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Effect of Raw, Germinated, and Fermented Chia Seeds on Blood Sugar and Lipid Profiles in Experimental Rats

Kamel, E. B. **; Frahat F.A.Foda*, Abd El-Aleem, I.M.* and Ali, M.A. M. **

*** Agric. Biochem. Dept., Fac. Agric., Benha Univ., Egypt.**

**** Food Technol. Res. Inst., Agric. Res. Center, Giza, Egypt.**

Corresponding author: farhat.fouda@fagr.bu.edu.eg

Abstract

Due to the high antioxidant and bioactive compounds, chia seeds have been used for controlling metabolic disorders, especially for diabetes mellitus and hyperlipidemia. To study the effect of chia seeds on blood glucose and lipids, Streptozotocin-induced diabetic rats were fed traditional kishk supplemented with 10% raw, germinated, and fermented chia seeds. Thirty adult male rats were divided into five groups; the normal control group that fed on a basal diet(G1), the diabetic that fed on a basal diet (G2), diabetic rats fed on kishk supplemented with 10% raw chia seeds (G3, KRC), 10% germinated chia seeds (G4, KGC), and 10% fermented chia seeds (G5, KFC). Body weight loss was observed in G2, G3, and G4, while weight gain was observed in G1 and G5. Feeding with 10% chia seeds (raw, germinated, and fermented) showed reduced in blood glucose serum, triglyceride, and total cholesterol levels. Fermented chia had a higher effect in lowing blood glucose and serum lipids profiles compared with raw and germinated chia seeds. Histopathological alterations were observed in the liver and kidney tissues of diabetic rats fed kishk supplemented with raw, germinated and fermented chia seeds. The Pancreas of rats from G5 showed vacuolations of sporadic cells of islets of Langerhans.

Keywords: Salvia hispanica, germination, fermentation Kishk, and streptozotonized rats.

Introduction

Numerous people are struggling with the health issue of diabetes mellitus According to the World Health Organization and the International Diabetes Federation **(Atlas, 2015 and WHO, 2016)**. According to **Ismaiel and Dumitraşcu (2019)**, diabetes type 2 is thought to be a major contributing factor to other conditions such as atherosclerosis, cardiovascular disease, high blood pressure, and fatty liver disease. About 40 million people in North Africa and the Middle East region have diabetes. Egypt is among the world's top 10 countries with diabetes prevalence **(Powers *et al*.,2015)**. Long-term hyperglycemia results from diabetics' inability to keep their blood glucose levels within normal ranges **(Kilpatrick *et al*., 2007; Arumsari *et al*., 2020)**.

Chia seeds (*Salvia hispanica L.*) are one of the herbaceous plants with promise for health and can be utilized as an alternative in the production of functional food products. Chia seeds and the products that are derived from them are potential sources of functional foods **(Safari *et al*., 2016)**. Due to their ability to lower blood sugar levels, chia seeds can lower the risk of developing the disease.

According to **Ayerza and Coates (2005)** reported that the rats given chia seed had serum levels of total cholesterol that were lower than those of rats given either the control diet or chia oil. Compared to animals fed the control food, animals fed chia diets had greater serum levels of HDL. Also, rats' blood glucose levels were observed to be lower after consuming chia seeds for six and twelve weeks **(Oliva *et al*., 2013)**. After three months of consuming black chia seeds, insulin-resistant rats were found to have significantly lower blood glucose levels, according to **Creus *et al*. (2016)**. **Da Silva *et al*. (2016)** Compared to casein-fed rats, those given various chia diets displayed reduced blood glucose levels.

The germination process decomposes large elements into simpler compounds such as carbohydrates, proteins, and fatty acids. It also reduces anti-nutrients and indigestible factors (**Aguilera *et al*., 2013)**.
Sattar *et al*. (2017) studied the antioxidant activity of germinated seeds and their potential benefits on health. Additionally, the seeds bioactive phenolic compounds increased during the germination period **(Abdel-Aty *et al*., 2019)**.

According to **Xu *et al*. (2020)**, fermented foods containing lactic acid bacteria (LAB) enhance weight gain while reducing serum glucose and total cholesterol levels. LAB liberates phenolic compounds during the hydrolysis of cell components, increasing the antioxidant activity of fermented products **(Adebo and Medina-Meza,2020)**. Kishk was a traditional fermented food from Upper Egypt that has a high nutritional value. The primary components of kishk are fermented milk and parboiled wheat, which make it a readily available and abundant source of fiber, vitamins, and minerals **(Abd El-Ghani *et al*., 2014)**. Kishk was rich in necessary fatty acids (ω-3 and ω-6), and essential amino acids (such as lysine, tyrosine, and proline), **(Bahgaat and Ghani 2017)**.

This work aims to evaluate the effectiveness of chia seeds (raw, germinated, and fermented) added to kishk to help type 2 diabetics maintain. Also, the effects of chia seeds on lipids profiles, liver and kidney functions and histopathological examination were evaluated.

Materials and methods

1. Materials

Chia seeds were obtained from the Agricultural Seeds, Spices and Medicinal Plants Company (Haraz), Cairo, Egypt. Streptozotocin was purchased from Sigma/Aldrich Chemical Company, USA. Casein, cellulose, and choline chloride were obtained from Modern Lab Giza, Egypt. The components of the vitamin and salt mixture were purchased from Techno-Gene, Chemical Co., El Doki, Egypt. Kits of glucose (Glu) were obtained from Bicon Diagnosemittel GmbH and Co.KG Hecke 8 made in Germany. Kits (AST), (ALT), Total Protein (TP), (HDL-cholesterol), (LDL–cholesterol), urea and creatinine were obtained from Bio diagnosis Co. (T.C) and (T.G) were obtained from El– Gomhoryia Co.

Chia seeds were processed and ground into whole meal flour using a laboratory mill (IKA-Laboratechnic, Janke and Kunkel Type: MFC, Germany) and stored at -20°C until analysis. Germinated seeds were processed by germination conditions laid out based as indicated by **(Beltrán-Orozco *et al*.,2020)**. To process fermented seeds, fermentation conditions were established based on the information provided **(Tamime *et al*., 1997)**.

