05) increase in body weight in non-diabetic rats (negative controls, G1), diabetic rats treated with metformin (G3) and diabetic rats fed a basal diet containing PS leaf powder (Table 2). The highest increment (24.99%) was recorded for the non-diabetic rats (G1).

Table 2: Changes in body weight of STZ-induced diabetic rats after 40 days of feeding basal diet supplemented with either purslane, chard or chicory leaf powders

Groups	Initial Weight	Final Weight	% Change*
G1	257.33 ^{abA} ±6.50	321.66 ^{aB} ±9.60	+24.99
G2	257 ^{abA} ±6.24	195.33 ^{dB} ±5.03	-23.99
G3	262.33 ^{abA} ±10.69	264 ^{bA} ±20.42	+0.63
G4	248 ^{bA} ±1.73	255.66 ^{bA} ±4.61	+3.08
G5	271 ^{aA} ±7.93	224 ^{cB} ±5.50	-17.43
G6	270.66 ^{aA} ±10.01	255 ^{bA} ±9.16	-5.78

-Data are the mean of triplicate with standard deviation.
-Means with different superscript capital letters in each row are significantly different (p<0.05). Means with different superscript small letters in each column differ significantly (p<0.05).
- % Change = [(Final weight- Initial weight)/(Initial weight)] x100
-G1: normal control group; G2: diabetic untreated group; G3: diabetic rats fed a basal diet and treated with the drug metformin at 100 mg/Kg b.w; G4: diabetic rats fed a basal diet supplemented with purslane leaf powder at 1% level; G5: diabetic rats fed a basal diet supplemented with chard leaf powder at 1% level; G6: diabetic rats fed a basal diet supplemented with chicory leaf powder at 1% level

On the other hand, a significant (p<0.05) decrease in body weight was noticed in non-treated diabetic rats (positive control group, G2), and diabetic rats fed a basal diet containing CA leaf powder (G5). The highest decrement (23.99%) was recorded for the non-treated diabetic rats (G2) while the lowest decrement (5.78%) was noticed for the CI leaf powder group (G6). A significant (p<0.05) weight loss is linked to STZ-induced diabetes, which may be caused by the catabolic consequences of acute hyperglycemia and insulin insufficiency as well as the volume depletion brought on by osmotic diuresis [54].

Organs' relative weight

The liver relative weight percentage in diabetic rats fed a basal diet (G2) for 40 days was significantly (p<0.05) higher than that of the other investigated groups (G1, and G3-G6) (Figure 5a).

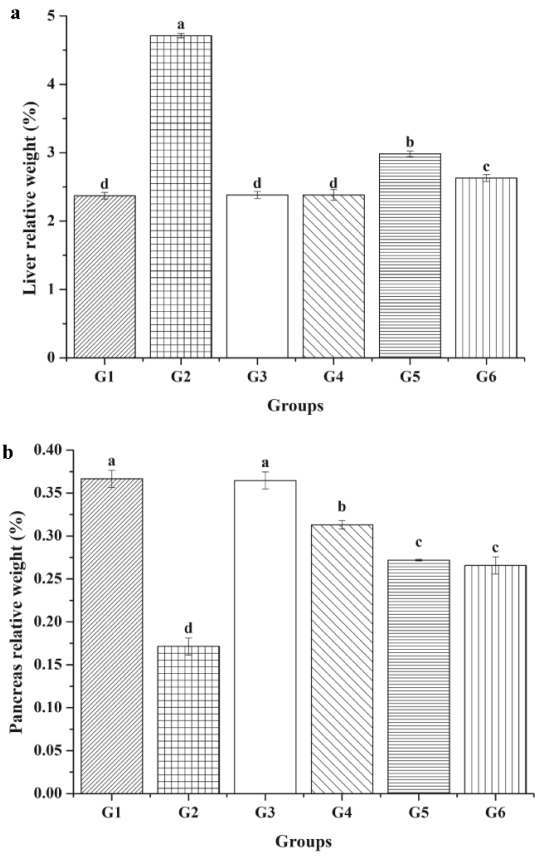

Fig. 5. The liver (a) and pancreas (b) relative weight (organ to body weight ratio) of diabetic rats after 40 days of feeding a basal diet supplemented with 1% leaf powder of purslane (G4), chard (G5) and chicory (G6) compared with a non-diabetic control group (G1), diabetic non-treated group (G2), and diabetic rats treated with 100 mg drug (metformin)/Kg b.w (G3). Groups (1, 2 and 3) were fed a basal diet. Data are presented as the mean ±standard deviation of the mean (SD). Bars with different letters indicate significant difference (p<0.05, n = 5) by Tukey's test

Diabetes is accompanied by an increase in liver-relative weight [55]. There is no significant (p>0.05) difference in the liver relative weight between G1, G3, and G4.

