J Herbmed Pharmacol. 2023; 12(2): 250-261.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2023.27

Journal of Herbmed Pharmacology

The modulatory effects of black chia (*Salvia hispanica*) and garden cress (*Lepidium sativum*) seeds on Nε-CML formation in streptozotocin-injected rats

Hadeer Saad Mohamed, Tahany El-Sayed Kholief, Radwa Wahid Mohamed*, Amira Abd El-Rhman

Department of Biochemistry and Nutrition, Faculty of women for Art, Science and Education, Ain Shams University, Egypt

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 11 October 2022
Accepted: 10 January 2023

Keywords:
Hyperglycemia
Oxidative stress
Inflammation
Glycation end products

ABSTRACT

Introduction: Antioxidant and hypoglycemic properties of some plant seeds are considered natural preventives for diabetic-associated glycation. This study is concerned with the evaluation of the bioactive components of black chia and garden cress seeds and the examination of their modulatory effects on hyperglycemia and associated glycation induced by streptozotocin (STZ) injection.

Methods: Forty male rats were divided into 4 groups, 10 rats each: Group 1 (healthy control group); group 2 (diabetic group): rats injected STZ intraperitoneally to induce hyperglycemia; group 3 and group 4: rats treated (after diabetic induction) with 1 mL (20% w/w) black chia and garden cress seed extract, respectively.

Results: STZ injection caused marked hyperglycemia, oxidative stress, glycation, and inflammation condition with disturbance in organs functions and structural alterations in pancreatic tissue, while; treatment with black chia and garden cress seed extracts showed remarkable ($P<0.05$) modulatory effects on hyperglycemia and associated disorders.

Conclusion: Black chia and garden cress seeds might be used in the management of diabetes and associated glycation.

Implication for health policy/practice/research/medical education:
The use of black chia and garden cress seeds improves lesions of hyperglycemia and reduces the related side effects. These seeds might be recommended to physicians for the treatment of diabetes.

Please cite this paper as: Mohamed HS, Kholief TE, Mohamed RW, Abd El-Rhman A. The modulatory effects of black chia (*Salvia hispanica*) and garden cress (*Lepidium sativum*) seeds on Nε-CML formation in streptozotocin-injected rats. J Herbmed Pharmacol. 2023;12(2):250-261. doi: 10.34172/jhp.2023.27.

Introduction

The most significant metabolites of diabetic glucose toxicity, advanced glycated end products (AGEs), play a role in several stages of diabetes complications. Individuals with type 2 diabetes mellitus have a much higher rate of AGEs production than patients with type 1 diabetes. The amino acid Nε-carboxy methyl lysine (Nε-CML) is regarded as the essential part of AGEs. Nε-CML is a precursor to the development of diabetes problems (1-3). Additionally, non-enzymatic glycosylation is crucial in the pathophysiological and physiological development of processes, including aging, atherosclerosis, neurological disorders, and chronic renal failure. Glycation control can reduce the severity of diabetes consequences. The body's natural defense mechanism, synthetic inhibitors, and natural inhibitors can all stop glycation. In comparison to manufactured molecules, naturally occurring phytochemical products are thought to be non-toxic, affordable, and readily available in ingestible forms (2). Numerous plants and organic macromolecules prevent glycation and have anti-diabetic properties. Antioxidant properties may be found in some hypoglycemic substances. A highly efficient and all-natural way to prevent glycation in diabetes is to maintain a healthy blood sugar level. Additionally, plasma amines, antioxidant enzymes, and compounds, as well as liver enzymes that aid in detoxification, promote this defense.

Black Chia seeds *(Salvia hispanica* L.*)* belongs to the *Salvia* genus. Due to their high dietary fiber content, black chia seeds are said to be good hypoglycemic and hypolipidemic agents. It is distinguished by having large concentrations of polyunsaturated fatty acids, particularly

***Corresponding author**: Radwa Wahid Mohamed, Email: radwa.wahid@women.asu.edu.eg

α-linolenic acid (ALA), which makes up around 60% of all fatty acids. The seeds contain more omega-3 acids than flaxseed (3). Additionally, seeds are a plentiful source of phytocompound groups with significant biological activity. Gallic, caffeic, chlorogenic, cinnamic, and ferulic acids, as well as quercetin, kaempferol, epicatechin, rutin, apigenin, and p-coumaric acid, are some of the polyphenols that are particularly noteworthy (4).

The garden cress *(Lepidium sativum* L.*)* plant belongs to the Brassicaceae family. Due to its high phytochemical and flavonoid content, garden cress is widely recognized for its nutritional and therapeutic benefits. Flavonoid molecules have anti-inflammatory, antioxidant, and hypoglycemic effects (5). Additionally, the seeds have a healthy fatty acid profile and offer 7-8 mg of fiber per 100 g. It has a significant quantity of linolenic acid (26-34 percent). Arachidic acid (2–3.5%), oleic acid (26–30%), and linoleic acid (7–11%) are also present in reasonable amounts (6).

This work aimed to study the bioactive components of black chia and garden cress seeds and their hypoglycemic effects in diabetic type 2 with the prevention of the associated glycation process.

Materials and Methods

Materials

Streptozotocin (STZ) was purchased from Sigma Aldrich Company for Chemicals, Cairo, Egypt. Black chia seeds and Garden cress seeds were purchased from the Ministry of Agriculture, Cairo, Egypt.

Animals

For the experimental investigation, 40 healthy adult male Wistar rats, aged 6 weeks and weighing 180 ± 20g, were purchased from the National Research Center in Cairo, Egypt. Rats were fasted for 12 hours prior to receiving a single intraperitoneal injection of freshly produced STZ solution (in citrate buffer, pH = 4.5) for the purpose of inducing diabetes (7).

Animal grouping and experimental design

Ten rats apiece from each of the 4 groups of rats were used. Rats in group 1 (the healthy control group) were given commercial pellet diets and intraperitoneal injections of 0.1 mol/L citrate buffer and 1 mL distilled water once daily for 28 days (8). Group 2 (diabetic group): diabetic control rats received a commercial pellet diet after STZ induced hyperglycemia in rats. Group 3 (black chia seeds group) and group 4 (Garden cress seeds group): After diabetes induction, rats received a commercial pellet diet and were treated with 1 mL (20% (w/w)) black chia and garden cress seed extract, respectively (Orally using stomach tube daily for 28 days) (8).

Determination of bioactive components of black chia and garden cress seeds

The bioactive components of the tested seeds' methanolic extract were determined using gas chromatography according to the method described before (9).

Evaluation of glucose metabolic dysfunction and glycated markers

Serum glucose was determined by colorimetric assay (10) using Bio-Med kits, serum insulin was determined by immunosorbent assay (11) using RayBio Rat insulin ELISA Kit. Serum glycated hemoglobin A1c (HbA1c) was determined by colorimetric method (12) using BioSource Rat HbA1c ELISA kit and Nε-CML was determined by colorimetric method (13) using BioSource Rat Nε-CML ELISA kit, respectively. Quantitative insulin sensitivity check index (QUICKI) and homeostatic model assessment of insulin resistance (HOMA-IR) were calculated according to previously performed equations (14).

Determination of lipogenesis factors and Atherogenic index (AI)

Total cholesterol (TC), triacylglycerols (TAGs), and high-density lipoproteins (HDL-C) were measured calorimetrically (15) using BIO-Med kits. Low-density lipoproteins (LDL-C) and very low-density lipoproteins (VLDL-C) were calculated using previously described equations (16). Free fatty acids were measured by using an ELIZA kit (17). AI and RF were also calculated using previously described equations (18)

Evaluation of oxidative stress status and antioxidants in the pancreas:

Superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA) were measured using a Bio-Diagnostic kit (19-21).