Preparation of Kishk wheat grains was cleaned with water before being air dried to remove any remaining bran or dust. then wheat grains were combined with fermented milk in a 1:4 ratio, The mixture was salted (0.85 g/100 g) and the resulting dough was kept at 20°C for 48 h. The dough was then placed into stainless steel trays and dried in a bakery oven at 50°C for 8 to 9 h. With the laboratory mill, the dried dough was ground into a powder (IKA-Laboratechnic, Janke, and Kunkel Type: MFC, Germany). The dried materials were kept in airtight containers and refrigerated while being kept in the dark until testing **(Tamime *et al*., 1997).**

The basic diet is prepared according to **(AIN, 1993)**. While, vitamins and salts mixture components were formulated according to **(Campbell, 1963 and Hegsted, 1941)**, respectively.

2. Experimental animals:

A total of thirty (30) animals of adult male albino rats weighing 140 to 183g were used in the present experiment. Animals were fed in the animal house, Food Technology Research Institute (FTRI), Giza, Egypt, under normal healthy conditions for one week and fed on a normal diet (basal diet). The animals were allowed free access to tap water and were fed on a uniformly basal diet.

3. Experiment Design:

For adaptation, the animals were fed for a week in the experimental animal cages. After the adaptation period, the rats were divided into five groups of six rats each. The experimental diets were fed to the animals for a total of five weeks.

(G1): Normal rats fed on a basal diet.

(G2): Diabetic rats fed on a basal diet

(G3): Diabetic rats fed on Kishk with 10% raw chia seeds (KRC).

(G4): Diabetic rats fed on Kishk with 10% germinated chia seeds (KGC).

(G5): Diabetic rats fed on Kishk with 10% fermented chia seeds (KFC).

Rats that had overnight fasted were given an intraperitoneal injection of 50 mg/kg STZ dissolved in (0.1M) citrate buffer. Blood samples from each rat were collected to measure serum glucose after two days of injections to guarantee the development of diabetes. Diabetic rats were those with blood glucose levels of 220-250 mg/dl. The total body weight of the animals was recorded at the beginning and during the experimental period. To determine body weight gain, the rats were weighed every week. Additionally, blood samples were taken from the orbital plexus using heparinized capillary glass tubes. Each sample was put into a centrifuge tube that had been dried off. After centrifuging blood samples for 10 min at 300 rpm, the supernatant was transferred to Eppendorf tubes and stored until analysis **(Schemer, 1967)**.

4. Biological analyses

The method described by **Passing and Bablok (1983)** was used to measure serum glucose. Triglycerides were determined in serum using the method of **Fossati and Prencipe (1982)**. High-density lipoprotein cholesterol was determined using the method of **Lopez-Virella *et al*. (1977)**. LDL – cholesterol was calculated for serum samples using the formula of Friedewald et al. (1972) LDL-C= T.C-(T.G/5+HDL-C). The total protein content was determined using the method of **Gornall *et al*. (1949)**. Determination of Alkaline phosphatase

(ALP) activity according to **Belfield *et al*. (1971)**. Serum aspartate transaminases (AST) and serum alanine transaminases (ALT) activities were measured colorimetrically according to the method described by **Reitman and Frankel (1957)**. Urea in serum was determined according to the method described by **Shephard and Mezzachi, (1983)**. Creatinine in serum was determined according to the method described by **Tietz (1986)**.

5. Histopathological examination

For histological examinations, tissue samples from the liver, kidney, and pancreas were obtained, cleaned in sterile saline, and fixed in 10% neutral formalin. The target organs were subsequently cleaned in xylene, gradually dehydrated in ethanol (50–99%), and finally embedded in paraffin. According to **Drury and Wallington (1986)**, sections were made and then stained with hematoxylin and eosin (H&S) dye for microscopic examination.

6. Statistical analysis

Each experiment's three collected data points were statistically examined in triplicate. Data were presented as mean values ±SD. Data were analyzed to one-way analysis of variance (ANOVA) and least significant difference (LSD) at $p<0.05$ followed by Duncan's new multiple-range tests to assess differences between the group's means **(Waller and Duncan, 1969)**.

Results and Discussion

To investigate the biological effects of kishk supplement with raw, germinated, and fermented chia seeds on serum lipid profiles, liver, and kidney functions, as well as blood sugar levels in diabetic rats.

Control groups were two groups (one normal G1 and one diabetic rat G2). The diabetic rat groups were fed on kishk with 10% raw chia seeds (G3), kishk with 10% germinated chia seeds (G4), and kishk with 10% fermented chia seeds (G5).

1. Body weight gain of the experimental rats.

The total body weight gain after 5 weeks is shown in Table (1). The G1 (normal control) fed on a basal diet exhibited the highest total body weight gain (30 g/5 week), according to the data. While G2 (the diabetic control) fed a basal diet saw a considerable loss in body weight of about 20.67 g/5 weeks due to STZ toxicity. The body weight of the other diabetes groups, G3 and G4, which were fed kishk supplemented with raw chia seeds and germinated chia seeds, decreased as well, but at a lower than that of G2 and there were minor differences between them. Concerning G5 (diabetic group) which fed on kishk with supplemented fermented chia seeds showed a significantly increased in body weight gain of 5.0 g/5 week.

According to **Mihafu *et al*. (2020)** the experimental and control groups' changes in body weight during the initial evaluation were compared. For the control group, there was a significant increase in body weight changes between the first and fourth weeks. The rats who consumed chia seeds have seen a gradual increase in body weight. **Khafagy and Hendawy (2022)** observed that the mean value of body weight gain of the normal control group was higher than the diabetic control group, being 1.14g and 0.71g respectively. In contrast, the group which fed on chia seeds (5%) was increased to 0.85g when compared to a control group of diabetics.