The lowest pancreatic relative weight was noticed in diabetic rats (G2) (Figure 5b). The highest pancreas relative weight was noticed in the normal group (G1) and drug group (G3), without significant difference, followed by diabetic rats fed a basal diet containing PS powder (G4). No significant (p>0.05) difference could be noticed in pancreas relative weight between CA powder (G5), and CI powder (G6) groups.

The highest spleen-relative weight was found in diabetic rats (G2) (Figure 6a).

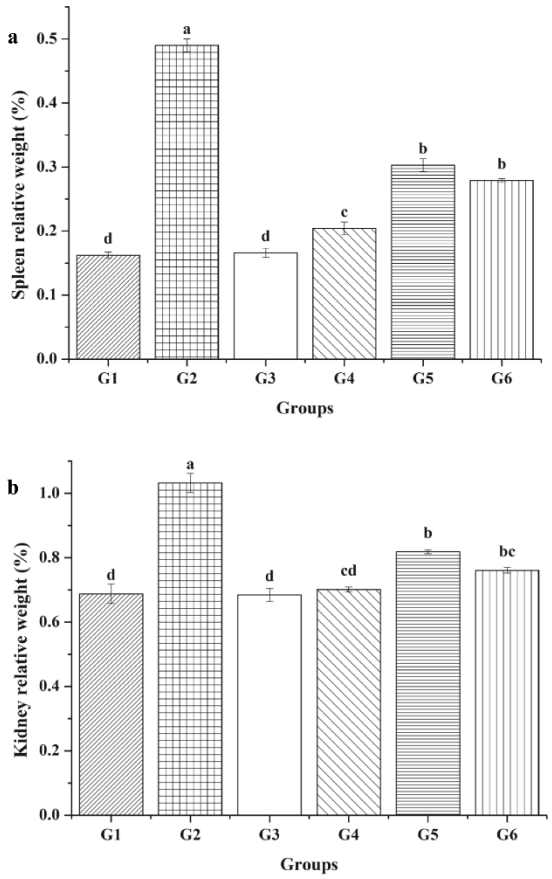

Fig. 6. The spleen (a) and kidney (b) relative weights (organ to body weight ratio) of diabetic rats after 40 days of feeding a basal diet supplemented with 1% leaf powder of purslane (G4), chard (G5) and chicory (G6) compared with a non-diabetic control group (G1), diabetic non-treated group (G2), and diabetic rats treated with 100 mg drug (metformin)/Kg b.w (G3). Groups (1, 2 and 3) were fed a basal diet. Data are presented as the mean ±standard deviation of the mean (SD). Bars with different letters indicate significant difference (p<0.05, n = 5) by Tukey's test.

On the other hand, the lowest spleen relative weight was recorded for the normal group (G1) and diabetic rats that were fed a basal diet containing PS powder (G4) without significant (p<0.05) difference between them followed by the drug group (G3). Though, there was no significant (p>0.05) difference in spleen relative weight between diabetic rats fed a basal diet containing either CA powder (G5) or CI powder (G6), both were significantly (p<0.05) lower than that of the diabetic group (G2).

The highest level of kidney relative weight was recorded in diabetic rats (G2) (Figure 6b). No significant (p>0.05) difference could be observed between kidney relative weight of diabetic rats fed a basal diet containing CA powder (G5) or CI powder (G6). The kidney relative weight of diabetic rats fed a basal diet containing PS powder (G4) was not significantly (p>0.05) different from those of the control group (G1) and drug group (G3).

Histopathological of liver sections

The central vein and hepatocytes in the non-diabetic rats' liver sections (control group, G1) were normal (Figure 7a).