Determination of inflammatory biomarkers:

Interferon-γ (INF-γ) and interleukin1-β (IL-1β) were determined by the colorimetric method using Rat ELISA kits of INF-γ and IL-1β (BioSource), respectively (22).

Evaluation of organs functions

To evaluate liver function, serum albumin and total bilirubin were measured calorimetrically (23) using Bio-Diagnostic kits. Kidney function tests were performed by the estimation of urea and creatinine calorimetrically using Diamond Diagnostic kits (24). For pancreatic function evaluation, pancreatic trypsin and pancreatic iso-amylase were determined using an ELISA kit (BioVision) (25).

Microscopic examination of the pancreas

For regular histological investigation using hematoxylin-eosin (H&E) stain, pancreas portions were carefully excised and preserved in neutral formalin solution at a 10% concentration.

Statistical analysis

Version 16.0 of the Statistical Package for Social Science

(Microsoft Windows and SPSS Inc.) were utilized to analyze the data. The data were expressed as mean ± standard deviation (SD). The one-way analysis of variance (ANOVA) was used to detect statistical differences between groups; the mean difference was considered significant at the $P < 0.05$ level (26).

Results

Determination of bioactive components in seed extracts by gas chromatography (GC)

The results presented in Tables 1 and 2 and Figures 1 and 2 give an overview of bioactive components in black chia and garden cress seed extracts. From the results, it was clear that, black chia seed extract contained 49.62% of linolenic acid, 18.26% of chlorogenic acid, 6.11% of 5-hydroxymethylfurfural, 5.85% of caffeic acid, 5.74% quercetin, 5.46% of 3,6-dimethyl-3,6-dihydro-pyran-2-one oxime, and other bioactive components in traces amounts (Table 1) while, the garden cress seed extract contained 74.79% of 7,10- hexadecadienoic acid, methyl ester, 21.31% of 11- octadecenoic acid, methyl ester, and other bioactive components in smaller amounts (Table 2).

The effect of black chia and garden cress seed extract on evaluation of glucose metabolic dysfunction markers and glycation process

The results presented in Table 3 showed that the induction of diabetes by STZ injection in the group 2 caused a significant increase ($P< 0.05$) in serum glucose level by 419.36% compared with the healthy control group (1). In addition, the administration of black chia and garden cress seed extracts as treatments caused a significant decrease ($P< 0.05$) in serum glucose levels by about 66.17% and 69.67%, respectively when compared with the diabetic control group 2. The results of serum insulin level showed a significant decrement ($P< 0.05$) in the diabetic group by 41.50% as compared with the healthy control group 1. In addition, the administration of seed extracts caused a significant elevation ($P< 0.05$) in serum insulin levels by about 31.73% and 48.13%, respectively when compared with diabetic control group 2. With respect to the results of glycated hemoglobin (HbA1c), the induction of diabetes caused the highest significant rise ($P< 0.05$) in HbA1c by 161.64% when compared with the healthy control group 1. While, the administration of the

Table 1. Bioactive components of black chia seeds

Name	Formula	Retention time (RT)	Area sum %
9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (Linolenic acid)	$C_{18}H_{30}O_2$	28.494	49.62
Chlorogenic acid	$C_{16}H_{18}O_9$	19.137	18.26
5-Hydroxymethylfurfural	$C_6H_6O_3$	15.463	6.11
Caffeic acid	$C_9H_8O_4$	21.465	5.85
Quercetin	$C_{15}H_{10}O_7$	15.612	5.74
3,6-Dimethyl-3,6-dihydro-pyran-2-one oxime	$C_7H_{11}NO_2$	11.85	5.46
Gamma-sitosterol (Clionasterol)	$C_{29}H_{50}O$	41.717	2.63
6-Acetyl-beta-d-mannose	$C_8H_{14}O_7$	13.518	2.33
Pterin-6-carboxylic acid	$C_7H_5N_5O_3$	13.136	2.14
1-(5-Bicyclo [2.2.1] heptyl) ethylamine	$C_9H_{17}N$	10.829	1.86

Table 2. Bioactive components of garden cress seeds

Name	Formula	Retention time (RT)	Area sum %
7,10- Hexadecadienoic acid, methyl ester	$C_{17}H_{30}O_2$	24.919	74.97
11- Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	24.979	21.31
D-(+)-Turanose, octakis(trimethylsilyl) ether	$C_{36}H_{86}O_{11}Si_8$	25.756	1.21
Benzenepropanoic acid, .alpha.-(hydroxyimino)-	$C_9H_9NO_3$	9.509	0.93
Silanol, trimethyl-, phosphate (3:1) (Tris(trimethylsilyl) phosphate)	$C_9H_{27}O_4PSi_3$	11.31	0.44
Trimethylsilyl ether of glycerol	$C_{12}H_{32}O_3Si_3$	11.212	0.36
Oleic acid, trimethylsilyl ester	$C_{21}H_{42}O_2Si$	21.182	0.27
Stearic acid, 2,3-bis(trimethylsiloxy)propyl ester	$C_{27}H_{58}O_4Si_2$	25.529	0.17
n-Pentadecanoic acid, trimethylsilyl ester	$C_{18}H_{38}O_2Si$	11.529	0.1
Palmitic acid, trimethylsilyl ester	$C_{19}H_{40}O_2Si$	19.629	0.09
1-Monopalmitin trimethylsilyl ether	$C_{25}H_{54}O_4Si_2$	24.135	0.09
Benzoic acid tri-methyl-silyl ester	$C_{10}H_{14}O_2Si$	10.843	0.08

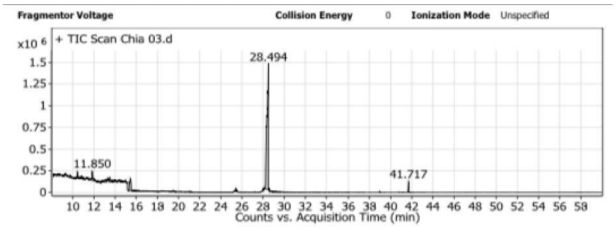

Figure 1. Gas chromatography/mass spectrometry chromatogram of bioactive components of black chia seed extract.

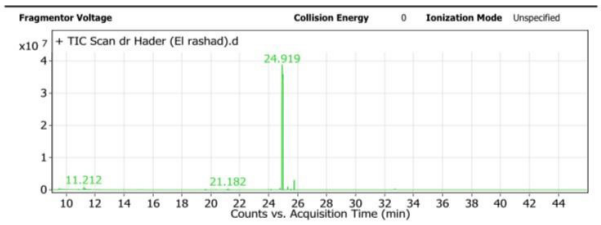

Figure 2. Gas chromatography/mass spectrometry chromatogram of bioactive components of garden cress seed extract.

tested seed extracts caused a significant decline ($P < 0.05$) in HbA1c by 28.05% and 50.26%, respectively when compared with the diabetic group 2. Regarding serum QUICKI, the induction of diabetes caused a significant decrease ($P < 0.05$) by 25.05% when compared with the healthy control group 1. In addition, the administration of the tested extracts caused a significant increase ($P < 0.05$) in QUICKI by about 20.47% and 23.32%, respectively compared with the diabetic control group 2. From Table 3 the results of HOMA-IR confirmed that the injection of STZ caused the highest significant increase ($p<0.05$) by 204.31% compared with the healthy control group 1. Moreover, the oral administration of the tested treatments caused a significant decrease ($P < 0.05$) in HOMA-IR by 53.30% and 55.19%, respectively when compared with the diabetic control group 2. According to the results of serum Nε-CML, the induction of diabetes caused a significant increase ($P < 0.05$) by 129.93% compared with the healthy control group 1. Additionally, the oral administration of different treatments caused significant decrements ($P < 0.05$) in Nε-CML by 45.15% and 47.64%, respectively when compared with the diabetic control group.