Table 1. Initial body weight, body weight gain of experimental rats

Groups	Initial body weight (g)	Final body weight (g)	body weight gain (g)
G1Normal control	201.67 ± 6.66^{ab}	231.67 ± 6.66^{a}	$+30 \pm 0^{a}$
G2Diabetic rats	211.33 ± 3.51^{a}	190.67 ± 3.51^{bc}	-20.67 ± 0.58^{d}
G3 KRC	176.67 ± 17.62^{c}	166.33 ± 18.01^{d}	-10.33 ± 2.52^{c}
G4 KGC	188.33 ± 11.02^{bc}	176.67 ± 12.06^{cd}	-11.67 ± 1.16^{c}
G 5 KFC	195.00 ± 14.18^{abc}	200.33 ± 14.84^{b}	$+5.33\pm 1.53^{b}$
LSD at 5%	21.36	22.24	2.62

G1: Normal control fed on a basal diet; G2: diabetic control fed on a basal diet; G3 (KRC): rats fed on kishk with 10% raw chia seeds; G4 (KGC): rats fed on kishk with 10% germinated chia seed; G5(KFC): rats fed on kishk with 10% fermented chia seeds, ± S.D: standard deviation.

2. Effect of feeding with diets containing kishk supplemented with raw, germinated and fermented chia seeds on the organ's weight of the experimental rats:

The weight of organs and the ratio of organs/body weight of normal and diabetic rats fed on different diets were measured and the results are shown in Table (2). From the obtained results, it can be seen that the liver and pancreas weight ratio was increased in G2 (diabetic control) (2.86 and 0.22%) for the liver and pancreas, respectively. The obtained data revealed that liver, kidney, heart, and pancreas weight increased with the decrease in body weight in diabetic rats compared with normal control. However, rats fed on different diets and normal control had very few changes in kidney and heart weight/body weight ratio. From the obtained data we found that there was a significant increase in liver weight/body weight ratio in all diabetic rats when compared with normal control.

Table 2. The weight of the liver, kidney, heart, and pancreas and their ratio to the body weight of the experimental rats

Groups	Final body weight(g)	Liver weight		Kidney weight		Heart weight		Pancreas weight	
		(g)	Ratio%	(g)	Ratio%	(g)	Ratio%	(g)	Ratio%
G1Normal control	231.67	5.87±0.47[ab]	2.53	2.40±0.14[a]	1.04	0.96±0.10[a]	0.41	0.34±0.04[a]	0.15
G2Diabetic rats	190.67	5.46±0.11[b]	2.86	1.92±0.17[b]	1.01	0.80±0.10[b]	0.42	0.41±0.02[a]	0.22
G3 KRC	166.33	6.04±0.81[ab]	3.63	1.60±0.37[b]	0.96	0.78±0.11[b]	0.47	0.34±0.08[a]	0.20
G4 KGC	176.67	6.38±0.40[ab]	3.61	1.87±0.12[b]	1.06	0.85±0.03[b]	0.48	0.44±0.04[a]	0.25
G 5 KFC	200.33	6.67±0.30[a]	3.33	2.36±0.08[a]	1.18	0.91±0.05[b]	0.45	0.44±0.11[a]	0.22
LSD at 5%	22.24	0.87		0.37		0.15		0.12	

G1: Normal control fed on a basal diet; G2: diabetic control fed on a basal diet; G3 (KRC): rats fed on kishk with 10% raw chia seeds; G4 (KGC): rats fed on kishk with 10% germinated chia seeds; G5(KFC): rats fed on kishk with 10% fermented chia seeds, ± S.D: standard deviation.

3. Effect of feeding with diets containing kishk with raw, germinated and fermented chia seeds on serum blood glucose of the experimental rats.

The effect of kishk with raw, germinated and fermented chia seeds on the blood glucose of the experimental rats were evaluated and the results are summarized in Table (3).

The fermented group (G5) was more effective in reducing blood glucose levels than G3 and G4 in STZ-induced diabetic rats. Furthermore, the ability of G5 to lower blood glucose levels was more notable. As shown in Table (3) the degrees to which blood glucose levels were reduced in groups G5, G3, and G4 were 54, 35, and 32%, respectively. These results further illustrated that chia seeds (raw, germinated and fermented) produced hypoglycemic effects in STZ-induced diabetic rats.

According to **Weickert and Pfeiffer (2008)**, soluble dietary fiber increases the viscosity of the intestinal lumen, which reduces glucose's interaction with the enterocyte and decreases absorption. Rats' blood glucose levels were shown to be lower after consuming white chia seeds for six and twelve weeks, according to **Oliva *et al*. (2013)**. The same effects were reported by **(Da Silva Marineli *et al*., 2015)**. **Adams et al. (2018)** reported that the soluble fiber in chia seeds may have sped up the process of food digestion and increased the number of nutrients that intestinal cells were able to absorb. Additionally, fermentation may change the soluble fiber's physical and chemical properties as well as how well nutrients are absorbed. According to **Creus *et al*. (2016)**, insulin-resistant rats fed black chia seeds for three months had a significant decrease in blood glucose levels. Chia seeds have been found to affect diabetic rat serum glucose, according to **Khafagy and Hendawy (2022)**. It was clear that the normal control group's mean glucose value was (95 mg/dl), whereas the diabetes control group's mean glucose value was (380 mg/dl). When compared to a diabetic control group, rats fed on a basal diet containing 5% chia seeds displayed a mean value of (110 mg/dl) that was lower. Chia seeds and chia sprouts considerably reduced the hyperglycemia of induced obese rats, by **Gómez-Velázquez *et al*. (2022)**.

Table 3. The serum glucose level in normal and diabetic rats during experimental rats

Groups	Zero time	After 1week	After 2weeks	After 3 weeks	After 4 weeks	After 5 weeks	Serum glucose reduction %
G1Normal control	77.67±1.53[c]	75.67±2.52[d]	75.33±3.79[d]	75.33±3.06[d]	73.67±1.53 [d]	72±1.73[d]	-
G2Diabetic rats	292±2[a]	287.33±3.51[a]	265.67±2.89[a]	258±4.58 [a]	252.67±11.72[a]	245.33±14.57[a]	-
G3 KRC	281±9.64[b]	253.33±7.02[c]	246.33±8.08[b]	226.67±6.81[b]	189±7[b]	159.33±10.69[b]	35
G4 KGC	284.67±6.66[ab]	271.33±0.58[b]	258.67±6.43[a]	227±7.94[b]	179.33±8.51[bc]	168±4[b]	32
G 5 KFC	278.67±4.04[b]	255.67±9.07[c]	218.33±3.06[c]	195.67±4.16[c]	167.33±6.11[c]	113.67±6.43[c]	54
LSD at 5%	10.29	9.99	9.58	10.19	14.05	16.005	

G1: Normal control fed on a basal diet; G2: diabetic control fed on a basal diet; G3 (KRC): rats fed on kishk with 10% raw chia seeds; G4 (KGC): rats fed on kishk with 10% germinated chia seeds; G5(KFC): rats fed on kishk with 10% fermented chia seeds, ± S.D: standard deviation.