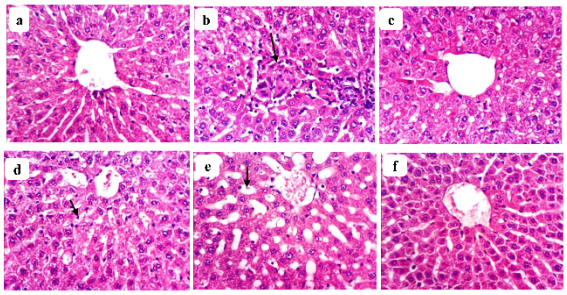

Fig. 7. Photomicrographs of the liver sections of : normal rats fed a basal diet (control negative group) (a), diabetic non-treated rats fed a basal diet (control positive group) (b), diabetic rats fed a basal diet and treated with 100 mg metformin/Kg b.w (c), diabetic rats fed a basal diet supplemented with 1% purslane leaf powder (d), diabetic rats fed a basal diet supplemented with 1% chard leaf powder (e) and diabetic rats fed a basal diet supplemented with 1% chicory leaf powder (f), for 40 days, (H&E x 200)

The examined sections from the diabetic group (G2) revealed Kupffer cell activation, fibroplasia in the portal triad, and sinusoidal leukocytosis (Figure 7b).

Moreover, mild changes were noticed in liver sections from the PS group (G4) described as slight activation of Kupffer cells (Figure 7d). PS ethanol extract (100 mg/kg, orally) significantly lessened liver damage in STZ-induced diabetic mice [2]. However, the liver from the CA group (G5) showed slight dilatation of the hepatic sinusoids (Figure 7e).

Chard aqueous extract (2 g/kg) ameliorated the liver injury caused by diabetes in female albino rats [56]. At the same time, other sections of the liver of diabetic rats of drug-treated group (G3) or CI group (G6) exhibited normal histology of the hepatic parenchyma with no histopathological alterations (Figures 7c and 7f).

Histopathological of pancreas sections

Figure 8 illustrates the pancreas' histopathological alterations. Normal pancreatic acini and Langerhans islets were observed in the pancreas of control animals (G1) (Figure 8a).

On the other hand, the pancreas of diabetic rats (G2) displayed cystic dilatation of the pancreatic duct, damage of pancreatic acini, inflammatory cell infiltration, and vacuolation and death of the islets of Langerhans (Figure 8b). This result is in agreement with Mahmoud et al. [57]. STZ-induced pancreatic

injury [58]. Moreover, sections from G4 and sections from G5 exhibited slight vacuolations of some cells of islets of Langerhan's (Figures 8d and 8e). PS extract alleviated hyperglycemia, and the effects might be due to reduced oxidative stress and inflammation in rats [59].

Furthermore, the pancreas of rats from G6 or drug group (G3) showed no histopathological alterations (Figures 8c and 8f).

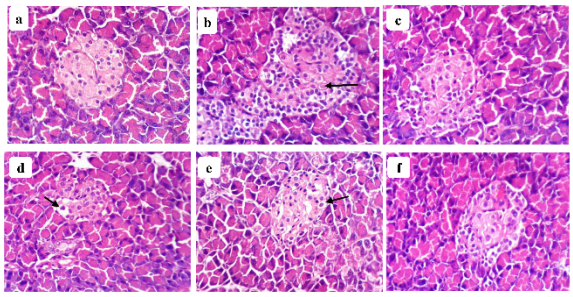

Fig. 8. Photomicrographs of the pancreas sections of : normal rats fed a basal diet (control negative group) (a), diabetic non-treated rats fed a basal diet (control positive group) (b), diabetic rats fed a basal diet and treated with 100 mg metformin/Kg b.w (c), diabetic rats fed a basal diet supplemented with 1% purslane leaf powder (d), diabetic rats fed a basal diet supplemented with 1% chard leaf powder (e) and diabetic rats fed a basal diet supplemented with 1% chicory leaf powder (f), for 40 days, (H&E x 200)

3. Conclusion

The present study demonstrated that dietary supplementation with CI or PS leaf powders was effective at suppressing and regulating blood glucose level, alleviated insulin resistance, body weight loss, and restoring histopathological changes in the liver and pancreas to a normal state or ameliorating these changes in streptozotocin-induced diabetic rats. Polyphenols were identified as the main contributor to the noticed bioactivity.

Conflict of interest

All authors declare no conflict of interest.

Formatting of funding sources

No competing financial interests exist.

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Ethics statement

All national guidelines for laboratory animals were followed. The study protocol was approved by the Cairo University ethics committee (approval number CUIIF120).

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