The effect of black chia and garden cress seed extracts on lipogenesis factors and atherogenic indices in all experimental groups

From the results of in Table 4, the induction of diabetes caused a significant increase ($P < 0.05$) in serum TAGs by 31.97% compared with the healthy control group, while the oral administration of the tested seed extracts caused a significant decrease ($P < 0.05$) in serum TAGs by about 16.83% and 13.53%, respectively when compared with the diabetic control group. Considering the serum level of total cholesterol (TC) in STZ injection, there was a significant elevation ($P < 0.05$) in serum TC by 60.13% compared with the healthy control rats. In addition, the oral administration of different treatments caused a significant decrement ($P < 0.05$) by about 35.82% and 21.62%, respectively when compared with the diabetic group. With respect to the results of HDL-C, the induction of diabetes caused a significant decrease ($P < 0.05$) in serum HDL-C by 45.83% as compared with the healthy control group. In addition, the oral administration of the tested seed extracts caused a significant elevation ($P < 0.05$) in serum HDL-C by 52.20%, 29.15%, and 24%, respectively as compared with the diabetic control group.

The results of LDL-C confirmed that the induction of diabetes caused a significant rise ($P < 0.05$) in serum LDL-C by 198.72% compared with the healthy control group, while the oral administration of different treatments caused a significant decrement ($P < 0.05$) in serum LDL-C by 58.52% and 33.92%, respectively when compared with the diabetic control rats. From the results of VLDL-C, the induction of diabetes caused a significant increase ($P < 0.05$) in serum VLDL-C compared with healthy control rats by 31.99%, while the oral administration of the seed extracts caused a significant decline ($P < 0.05$) in serum VLDL-C by about 16.83 and 13.55%, respectively when compared with the diabetic control rats. From the result of free fatty acids (FFAs)

Table 3. Effect of black chia and garden cress seed extracts on glucose metabolic dysfunction markers and glycation process in all experimental groups

Parameters/Groups	Glucose level (mg/dL)	Insulin level (µIU/mL)	(HbA1c) %	QUICKI	HOMA	Nε-CML (ng/mL)
G1: (HC) group	73.51^{a} ±6.79	12.82^{a} ± 0.45	5.11^{a} ± 0.72	0.515^{a} ±0.01	2.09^{a} ± 0.17	226.90^{a} ±7.78
G2: (DC) group	381.78^{b} ±42.60	7.50^{b} ± 0.49	13.37^{b} ± 1.50	0.386^{b} ±0.01	6.36^{b} ± 0.86	521.71^{b} ±10.47
G3: (BC) group	129.14^{c} ±6.97	9.88^{c} ± 0.75	9.62^{c} ± 1.20	0.465^{c} ±0.01	2.97^{c} ± 0.25	286.16^{c} ±14.17
G4: (GC) group	115.44^{d} ±3.32	11.11^{d} ± 0.84	6.65^{d} ± 0.51	0.476^{d} ±0.00	2.85^{c} ± 0.23	273.16^{d} ±18.80
LSD	12.42	0.402	0.651	0.0	0.27	8.47

HbA1c: Hemoglobin A1c; HOMA: Homeostatic model assessment of insulin resistance; QUICKI: Quantitative insulin sensitivity check index; Nε-CML: Nε-carboxy methyl lysine.
Values are mean ± SD.
There is a significant difference between the means having different letters in the same row ($P < 0.05$).
G1: Healthy control (HC) group; G2: Diabetic control (DC) group; G3: Black chia (BC) group; G4: Garden cress (GC) group.

Table 4. Effect of black chia and garden cress seed extracts on lipogenesis factors and atherogenic indices in all experimental groups

Groups/Parameters	G1:(HC)	G2:(DC)	G3:(BC)	G4:(GC)	LSD
TAGs (mg/dL)	132.57^{a} ± 4.78	174.96^{b} ± 9.03	145.52^{c} ± 10.7	151.29^{d} ± 12.60	6.66
TC (mg/dL)	116.9^{a} ± 7.49	187.19^{b} ± 9.24	120.14^{c} ± 3.12	146.72^{d} ± 13.96	6.15
HDL-C (mg/dL)	46.54^{a} ± 5.76	25.21^{b} ± 3.02	38.37^{c} ± 4.46	32.56^{d} ± 6.10	3.068
LDL-C (mg/dL)	42.51^{a} ± 15.87	126.99^{b} ± 10.31	52.67^{c} ± 6.40	83.91^{d} ± 9.60	6.94
VLDL-C (mg/dL)	26.51^{a} ± 0.96	34.99^{b} ± 1.80	29.10^{c} ± 2.17	30.25^{c} ± 2.52	1.33
FFAs (ng/mL)	94.52^{a} ± 6.81	239.77^{b} ± 14.83	128.32^{c} ± 7.67	124.28^{c} ± 5.87	6.15
AI)	2.986^{a} ± 0.41	6.801^{b} ± 1.25	3.829^{c} ± 0.48	4.768^{d} ± 0.84	0.48
RF	0.95^{a} ± 0.45	5.13^{b} ± 0.95	1.06^{a} ± 0.46	2.64^{c} ± 0.44	0.38

TC: Total cholesterol; HDL-C: high-density lipoproteins; LDL-C: Low-density lipoproteins; VLDL-C: very low-density lipoproteins; FFAs: Free fatty acids; RF: Risk factor; AI, Atherogenic index.
Values are mean ± SD.
There is a significant difference between the means having different letters in the same row ($P < 0.05$).
G1: Healthy control (HC) group; G2: Diabetic control (DC) group; G3: Black chia (BC) group; G4: Garden cress (GC) group.

tabulated in table (4), the induction of diabetes caused a significant increase ($P < 0.05$) in serum FFAs by about 153.67% compared with the healthy control rats, while the oral administration of the tested seed extracts caused a significant decrement ($P < 0.05$) in serum FFAs by about 46.48% and 48.17%, respectively when compared with the diabetic control rats. Considering the value of AI, the injection of STZ caused a significant increase ($P < 0.05$) in serum AI by 127.76% compared with the healthy control rats. On the other hand, the oral administration of the tested seed extracts caused a significant decline ($P < 0.05$) in serum AI by about 43.70% and 29.89%, respectively when compared with the diabetic control rats. From the results of risk factor (RF), the induction of diabetes caused a significant increase ($P < 0.05$) in serum RF by 440% compared with the healthy control rats. In addition, the oral administration of different treatments caused a significant decrease ($P < 0.05$) in serum RF by about 79.34% and 48.54%, respectively when compared with the diabetic control rats.

The effect of black chia and garden cress seed extracts on antioxidant and lipid peroxidation status in pancreatic tissue in all experimental groups

The data presented in Table 5 showed that pancreatic SOD activity significantly decreased ($P < 0.05$) following STZ injection by about 56.74% when compared with the healthy control rats. In addition, the administration of black chia and garden cress seed extracts caused a significant increase ($P < 0.05$) in SOD activity by 95.73% and 103.23%, respectively compared with the diabetic control rats. Additionally, pancreatic GSH levels showed a significant decrease ($P < 0.05$) with diabetic induction by about 34.12% when compared with the healthy control rats. Administration of different treatments caused a significant increase ($P < 0.05$) in GSH levels by 50.37% and 44.24%, respectively compared with the diabetic control rats. Considering the level of pancreatic MDA, the injection of STZ caused a significant increase ($P < 0.05$) in MDA level by 257.02% compared with the healthy control group. In addition, the oral administration of the tested seed extracts caused a significant decrease ($P < 0.05$) in MDA level by about 50.02% and 42.80%, respectively when compared with the diabetic control rats.