4. Effect of feeding with diets containing kishk with raw, germinated and fermented chia seeds on serum lipid fractions of the experimental rats:

Table (4) represent the levels of total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol in normal and diabetic rats fed on kishk with raw, germinated and fermented chia seeds.

From the obtained data, it could be observed that total cholesterol content was significantly increased in G2 (diabetic control) to 285% compared to G1 (normal control). However, the treatments G3 which fed on kishk with raw chia seeds (KRC), G4 kishk with germinated chia seeds (KGC), and G5 kishk with fermented chia seeds (KFC) significantly lowered serum total cholesterol of the diabetic rats to 161,194 and 120 % related to the normal control G1.

The data in the same Table (4) revealed that serum triglycerides were significantly increased in G2 (diabetic control) to 337 % compared to G1 (normal control). Treatments of G3 and G4 which were fed on kishk with raw chia seeds (KRC) and kishk with germinated chia seeds (KGC) significantly lowered to 212 and 232 % for triglycerides, respectively. Concerning G5 which fed on kishk with fermented chia seeds (KFC) significantly lowered triglycerides to 124 %. It is clear that the levels of triglycerides of all treated rats were improved relative to the diabetic control G2 but these improved values of diabetic rats were still higher than normal control G1.

According to **Ayerza and Coates (2005)** the chia diet resulted in a considerable reduction in serum triglycerides, a three-fold decrease in total cholesterol, and a significant increase in serum HDL cholesterol concentration when compared to the control. After 30 days of intake, both whole and ground chia seeds had a positive effect on the lipid profiles of the rats **(Ayerza and Coates 2007)**. However, **Oliva *et al*. (2013)** found that rats fed chia seeds for three weeks had significantly reduced levels of TG than the control groups. **Alamri (2019)** found that in treated groups of diabetic rats compared to control groups, chia seeds (both white and black) decreased total cholesterol and triglycerides. Also, **Khafagy and Hendawy (2022)** found that the mean values of total cholesterol for diabetes and normal control groups were 256 and 105 mg/dl, respectively. Rats given a base meal containing chia seeds (5%) had 195mg/dl less than the control group. The difference between the mean triglyceride values of the diabetes control group and the normal control group were 248 and 123 mg/dl, respectively, could be observed when compared to the diabetic control group, rats fed a basal meal containing chia seeds (5%) had a triglyceride level of 122 mg/dl.

Regarding HDL, it was observed that serum HDL of G2 (diabetic control) significantly decreased to 51% compared to G1 (normal control). The treatments of G3 and G4 rats fed on kishk with raw chia seeds (KRC) and kishk with germinated chia seeds (KGC) significantly increased HDL of the diabetic animals and the values were 99 and 92% relatives to normal control G1 but not significantly changes were found between them. The present data showed that HDL in G5 (diabetic rats) fed on kishk with fermented chia seeds (KFC) exhibited a higher improvement for diabetic rats by 122 %.

Concerning LDL, it could be noticed that serum LDL was highly significantly increased in G2 (diabetic control) to 659% compared to normal control. Treatments of G3 and G4 which diabetic rats fed on kishk with raw chia seeds (KRC) and kishk with germinated chia seeds (KGC) significantly decreased serum LDL levels to 246 and 349 % relative to normal control G1. From the present data, we found that the treatment G5 which diabetic rats fed on kishk with fermented chia seeds (KFC) slightly significantly decreased LDL to 115 % compared to normal control G1. It is clear that the LDL of all treated diabetic rats was improved relative to the diabetic control but these treated rats are still more than normal control animals.

According to **Ayerza and Coates (2005)** rats fed on chia seed had serum levels of total cholesterol that were less than those of rats fed on either the control diet or chia oil. In comparison to animals fed the control food, animals fed chia diets had higher serum levels of HDL. Rats fed chia showed considerably lower TC/HDL ratios than the control group. Also, **Da Silva et al. (2016)** found the groups fed chia seed or flour had lower triglyceride and LDL levels and higher HDL levels. Short-term consumption of chia in the form of seeds or flour demonstrated better lipid profiles, good protein digestion, and a hypoglycemic effect. Compared to the control group, the chia seed-fed group had lower total cholesterol. Therefore, consuming chia for a short period was able to improve lipid homeostasis in rats.

Khafagy and Hendawy (2022) observed that the mean values of (LDL) in the diabetes control group was higher than the normal control group were 168.4 and 19.4 mg/dl, respectively. Rats fed on a basal diet with chia seeds (5%) was 114.6 mg/dl when compared to a diabetic control group. It could be shown that the mean value of (HDL) of the normal control group was higher than the diabetic control groups were 61 and 38 mg/dl, respectively. The group fed on a basal diet with chia seeds (5%) was 56 mg/dl when compared to a diabetic control group.

Table 4. Serum total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol levels of experimental rats.

Groups	TC (mg/dl)	% at control	TG (mg/dl)	% at control	HDL (mg/dl)	% at control	LDL (mg/dl)	% at control
G1Normal control	89.07±3.96^{e}	100	60.33±2.44^{e}	100	48.37±4.36^{b}	100	28.63±1^{d}	100
G2Diabetic rats	254.22±9.31^{a}	285	203.11±10.55^{a}	337	24.88±6.87^{c}	51	188.78±13.41^{a}	659
G3 KRC	143.7±3.34^{c}	161	127.7±3.62^{c}	212	47.87±2.3^{b}	99	70.29±5.2^{c}	246
G4 KGC	172.43±1.46^{b}	194	139.77±3.6^{b}	232	44.6±2.4^{b}	92	99.88±2.28^{b}	349
G 5 KFC	107.07±2.08^{d}	120	74.93±2.35^{d}	124	59.13±0.67^{a}	122	32.95±2.27^{d}	115
LSD at 5%	8.91		9.92		7.17		12.02	

G1: Normal control fed on a basal diet; G2: diabetic control fed on a basal diet; G3 (KRC): rats fed on kishk with 10% raw chia seeds; G4 (KGC): rats fed on kishk with 10% germinated chia seeds; G5(KFC): rats fed on kishk with 10% fermented chia seeds, ± S.D: standard deviation.