The effect of black chia and garden cress seed extracts on inflammatory biomarkers levels in serum in all experimental groups

The data presented in Table 5 confirmed a significant increase ($P < 0.05$) in serum INF-γ by 196.31% in diabetes group compared with the healthy control rats. On the other hand, the oral administration of the tested seed

Table 5. Effect of back chia and garden cress seed extracts on antioxidant, lipid peroxidation status, and inflammatory biomarkers in all experimental groups

Parameters/groups	SOD (U/g)	GSH (mmol/g)	MDA (nmol/g)	INF-γ (pg/ML)	IL-1β (pg/ML)
G1: (HC) group	20.02^{a} ± 1.63	58.76^{a} ± 2.05	32.48^{a} ± 2.84	51.46^{a} ± 2.95	115.07^{a} ± 3.89
G2: (DC) group	8.66^{b} ± 0.52	29.50^{b} ± 3.71	115.96^{b} ± 4.59	152.48^{b} ± 7.35	276.35^{b} ± 6.20
G3: (BC) group	16.95^{cd} ± 0.92	44.36^{c} ± 3.74	57.96^{c} ± 5.03	81.04^{c} ± 10.15	135.80^{c} ± 4.56
G4: (GC) group	17.60^{d} ± 1.29	42.55^{cd} ± 3.08	66.33^{d} ± 6.91	74.75^{d} ± 1.58	142.34^{d} ± 4.45
LSD	0.725	1.95	3.05	4.012	3.07

SOD: Superoxide dismutase; GSH: Reduced glutathione; MDA: Malondialdehyde; INF-γ: Interferon-γ.
Values are mean ± SD.
There is a significant difference between the means having different letters in the same row ($P < 0.05$).
G1: Healthy control (HC) group; G2: Diabetic control (DC) group; G3: Black chia (BC) group; G4: Garden cress (GC) group.

extracts caused a significant decrease ($P<0.05$) in serum INF-γ by about 46.85% and 50.98%, respectively when compared with the diabetic control group. Considering the results of IL-1β, the induction of diabetes caused a significant increase ($P<0.05$) in serum IL-1β by 140.16% compared with the healthy control rats. In addition, the oral administration of the tested seed extracts caused a significant decrease ($P<0.05$) in serum IL-1β by about 50.68% and 48.49%, respectively when compared with the diabetic control group.

The effect of black chia and garden cress seed extracts on liver function tests in all experimental groups

From the results presented in Table 6, the induction of diabetes mellitus caused a significant decrease ($P<0.05$) in serum albumin by 24.40% when compared with the healthy control group. In addition, the oral administration of tested treatments seed extracts caused a significant increase ($P<0.05$) in serum albumin by 16.73% and 19.92%, respectively compared with the diabetic control rats. Considering the serum total bilirubin level, the injection of STZ caused a significant increase ($P<0.05$) by 30.99% compared with the healthy control rats, while the oral administration of the tested seed extracts caused a significant decrease ($P<0.05$) in serum total bilirubin by about 20.24% and 20.09%, respectively when compared with the diabetic control rats.

The effect of black chia and garden cress seed extracts on kidney function in all experimental groups

The kidney function presented in Table 6 showed the induction of diabetes caused a significant increase ($P<0.05$) in serum urea by 102.54% compared with the healthy control rats, while the oral administration of the tested treatments with seed extracts caused a significant decrease ($P<0.05$) in serum urea by about 33.13% and 26.15%, respectively when compared with the diabetic rats. With respect to the level of serum creatinine, the induction of diabetes caused a significant increase ($P<0.05$) in serum creatinine by 71.10% compared with the healthy control rats, while the oral administration of the tested seed extracts caused a significant decrease ($P<0.05$) in serum creatinine by about 32.50% and 40.20%, respectively when compared with the diabetic control rats.

The effect of black chia and garden cress seed extracts on pancreatic function in all experimental groups

The data presented in Table 6 showed that the induction of diabetes caused a significant increase ($P<0.05$) in pancreatic trypsin concentration by 270.41% compared with the healthy control rats. In addition, the oral administration of the tested seed extracts caused a significant decrease ($P<0.05$) in pancreatic trypsin concentration by about 54.80% and 55.21%, respectively when compared with the diabetic control rats. With respect to the activity of pancreatic amylase enzyme, the injection by STZ caused a significant increase ($P<0.05$) by 168.79% compared with the healthy control group. But the oral administration of the tested treatments of seed extracts caused a significant decrease ($P<0.05$) in pancreatic amylase enzyme activity by about 49.64% and 54.19%, respectively when compared with the diabetic control.

The effect of black chia and garden cress seed extracts on histological examination of the pancreas in all experimental groups

The data illustrated in Figure 3 showed the effect of black chia and garden cress seed extracts on the microscopic examination of pancreatic tissues in all experimental rat groups. From the data, the healthy control rats showed normal histological structure of the islet of Langerhans as well as the acini and duct system as exocrine ones (Figures 3a and 3b). However, the induction of diabetes by STZ injection caused the atrophy of Langerhans islets with decline in the size and number of the cells associated with periductal inflammatory cells infiltration surrounding the cystic ducts of the exocrine portion (Figures 3c and 3d). In addition, the oral administration of black chia and garden cress seed extracts caused significant histopathological improvement in the morphological cellular features of pancreatic tissues (Figures 3e, 3f, 3g and 3h, respectively).

Discussion

Hyperglycemia is assumed to be the primary cause of both

Table 6. Effect of black chia and garden cress seed extracts on the liver, kidney, and pancreatic functions in all experimental groups

Parameters/Groups	Albumin (g/dL)	Total Bilirubin (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)	Trypsin (ng/g)	Amylase (µIU/g)
G1: (HC) group	3.32^{a}± 0.35	1.026^{a}± 0.01	24.77^{a}± 1.78	0.865^{a}±0.06	2.67^{a}± 0.33	65.44^{a}± 3.64
G2: (DC) group	2.51^{b}± 0.31	1.344^{b}±0.10	50.17^{b}± 5.08	1.48^{b}±0.12	9.89^{b}± 1.07	175.90^{b}± 8.38
G3: (BC) group	2.93^{c}± 0.08	1.072^{c}±0.02	33.55^{c}± 3.59	0.999^{c}±0.11	4.47^{c}± 0.44	88.58^{c}± 4.73
G4: (GC) group	3.01^{c}± 0.07	1.074^{c}±0.01	37.05^{d}± 3.22	0.885^{a}±0.07	4.43^{c}± 0.37	80.57^{d}± 4.85
LSD	0.14	0.03	2.20	0.07	0.363	3.375

Values are mean ± SD.
There is a significant difference between the means having different letters in the same row ($P<0.05$).
G1: Healthy control (HC) group; G2: Diabetic control (DC) group; G3: Black chia (BC) group; G4: Garden cress (GC) group.

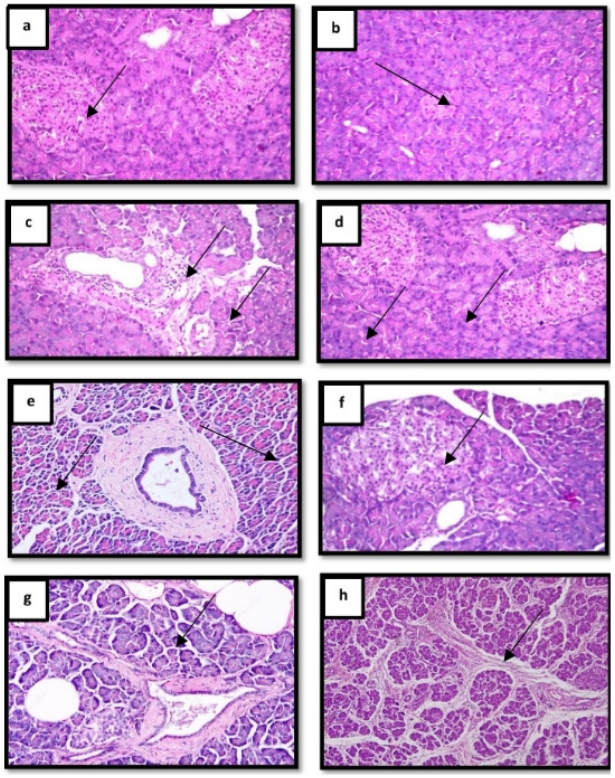

Figure 3. Microscopic examination of pancreatic tissues in all experimental groups. (a and b) The healthy control rats (G1) showed no histopathological alteration and the normal histological structure of the islet of Langerhans. (c and d) The diabetic rats (G2) showed atrophy in the islet of Langerhans with a decline in the size and number of the cells associated with periductal inflammatory cells infiltration surrounding the cystic ducts of the exocrine portion. (e and f) Chia seed group (G3) showed histopathological improvement in morphological cellular features of pancreatic tissues. (g and h) Garden cress seed group 4 showed small inflammatory cells with less infiltration surrounding the duct. (H & E ×400).

glycation and the gradual buildup of AGEs in physiological tissues. Despite the possibility that intracellular AGEs may be involved in the activation of intracellular signaling pathways and modification of the functions of intracellular proteins, there are numerous indications that the accumulation of AGEs is a significant pathogenic factor for the development of diabetes, cataracts, atherosclerosis, diabetic nephropathy, and neurodegenerative diseases (2). Anti-diabetic drugs can interrupt glycation by reducing blood glucose levels or by inhibiting the third phase of the synthesis of AGEs because glycation is an irreversible reaction.

Black chia seeds are a rich source of bioactive chemicals due to their high concentration of polyphenolic compounds, including chlorogenic acid, caffeic acid, myricetin, quercetin, and kaempferol, as well as lipophilic substances like tocopherols, phytosterols, carotenoids, and phospholipids. Polyunsaturated fatty acids (PUFAs), particularly the beneficial linolenic acid, are very abundant in black chia seeds, which are favorable for human health (27,28). In addition, hexadecanoic acid, palmitic acid, benzenepropanoic acid, methyl ester, and other substances are the primary chemicals discovered in garden cress seeds. Garden cress also includes oleic acid (30.5%), palmitic acid (10.3–0.12), and other fatty acids. These findings demonstrated the value of garden cress as an omega -3 fatty acid source (29).

Induction of diabetes by STZ injection was found to be associated with high serum glucose levels, a lack or absence of the hormone insulin, increased glycation products, HbA1c and Nε-CML, and increased insulin resistance indices. These findings were based on the evaluation of glucose metabolic dysfunction and glycated markers. Oral administration of black chia and garden cress seed extracts exhibited an anti-diabetic effect because of their ability to preserve beta-cell and pancreatic cell proliferation. In contrast to the diabetic group, this resulted in a rise in blood insulin levels and a drop in serum glucose levels, which enhanced the cells' sensitivity to insulin and glucose absorption. Our results confirmed previous studies that demonstrated chia seeds enhanced biological markers for dyslipidemia, inflammation, cardiovascular disease, glucose homeostasis, insulin resistance, and glycation without causing adverse effects (30). Additionally, in insulin-resistant rats given black chia seeds for two months, a considerable drop in blood glucose levels was seen along with a decrease in serum Nε-CML. This could be as a result of the large amount of dietary fiber included in chia seeds, which works to reduce the absorption of intestinal glucose (31). Consistent with our results a research (32), found that the consumption of chia seeds decreased blood glucose levels. Their high phenolic content and increased free radical scavenging activity had the effect of inhibiting stage 3 of Nε-CML synthesis. Because garden cress seeds' physiologically active chemicals promoted glucose absorption via inducing insulin receptor kinase activity, insulin receptor autophosphorylation, and eventually enhanced glycogen synthase activity, they supported prior results that indicated these seeds contained insulin-mimetic characteristics (33). Additionally, using the aqueous garden cress extract might significantly reduce blood glucose levels. According to Hamidpour et al study (34), the presence of benzyl isothiocyanate flavonoid in garden cress seed extract, which prevented Amadori reaction, an organic reaction of hemoglobin glycation, accounted for the powerful glycation inhibition effect of garden cress seed extract on synthesis of HbA1c. Antioxidant compounds may be useful preventative agents for the production of glycation and AGE since oxidative stress followed and accelerated the synthesis of glycation and Nε-CML (35). The bulk of studies on polyphenolic compounds with regard to possible anti-diabetic effects focused on flavonoids in particular. In STZ-induced diabetic rats, oral administration of polyphenolic compounds extract resulted in a significant reduction in HbA1c and Nε-CML production, fasting blood glucose levels, and an improvement in lipid profile status, indicating that garden cress has strong antioxidant

properties in addition to anti-diabetic and cholesterol-lowering effects.

Lipogenesis factors and atherogenic indices results showed that hyperlipidemia and oxidative stress are the disorders of diabetes mellitus. They also elevated the serum levels of TAGs, TC, LDL-C, VLDL-C, FFAs, AI, and RF and decreased the level of HDL-C. In contrast, administering black chia and garden cress seed extracts caused a decrease in serum levels of TAGs, TC, LDL-C, VLDL-C, FFAs, AI, and RF and an increase in HDL-C serum level. The ability of black chia and garden cress seeds to improve lipid metabolism and decrease atherogenicity might be due to the high content of omega-3 fatty acids, which helps to increase HDL-C "good", decrease LDL-C "bad", and reduce blood TAGs. In addition to highly content of vitamin C, which help to protect the arteries from the increased level of cholesterol. Additionally, our findings supported those of (36) who discovered that chia seed, the richest vegetal source of linolenic fatty acids, significantly reduced TAGs, LDL-C, TC, and FFAs, while considerably raising HDL-C. After consuming both black and white chia seeds, an improvement in lipid profiles was seen, which might be related to the high concentration of omega-3 fatty acids in chia seeds (37). Dietary chia supplementation increased dietary fiber intake by 55% and plasma-linolenic acid content by 75% (38). Additionally, as compared with the matching control group, TC, HDL-C, and FFAs all dramatically decreased by roughly 25%, 32%, and 38%, respectively. In a previous research (39), rats received diets enriched with garden cress seeds powder at 5% and 10%, and their mean levels of LDL-C and VLDL-C were lower than those of the positive control group. Garden cress seeds were also given to hypercholesterolemic rats, improving their lipid profiles because they contained large amounts of vitamin C, which protected the arteries from excessive cholesterol levels (40). It was evident from the findings of the antioxidant and lipid peroxidation status in pancreatic tissue that the induction of diabetes mellitus led to oxidative stress in pancreatic cells, which decreased the antioxidant enzyme SOD, reduced GSH molecule, and increased the lipid peroxidation product MDA. Due to their antioxidant properties, administration of black chia and garden cress seed extracts increased SOD activity and GSH level, and decreased MDA level in pancreatic tissues, enabling cell production of insulin and glucagon to regulate blood sugar levels. Our findings are consistent with an earlier research (41) showing that diabetes mellitus is related to reduced antioxidant enzyme activity and lipid peroxidation caused by free radicals. These facts manifested as a marked decrease in SOD activity and GSH level, as well as an increase in MDA in pancreatic tissue. In addition, another study (42) verified that adding chia and garden cress seeds to the diet significantly reduced inflammation and lipid peroxidation as seen by lower levels of MDA, nitric oxide (NO), and tumor necrosis factor alpha (TNF-α) in the rat liver tissues. The primary antioxidants in chia seeds, caffeic acid, and chlorogenic acid were capable of preventing lipid peroxidation. These compounds are substantially stronger and more potent antioxidants than other antioxidants like vitamin E and vitamin C. Another antioxidant present in chia seeds was quercetin, which could prevent the oxidation of lipids. Additionally, the antioxidant properties of quercetin were considered to be stronger than those of some flavanol compounds (3). Our results were consistent with those of (8) who found that administering garden cress methanol extract to diabetic rats significantly ($P < 0.001$) increased the mean value of SOD in comparison to the positive control. In accordance with these results, garden cress included a substantial quantity of phenolic groups, which resulted in the scavenging of free radicals, one of the primary antioxidation techniques for stopping the chain reaction of lipid peroxidation. The antioxidant activity of garden cress was discovered to inhibit the glycation process, reduced HbA1c levels, and stop the production of Nε-CML (35).