5. Effect of feeding with diets containing kishk with raw, germinated and fermented chia seeds on kidney function of the experimental rats.

The kidney function such as urea and creatinine values are presented in Table (5). The data showed that urea was significantly increased in G2 (diabetic control) by 188 % compared with G1 (normal control). However, treatments of G3, G4, and G5 which fed on kishk with raw chia seeds (KRC), kishk with germinated chia seeds (KGC), and kishk with fermented chia seeds (KFC) significantly decreased serum urea of diabetic rats to 144,148 and 151%, respectively. The results in the same Table (5) revealed that creatinine content was significantly increased in G2 (diabetic control) to 297 % compared to normal control but all diabetic-treated rats had low creatinine content with significant differences between them, while the percent (%) values were 145,138 and 131%, respectively.

According to **Khafagy and Hendawy (2022)** Found that the mean values of creatinine in the diabetes control group and the normal control group, respectively were 1.54 and 0.70 mg/dl. When compared to a diabetic control group, treated rats fed a basal diet containing chia seeds (5%) had creatinine levels that were 0.56 mg/dl lower. It was clear that the mean urea values for the diabetic and normal control groups, 82 and 17 mg/dl, respectively, were higher. Compared to a diabetic control group, rats fed a basal diet containing chia seeds (5%) had 36 mg/dl when compared to a diabetic control group.

Table 5. Serum urea and creatinine values of the experimental rats

Groups	Urea (mg/dl)	% at control	Creatinine (mg/dl)	% at control
G1Normal control	24.8±0.4^{c}	100	0.64±0.02^{c}	100
G2Diabetic rats	46.63±0.65^{a}	188	1.90±0.06^{a}	297
G3 KRC	35.73±0.9^{b}	144	0.97±0.03^{a}	145
G4 KGC	36.67±1.15^{b}	148	0.88±0.56^{b}	138
G 5 KFC	37.33±3.36^{b}	151	0.84±0.05^{b}	131
LSD at 5%	3.04		0.08	

G1: Normal control fed on a basal diet; G2: diabetic control fed on a basal diet; G3 (KRC): rats fed on kishk with 10% raw chia seeds; G4 (KGC): rats fed on kishk with 10% germinated chia seeds; G5(KFC): rats fed on kishk with 10% fermented chia seeds, ± S.D: standard deviation.

6. Effect of feeding with diets containing kishk with raw, germinated and fermented chia seeds on liver function of the experimental rats.

The results of liver functions (ALT, AST and ALP activities) in serum are presented in Table (6). The data showed that ALT and AST activities were significantly stimulated in G2 (diabetic control) by 305 % and 252 % relative to G1 (normal control).

However, all treated groups had improved ALT and AST activities relative to G2 (diabetic control) but these activities were still higher than that of normal control. From the present data, we found that ALT and AST activities were significantly inhibited in diabetic rats by the treatments of G4 and G5 which were (143 and 141%) for ALT and (118 and 173%) for AST compared with G2 (diabetic control). Also, the data show that treatment G3 slightly decreased ALT and AST activities which were 224 % and 176 % respectively relative to normal control.

The data in the same Table (6) revealed that serum alkaline phosphatase (ALP) was significantly increased in G2 (diabetic control) to 263 % compared to G1 (normal control). While the treatment of G3 which fed on kishk with raw chia seeds (KRC) significantly inhibited ALP activity to a value of 231 %. Concerning treatments of G4 which fed on kishk with germinated chia seeds (KGC) and G5 which fed on kishk with fermented chia seeds (KFC) significantly lowered to 177 and 128 % for ALP, respectively.

According to **Mohd Ali *et al.* (2012), Sargi *et al.* (2013), and Valdivia-López and Tecante (2015)** reported that when feeding chia seeds has been associated with improvements in lipid profiles and liver function. This is because chia seeds contain a lot of ω-3 fatty acids. **da Silva Marineli *et al.* (2015) and Da Silva *et al.* (2016)** reported that chia seeds were effective in reducing markers of liver damage by reducing the levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). According to **Khafagy and Hendawy (2022**), the mean AST values for diabetes and normal control groups were 162 and 128 (U/L), respectively. Rats fed a basal diet containing chia seeds (5%) were 111 (U/L) as compared to a control group of diabetes individuals. It was noticeable that the mean ALT values for the diabetes control group were 128 and 38 (U/L), respectively, when higher than those for the normal control group.

Table 6. Serum Alanine transferase (ALT) and aspartate transferase (AST) activities of the experimental rats.

Groups	GPT/ALT (U/L)	% at control	GOT/AST (U/L)	% at control	ALP (U/L)	% at control
G1Normal control	$14{\pm}1^{d}$	100	$29.33{\pm}2.08^{c}$	100	$67.67{\pm}5.03^{e}$	100
G2Diabetic rats	$42.67{\pm}2.52^{a}$	305	$74{\pm}4^{a}$	252	$178{\pm}4.58^{a}$	263
G3 KRC	$31.33{\pm}1.53^{b}$	224	$51.67{\pm}3.22^{b}$	176	$156{\pm}6.08^{b}$	231
G4 KGC	$20{\pm}2.65^{c}$	143	$34.67{\pm}3.06^{c}$	118	$119.67{\pm}2.52^{c}$	177
G 5 KFC	$19.67{\pm}3.79^{c}$	141	$50.67{\pm}5.51^{b}$	173	$86.67{\pm}2.52^{d}$	128
LSD at 5%	4.53		6.82		7.97	

G1: Normal control fed on a basal diet; G2: diabetic control fed on a basal diet; G3 (KRC): rats fed on kishk with 10% raw chia seeds; G4 (KGC): rats fed on kishk with 10% germinated chia seeds; G5(KFC): rats fed on kishk with 10% fermented chia seeds, ± S.D: standard deviation.

7. Effect of feeding with diets containing kishk with raw, germinated, and fermented chia seeds on total protein and albumin of the experimental rats.

The contents of total protein (TP) and albumin for G2 (diabetic control) and different treatments were evaluated and the obtained data are presented in Table (7).

The data showed that they are a significant decrease in total protein in diabetic control and all diabetic treatments feeding on Kishk with raw, germinated and fermented chia seeds. However, Table (7) data showed a significant decrease in serum total protein in rats from 7.44 g/dl in normal control to 5.83 g/dl in diabetic control. In addition, there was no significant difference in total protein between diabetic control and diabetic groups feeding on Kishk with raw, germinated and fermented chia seeds.

Concerning albumin, it could be noticed that serum albumin was highly significantly decreased in G2 (diabetic control) to 92% compared to normal control. Treatment of G3 which diabetic rats fed on kishk with raw chia seeds (KRC) slightly decreased serum albumin levels to 91 % relative to normal control G1. From the present data, we found that the treatments G4 and G5 which diabetic rats fed on kishk with germinated chia seeds (KGC) and kishk with fermented chia seeds (KFC) same high significantly decreased serum albumin to 90 % compared to normal control G1. It is clear that albumin in all diabetic rats was significantly decreased compared to the normal group.

Saleem *et al.* (2020) found that the levels of total protein and albumin in the disease control group were substantially higher (6.14 g/dL and 3.74 g/dL, respectively) than the normal control values (5.44 g/dL and 3.06g/dL, respectively). But in standard and experimental groups of the chia seed extract, serum total protein, and albumin were parallel to normal control values.

Table 7. Serum Total protein and Albumin values of the experimental rats

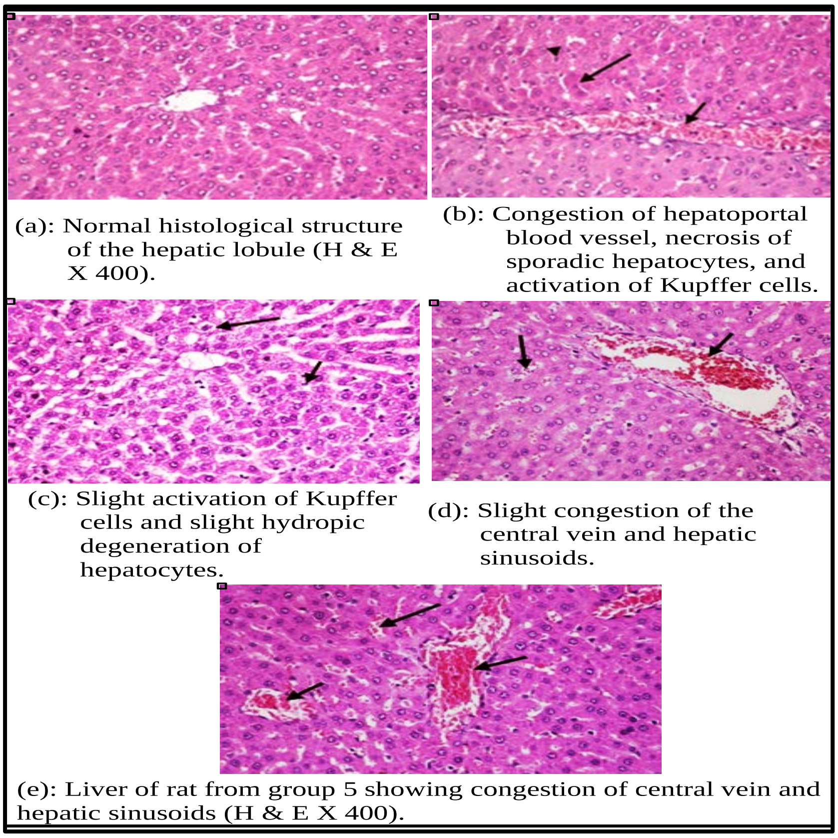
Groups	Total protein (g/dl)	% at control	Albumin (g/dl)	% at control
G1Normal control	$7.44{\pm}0.17^{a}$	100	$3.54{\pm}0.19^{a}$	100
G2Diabetic rats	$5.83{\pm}0.21^{b}$	78	$3.24{\pm}0.17^{b}$	92
G3 KRC	$6.50{\pm}1.26^{ab}$	87	$3.22{\pm}0.1^{b}$	91
G4 KGC	$6.63{\pm}0.33^{ab}$	89	$3.17{\pm}0.19^{b}$	90
G 5 KFC	$6.97{\pm}0.57^{ab}$	94	$3.17{\pm}0.09^{b}$	90
LSD at 5%	1.18		0.28	

G1: Normal control fed on a basal diet; G2: diabetic control fed on a basal diet; G3 (KRC): rats fed on kishk with 10% raw chia seeds; G4 (KGC): rats fed on kishk with 10% germinated chia seeds; G5(KFC): rats fed on kishk with 10% fermented chia seeds, ± S.D: standard deviation.

8- Histopathology:

a. Histopathological examination of the liver:

Microscopically, the liver of rats from (G1, normal control) revealed the normal histological structure of the hepatic lobule (Figs. 1a). On the contrary, the liver of rats from (G2, diabetic control) showed congestion of hepatoportal blood vessels, necrosis of sporadic hepatocytes, activation of Kupffer cells, hyperplasia of biliary epithelium, and oval cells proliferation (Fig. 1b). On the other hand, liver of rats from (G3, diabetic rats fed on Kishk with 10% raw chia seeds) revealed mild changes described as slight activation of Kupffer cells and slight hydropic degeneration of hepatocytes (Figs. 1c). Moreover, the liver of rats from (G4, diabetic rats fed on Kishk with 10% germinated chia seeds & (G5, diabetic rats fed on Kishk with 10% fermented chia seeds) revealed more or less similar mild changes. Examined sections from those groups exhibited slight activation of Kupffer cells and slight congestion of hepatic sinusoids as well as slight congestion of central vein and hepatic sinusoids (Figs. 1d and 1e). These results were in line with those of (El-Yamany, 2020) who observed that diabetic rats consuming 5% chia seeds displayed little hydropic hepatocyte degeneration. Additionally, these results are in agreement with those reported by Munir et al. (2021) and El-Feky et al. (2022).