Serum levels of inflammatory biomarkers showed that diabetes mellitus generated large amounts of free radicals and damaged the antioxidant system. An imbalance between the creation and removal of ROS, which was shown as an increase in the production of IFN-γ and IL-1β in diabetes, was linked to the inflammation of the pancreatic β-cells. These pro-inflammatory substances increased the activity of inducible nitric oxide synthase (iNOS), which produced NO and aided in the deterioration of β-cells. Due to the anti-inflammatory and antioxidant effects of black chia and garden cress seed extracts, the inflammatory biomarkers decreased. Following the injection of STZ, inflammatory cells were found inside the pancreatic islets and AGEs were produced. The interaction of these molecules with their cell-surface receptors led to oxidative stress and the start of an inflammatory cascade. The MAPK pathway was activated throughout the inflammatory cascade resulting in the production of IL-6, TNF-α, IL-1β, VCM-2, and INF-γ, and caused β-cell dysfunction (2). The intake of chia seeds had the ability to improve the antioxidant activity by increasing SOD activity, CAT activity, and PPAR-α expression. In addition, chia consumption decreased the concentrations of the inflammatory biomarkers IL-1β and INF-γ. The improvement may be due to the presence of phenolic compounds, vitamins, minerals, dietary fiber, and polyunsaturated fatty acids from omega-3-type (43). The intake of garden cress seeds resulted in decreased inflammation and oxidative stress due to their high contents of vitamins B complex, A & C that inhibited AGEs combination with their receptors. The anti-inflammatory effects resulted in decreased glycation process, HbA1c and Nε-CML formation (8).

The findings of liver function evaluation revealed that diabetes mellitus produced severe damage to the liver cells, which was shown as a significant rise in blood

total bilirubin and a significant fall in serum albumin. However, the group that received black chia and garden cress demonstrated enhanced liver function. Diabetes mellitus decreased serum albumin level and damaged the liver. Additionally, it has been shown that both white and black chia seeds can enhance liver function and reduce liver damage (44,45). Our findings corroborated those of a previous study (46) that found the levels of albumin were significantly higher in the chia seed extract treatment group compared to the control group. This might be because chia seeds contain a high amount of omega-3, which is considered an antioxidant, playing a role in stimulating protein synthesis throughout the body by decreasing lipid peroxide and increasing catalase activity, which and reduce lipid peroxide. The mechanism of the hepatoprotective action of garden cress might be related to the ability to inhibit lipid peroxidation in the liver. The presence of flavonoids triterpenes, alkaloids, tannins, and coumarins in garden cress seeds explain its role in hepatoprotection by inhibiting the free radicals mediated damage (47).

The evaluation of kidney function showed that renal failure was a diabetic complication due to the effect of glucose on the renal glomerulus and the oxidative stress present in diabetes mellitus causing the alternation in the kidney function and leading to increase in both serum urea and creatinine. Diabetes induction affected renal function by increasing serum urea and creatinine levels. This result was consistent with the fact that STZ-induced diabetes led to diabetic nephropathy (48). Treating the diabetic rats with garden cress with different concentrations of methanol extract caused a significant decrease in serum urea and creatinine. Flavonoids and phenolic compounds protected against diabetic nephropathy in STZ- induced diabetic rats improving blood urea nitrogen and creatinine as well as kidney tissue damage, with a reduction in mitochondrial damage (49). Moreover, the extract of garden cress had both renal protective and curative effects as it significantly reduced the blood levels of urea and creatinine indicating an increased glomerular filtration rate (42). In addition, chia seed methanolic extract had renal protective effects by decreasing blood levels of urea and creatinine in CCL4-induced renal toxicity. The authors explained their results by the high scavenging activity for free radicals of chia due to high level of flavonoids with excess hydroxyl groups in their structure (4).

From the results of pancreatic function evaluation, increased levels of trypsin and amylase occurred due to the inflammation of the pancreas. While after the oral administration of black chia and garden cress, the pancreatic function was improved to regulate the production of trypsin and amylase enzymes due to their antioxidant effect, which decremented the oxidative stress on the pancreatic cells and improved the sensitivity of insulin to uptake glucose by cells. Acute or chronic pancreatitis is often indicated by elevated amylase levels. Amylase levels rose four to six times beyond the upper limit of the normal range due to acute pancreatitis (50). When digestive enzymes get activated, irritate the pancreatic cells and result in inflammation. Acute pancreatitis can damage the pancreas over time, which can result in chronic pancreatitis. The pancreas may develop scar tissue, which would impair its functionality. Diabetes and digestive issues were brought on by an inefficient pancreas (51). When pancreatic inflammation developed, trypsin levels in all patients increased. In the small intestine, serum trypsin was created along with its precursor enzyme. The subsequent activation of other pancreatic enzymes by trypsin caused tissue damage and ultimately the auto-digestion of pancreatic cells or the pancreas. Both black chia and garden cress seeds had anti-inflammatory properties that improved pancreatic function, returned trypsin concentration and amylase enzyme activity to normal levels, and increased the pancreatic capacity to carry out its typical function (52).

The results of microscopic examination of the pancreatic tissue confirmed that diabetes mellitus induction caused oxidative stress in pancreatic cells, resulting in damage and atrophy in beta of Langerhans, while administration of different treatments led to a decrease in the oxidative stress on pancreatic tissue and improved pancreatic function. Our findings were consistent with earlier findings (53) that showed that treatment with garden cress seeds improved the pancreatic architecture and caused the islets of Langerhans to regenerate. Garden cress's potent ability to repair the histological damage caused by STZ and hyperglycemia was explained by increasing cell repair and proliferation, stimulation, and enhancing insulin secretion. This powerful action was likely a result of the garden cress's flavonoids, alkaloids, and polyphenol contents. Hexadecadienoic acid content of garden cress seeds was reported (42) to inhibit the combination of AGEs with their receptors and resulted in inhibiting the MAPK pathway of inflammatory cytokines production. Decreased inflammatory conditions regenerated the structure of pancreatic tissue. Moreover, Chia seeds have been reported to regenerate the normal structure of pancreatic tissue by decreasing free radicals and inflammatory conditions through their high omega-3 fatty acids content, which have anti-inflammatory effects by inhibiting NF-κB pathway, the pathway of inflammatory cytokines production (4).

Conclusion

Injection of STZ resulted in hyperglycemia, disturbed lipid metabolism, oxidative stress, glycation, inflammation, and disturbed liver, kidney, and pancreas functions, in addition to structural alteration of pancreas tissue. Being keen to consume food high in antioxidant contents and phytochemicals, such as polyphenols, carotenoids, and flavonoids seem to be sufficient in alleviating hyperglycemia and associated disorders. Black chia and

garden cress seeds can prevent hyperglycemia and the glycation process. In addition to their antioxidant and anti-inflammatory effects that appeared as a marked reduction of oxidative stress markers (MDA, SOD, and GSH) and inflammatory cytokines (IL-1β and INF-γ), black chia and garden cress seeds have hepatoprotective, renoprotective, and pancreatic protective effects. These protective effects appeared in liver, renal, and pancreatic function parameters (albumin, total bilirubin, urea, creatinine, trypsin, and amylase), as well as on the pancreatic tissue structure of STZ-injection animals.

Authors' contribution

Conception and design: TEK; Acquisition, analysis, and interpretation of data: AAE, RWM, and HSM; Drafting the work and revising: AAE and RWM; Final approval of the manuscript: TEK, AAE, and RWM.

Conflict of interests

No potential conflict of interest relevant to this article was reported.

Ethical considerations

All authors declare that principles of laboratory animal care (National Institute of health guide for care and use of laboratory animal) were followed. All experiments were approved by The Women's Faculty for Arts, Science, and Education at Ain Shams University's Institutional Animal Care Use Committee (IACUC) (Approval No. 1617 in 1/9/2020).

Funding/Support

This research (MSc project) was without any financial assistance, and the sponsor of this project was Hadeer Saad Mohammed.

References

1. da Silva Marineli R, Lenquiste SA, Moraes É A, Maróstica MR Jr. Antioxidant potential of dietary chia seed and oil (*Salvia hispanica* L.) in diet-induced obese rats. Food Res Int. 2015;76(Pt 3):666-74. doi: 10.1016/j.foodres.2015.07.039.
2. Oliveira-Alves SC, Vendramini-Costa DB, Betim Cazarin CB, Maróstica Júnior MR, Borges Ferreira JP, Silva AB, et al. Characterization of phenolic compounds in chia (*Salvia hispanica* L.) seeds, fiber flour and oil. Food Chem. 2017;232:295-305. doi: 10.1016/j.foodchem.2017.04.002.
3. Villanueva-Bermejo D, Calvo MV, Castro-Gómez P, Fornari T, Fontecha J. Production of omega 3-rich oils from underutilized chia seeds. Comparison between supercritical fluid and pressurized liquid extraction methods. Food Res Int. 2019;115:400-7. doi: 10.1016/j.foodres.2018.10.085.
4. Parker J, Schellenberger AN, Roe AL, Oketch-Rabah H, Calderón AI. Therapeutic perspectives on chia seed and its oil: a review. Planta Med. 2018;84(9-10):606-12. doi: 10.1055/a-0586-4711.
5. Jain T, Grover K. A comprehensive review on the nutritional and nutraceutical aspects of garden cress (*Lepidium sativum* Linn.). Proc Natl Acad Sci India Sect B Biol Sci. 2018;88(3):829-36. doi: 10.1007/s40011-016-0775-2.
6. Jain T, Grover K, Kaur G. Effect of processing on nutrients and fatty acid composition of garden cress (*Lepidium sativum*) seeds. Food Chem. 2016;213:806-12. doi: 10.1016/j.foodchem.2016.07.034.
7. Alamri E. The influence of two types of chia seed on some physiological parameters in diabetic rats. Int J Pharm Res Allied Sci. 2019;8(3):131-6.
8. Qusti S, El Rabey HA, Balashram SA. The hypoglycemic and antioxidant activity of cress seed and cinnamon on streptozotocin induced diabetes in male rats. Evid Based Complement Alternat Med. 2016;2016:5614564. doi: 10.1155/2016/5614564.
9. Kweon MH, Hwang HJ, Sung HC. Identification and antioxidant activity of novel chlorogenic acid derivatives from bamboo (*Phyllostachys edulis*). J Agric Food Chem. 2001;49(10):4646-55. doi: 10.1021/jf010514x.
10. Trinder P. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. J Clin Pathol. 1969;22(2):158-61. doi: 10.1136/jcp.22.2.158.
11. Kao PC, Taylor RL, Service FJ. Proinsulin by immunochemiluminometric assay for the diagnosis of insulinoma. J Clin Endocrinol Metab. 1994;78(5):1048-51. doi: 10.1210/jcem.78.5.8175958.
12. John WG, Gray MR, Bates DL, Beacham JL. Enzyme immunoassay--a new technique for estimating hemoglobin A1c. Clin Chem. 1993;39(4):663-6.
13. Monnier VM, Cerami A. Nonenzymatic browning in vivo: possible process for aging of long-lived proteins. Science. 1981;211(4481):491-3. doi: 10.1126/science.6779377.
14. Lath R, Shendye R, Jibhkate A. Insulin resistance and lipid profile in polycystic ovary syndrome. Asian J Biomed Pharm Sci. 2015;5(47):30-5.
15. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem. 1982;28(10):2077-80.
16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502.
17. Boden G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes. 1997;46(1):3-10.
18. Notarnicola M, Altomare DF, Correale M, Ruggieri E, D'Attoma B, Mastrosimini A, et al. Serum lipid profile in colorectal cancer patients with and without synchronous distant metastases. Oncology. 2005;68(4-6):371-4. doi: 10.1159/000086977.
19. Nishikimi M, Appaji N, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun. 1972;46(2):849-54. doi: 10.1016/s0006-291x(72)80218-3.
20. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963;61:882-8.
21. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-8. doi: 10.1016/0003-2697(79)90738-3.
22. Wang B, André I, Gonzalez A, Katz JD, Aguet M, Benoist C, et al. Interferon-gamma impacts at multiple points during