(a): Normal histological structure of the hepatic lobule (H & E X 400).

(b): Congestion of hepatoportal blood vessel, necrosis of sporadic hepatocytes, and activation of Kupffer cells.

(c): Slight activation of Kupffer cells and slight hydropic degeneration of hepatocytes.

(d): Slight congestion of the central vein and hepatic sinusoids.

(e): Liver of rat from group 5 showing congestion of central vein and hepatic sinusoids (H & E X 400).

Fig. 14 (a-e). Histopathological examination of livers: G1: Normal rats, G2: diabetic rats, G3: rats fed on kishk with 10% raw chia seeds, G4: rats fed on kishk with 10% germinated chia seeds G5: rats fed on kishk with 10% fermented chia seeds. The cuts were H & E and magnification power is X 400.

b. Histopathological examination of kidneys:

Microscopically, the kidneys of rats from (G1, normal control) revealed the normal histological structure of renal parenchyma (Figs.2a). On contrary, the kidneys of rats from (G2, diabetic control) showed cytoplasmic vacuolization of epithelial lining renal tubules, proteinaceous material in the lumen of renal tubules, thickening of the parietal layer of Bowman's capsule, and necrobiosis of epithelial lining renal tubules (Fig. 2b). However, kidneys of rats from (G3, diabetic rats fed on Kishk with 10% raw chia seeds) showed slight congestion of glomerular tuft and intratubular blood capillaries as well as cytoplasmic vacuolization of epithelial lining some renal tubules (Figs.2c). On the other hand, the kidneys of rats from (G4, diabetic rats fed on Kishk with 10% germinated chia seeds) revealed mild changes, and examined sections showed only slight congestion of glomerular tufts (Figs. 2d). Moreover, the renal tissue of rats from (G5) diabetic rats fed on Kishk with 10% fermented chia seeds was exhibited no histopathological alterations (Figs. 2e). These results are in agreement with those reported by (Elebeedy et al., 2022).

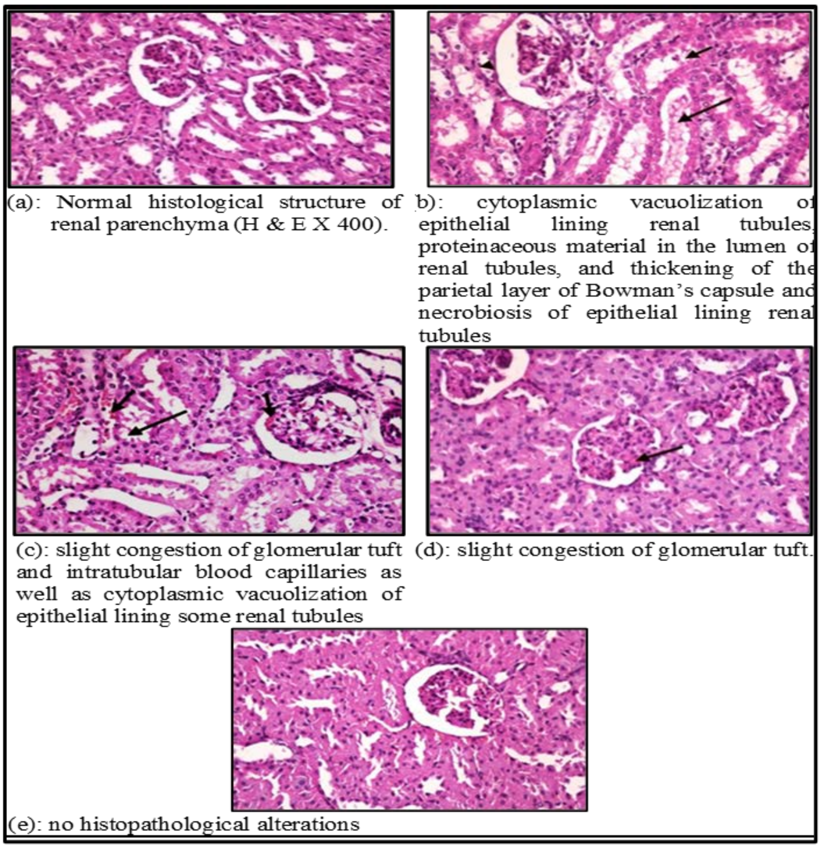


Fig. 2 (a-e). Histopathological examination of the Kidneys: G1: Normal rats, G2: diabetic rats, G3: rats fed on kishk with 10% raw chia seeds, G4: rats fed on kishk with 10% germinated chia seeds G5: rats fed on kishk with 10% fermented chia seeds. The cuts were H & E and magnification power is X 400.

c. Histopathological examination of the pancreas:

Microscopical examination of the Pancreas of rats from (G1, normal control) revealed normal pancreatic acini and normal islets of Langerhans (Fig. 3a). On the other hand, the pancreas of rats from (G2, diabetic control) showed marked vacuolations of cells of islets of Langerhans's (Fig. 3b). Meanwhile, the pancreas of rats from (G3, diabetic rats fed on Kishk with 10% raw chia seeds and G4, diabetic rats fed on Kishk with 10% germinated chia seeds) revealed an improved picture, as examined sections showed vacuolations of some cells of islets of Langerhans's (Figs. 3c, d). Moreover, marked regression of changes was observed in the pancreas of rats from (G5, diabetic rats fed on Kishk with 10% fermented chia seeds). Examined sections showed only vacuolations of sporadic cells of islets of Langerhans (Figs. 3e). These results were in line with those of (Elebeedy et al., 2022) who observed that diabetic animals treated with chia seeds displayed normal pancreatic parenchyma.