the progression of autoimmune diabetes. Proc Natl Acad Sci U S A. 1997;94(25):13844-9. doi: 10.1073/pnas.94.25.13844.
23. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta. 1971;31(1):87-96. doi: 10.1016/0009-8981(71)90365-2.
24. Kaplan A. Urea. Clin Chem. 1984;1257-60.
25. Rejzek M, Stevenson CE, Southard AM, Stanley D, Denyer K, Smith AM, et al. Chemical genetics and cereal starch metabolism: structural basis of the non-covalent and covalent inhibition of barley β-amylase. Mol Biosyst. 2011;7(3):718-30. doi: 10.1039/c0mb00204f.
26. Levesque R. SPSS Programming and Data Management: A Guide for SPSS and SAS Users. 3rd ed. USA: SPSS; 2007.
27. Alam S, Sarker MMR, Sultana TN, Chowdhury MNR, Rashid MA, Chaity NI, et al. Antidiabetic phytochemicals from medicinal plants: prospective candidates for new drug discovery and development. Front Endocrinol (Lausanne). 2022;13:800714. doi: 10.3389/fendo.2022.800714.
28. Martínez-Cruz O, Paredes-López O. Phytochemical profile and nutraceutical potential of chia seeds (*Salvia hispanica* L.) by ultra high performance liquid chromatography. J Chromatogr A. 2014;1346:43-8. doi: 10.1016/j.chroma.2014.04.007.
29. Abu-Rumman AM. Gas chromatography-mass spectrometry (GC-MS) analysis of extracted oil from whole garden cress (Rashaad) seeds. Am J Eng Res. 2018;7(4):1-8.
30. da Silva BP, Dias DM, de Castro Moreira ME, Toledo RC, da Matta SL, Lucia CM, et al. Chia seed shows good protein quality, hypoglycemic effect and improves the lipid profile and liver and intestinal morphology of Wistar rats. Plant Foods Hum Nutr. 2016;71(3):225-30. doi: 10.1007/s11130-016-0543-8.
31. Creus A, Ferreira MR, Oliva ME, Lombardo YB. Mechanisms involved in the improvement of lipotoxicity and impaired lipid metabolism by dietary α-linolenic acid rich *Salvia hispanica* L (Salba) seed in the heart of dyslipemic insulin-resistant rats. J Clin Med. 2016;5(2):18. doi: 10.3390/jcm5020018.
32. Xu SN, Zhou X, Zhu CJ, Qin W, Zhu J, Zhang KL, et al. Nϵ-Carboxymethyl-lysine deteriorates vascular calcification in diabetic atherosclerosis induced by vascular smooth muscle cell-derived foam cells. Front Pharmacol. 2020;11:626. doi: 10.3389/fphar.2020.00626.
33. Hamidpour R, Hamidpour M, Hamidpour S, Shahlari M. Cinnamon from the selection of traditional applications to its novel effects on the inhibition of angiogenesis in cancer cells and prevention of Alzheimer's disease, and a series of functions such as antioxidant, anticholesterol, antidiabetes, antibacterial, antifungal, nematicidal, acaracidal, and repellent activities. J Tradit Complement Med. 2015;5(2):66-70. doi: 10.1016/j.jtcme.2014.11.008.
34. Abdelwahab SI, Mariod AA, Taha MM, Zaman FQ, Abdelmageed AH, Khamis S, et al. Chemical composition and antioxidant properties of the essential oil of *Cinnamomum altissimum* Kosterm. (Lauraceae). Arab J Chem. 2017;10(1):131-5. doi: 10.1016/j.arabjc.2014.02.001.
35. Younus H, Anwar S. Prevention of non-enzymatic glycosylation (glycation): implication in the treatment of diabetic complication. Int J Health Sci (Qassim). 2016;10(2):261-77.
36. Prajapati VD, Maheriya PM, Jani GK, Patil PD, Patel BN. *Lepidium sativum* Linn.: a current addition to the family of mucilage and its applications. Int J Biol Macromol. 2014;65:72-80. doi: 10.1016/j.ijbiomac.2014.01.008.
37. Sargi SC, Silva BC, Santos HM, Montanher PF, Boeing JS, Santos Júnior OO, et al. Antioxidant capacity and chemical composition in seeds rich in omega-3: chia, flax, and perilla. Food Sci Technol. 2013;33(3):541-8. doi: 10.1590/s0101-20612013005000057.
38. Medina-Urrutia A, Lopez-Uribe AR, El Hafidi M, González-Salazar MDC, Posadas-Sánchez R, Jorge-Galarza E, et al. Chia (*Salvia hispanica*)-supplemented diet ameliorates non-alcoholic fatty liver disease and its metabolic abnormalities in humans. Lipids Health Dis. 2020;19(1):96. doi: 10.1186/s12944-020-01283-x.
39. Behrouzian F, Razavi SM, Phillips GO. Cress seed (*Lepidium sativum*) mucilage, an overview. Bioact Carbohydr Diet Fibre. 2014;3(1):17-28. doi: 10.1016/j.bcdf.2014.01.001.
40. Doke S, Guha M. Garden cress (*Lepidium sativum* L.) seed-an important medicinal source: a Review. J Nat Prod Plant Resour. 2014;4(1):69-80.
41. Saddala RR, Thopireddy L, Ganapathi N, Kesireddy SR. Regulation of cardiac oxidative stress and lipid peroxidation in streptozotocin-induced diabetic rats treated with aqueous extract of *Pimpinella tirupatiensis* tuberous root. Exp Toxicol Pathol. 2013;65(1-2):15-9. doi: 10.1016/j.etp.2011.05.003.
42. Ali LH, Rajab WJ. The effect of *Lepidium sativum* seeds extract on some oxidative stress, antioxidants and histological changes in rat treated with CCl4. Pak J Biotechnol. 2019;16:145-51. doi: 10.34016/pjbt.2019.16.1.22.
43. da Silva BP, Anunciação PC, Matyelka J, Della Lucia CM, Martino HSD, Pinheiro-Sant'Ana HM. Chemical composition of Brazilian chia seeds grown in different places. Food Chem. 2017;221:1709-16. doi: 10.1016/j.foodchem.2016.10.115.
44. El Bialy B, Thabet Saleh N, Abou-Elkhair SR. Potential hazards of feeding albino rats on diet containing repeatedly boiled cooking oil: clinicopathological and toxicological studies. Int J Adv Res. 2015;3(3):134-47.
45. da Silva Marineli R, Moura CS, Moraes É A, Lenquiste SA, Lollo PC, Morato PN, et al. Chia (*Salvia hispanica* L.) enhances HSP, PGC-1α expressions and improves glucose tolerance in diet-induced obese rats. Nutrition. 2015;31(5):740-8. doi: 10.1016/j.nut.2014.11.009.
46. Habib HG, Al-Hilali AH, Al-Gharawi JK. Effect of addition of iron and copper elements on some blood and immune traits of broilers. Biochem Cell Arch. 2020;20(1):505-8.
47. El-Hashash SA, Elmoslemany AM, Abd El-Mageed AA. Effect of some medicinal plant seeds on CCl4-induced hepatotoxicity in experimental rats. Egypt J Nutr Health. 2020;15(1):101-20. doi: 10.21608/ejnh.2020.117337.
48. Al-Malki AL, El Rabey HA. The antidiabetic effect of low doses of *Moringa oleifera* Lam. seeds on streptozotocin induced diabetes and diabetic nephropathy in male rats. Biomed Res Int. 2015;2015:381040. doi: 10.1155/2015/381040.
49. Xu X, Zheng N, Chen Z, Huang W, Liang T, Kuang H. Puerarin, isolated from *Pueraria lobata* (Willd.), protects against diabetic nephropathy by attenuating oxidative stress.

Gene. 2016;591(2):411-6. doi: 10.1016/j.gene.2016.06.032.
50. Ismail OZ, Bhayana V. Lipase or amylase for the diagnosis of acute pancreatitis? Clin Biochem. 2017;50(18):1275-80. doi: 10.1016/j.clinbiochem.2017.07.003.
51. Richardson A, Park WG. Acute pancreatitis and diabetes mellitus: a review. Korean J Intern Med. 2021;36(1):15-24. doi: 10.3904/kjim.2020.505.
52. Sharma H, Vyas RK, Vyas S. Role of serum trypsin level in diagnosis and prognosis of pancreatitis and compared with healthy subjects of Rajasthan. Am J Biochem. 2018;8(5):93-9. doi: 10.5923/j.ajb.20180805.02.
53. Desai SS, Walvekar MV, Shaikh NH. Cytoprotective effects of *Lepidium sativum* seed extract on liver and pancreas of HFD/STZ induced type 2 diabetic mice. Int J Pharmacogn Phytochem Res. 2017;9(4):502-7. doi: 10.25258/phyto.v9i2.8121.