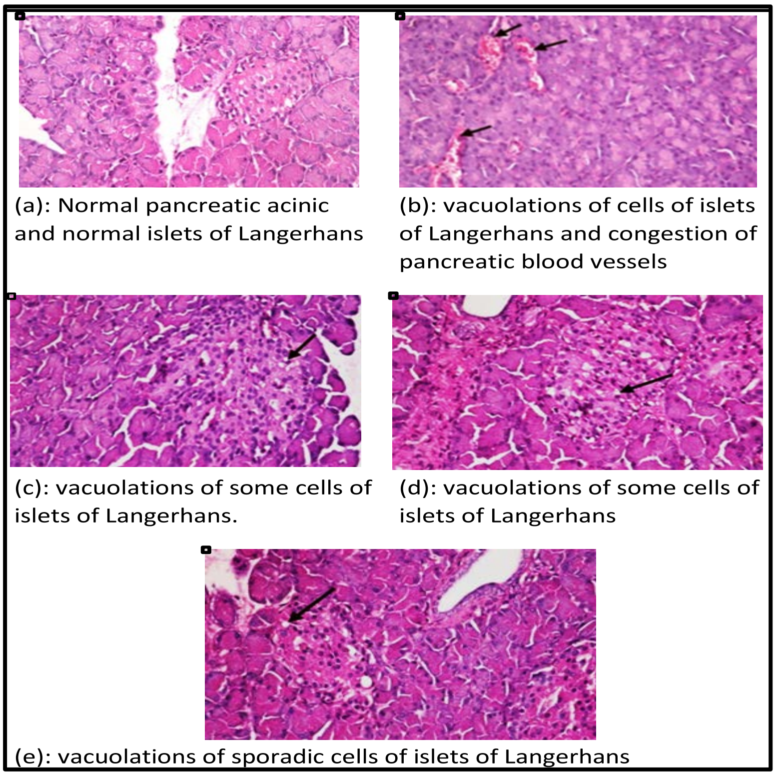


Fig. 3 (a-e). Histopathological examination of the pancreas: G1: Normal rats, G2: diabetic rats, G3: rats fed on kishk with 10% raw chia seeds, G4: rats fed on kishk with 10% germinated chia seeds G5: rats fed on kishk with 10% fermented chia seeds. The cuts were H & E and magnification power is X 400.

Conclusions

Based on the above results, it can be concluded that feeding with 10% chia seeds is effective for control blood glucose and lipids in STZ, induced diabetic rats. The fermented chia group was more effective in reducing blood glucose levels than raw and germinated groups in diabetic rats. Feeding with chia seeds has been associated with improvement in lipid profiles and liver functions. Microscopical examination of the pancreas in diabetic rats fed on kishk containing 10% fermented chia seeds showed marked regression of changes in the pancreas.

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تأثير بذور الشيا الخام والمنبتة والمخمرة على مستوي سكر ودهون الدم الكلية في فئران التجارب

****إشراق بدوي كامل– *فرحات فوده على فوده– *إبراهيم محمد عبد العليم– **مني عبد المنعم محمد علي**

*** قسم الكيمياء الحيوية الزراعية – كلية الزراعة – جامعة بنها –مصر**

**** معهد بحوث تكنولوجيا الاغذية– مركز البحوث الزراعية – الجيزة–مصر**

يهدف هذا البحث إلى تقييم تأثيرالكشك المدعم ب١٠% من بذور الشيا (الخام والمنبتة والمخمرة) علي ذكور الفئران المصابة بمرض السكري الناجم عن مادة الاستربنوسيوزين (STZ). تم تقسيم ثلاثون من ذكور الفئران البالغين الي خمس مجموعات . كل مجموعة تحتوي علي عدد 6 فئران . (المجموعة الأولي) الضابطة العادية التي تتغذى على النظام الغذائي الأساسي ، (المجموعة الثانية) مجموعة الضابطة المصابة بمرض السكري ، تتغذى على النظام الغذائي الأساسي ، (المجموعة الثالثة) مصابة بداء السكري تتغذى على الكشك مع 10٪ بذور الشيا خام ، (المجموعة الرابعة) فئران مصابة بداء السكري تتغذى على الكشك. مع 10٪ بذور الشيا المنبتة و (المجموعة الخامسة) مصابة بداء السكري تتغذى على الكشك مع 10٪ بذور الشيا المخمرة. تم دراسة تأثير بذور الشيا على نسبة مستوي الجلوكوز والدهون الدم كما تم قياس معدلات انزيمات الكبد ووظائف الكلي في سيرم الدم . أظهرت النتائج المتحصل عليها انه حدث إنخفاض معنوي في وزن الفئران في كل من المجموعة الثانية و الثالثة و الرابعة بينما لوحظ زيادة معنوية في الوزن في المجموعتين الاولي والخامسة. اظهرت نتائج تحاليل الدم للفئران المصابة بداء السكري الضابطة ارتفاع مستوي الجلوكوز 292ملجم/ديسيلتر ودهون الدم (الكوليستيرول الكلي و الدهون الثلاثية و الكوليسترول الضار (الليبوبروتين منخفض الكثافة كانت 254,22و203,11و188,78 ملجم/ديسليتر علي التوالي . بينما كانت قيم الليبوبروتين مرتفع الكثافة 24,88 ملجم/ديسليتر وذلك عند مقارنتها بالمجموعة الضابطة حيث كانت القيم على التوالى 89,07 الكوليستيرول الكلي و 60,33 الدهون الثلاثية و 28,63 ملجم/ديسيلتر لليبوبروتين منخفض الكثافة و الليبوبروتين مرتفع الكثافة 48,37 ملجم/ديسيلتر. بينما نتائج تحاليل سيرم الدم أوضحت أن التغذية علي الكشك المدعم ب10% من بذور الشيا الخام و المنبتة والمخمرة ادت الي حدوث انخفاض معنوى فى كل المجاميع المعالجة في مستوي دهون الدم لفئران التجارب تحت الدراسة وذلك بالمقارنة بالمجموعة الضابضة المصابة . كما أوضحت النتائج ان المجموعات التى تم تغذيتها على وجبات من لكشك المدعم ب10% من بذور الشيا الخام و المنبتة والمخمرة أدت إلى حدوث إنخفاض معنوى فى نشاط إنزيمات الكبد وذلك عند مقارنتها مع المجموعة الضابطة المصابة (+). أظهرت النتائج عند تقدير محتوى كل من اليوريا والكرياتين فى سيرم الالدم لفئران التجارب للمجاميع تحت الدراسة انخفاضا معنويا عند مقارنتها بالمجموعة الضابطة المصابة . كما اظهرت النتائج انخفاضا معنويا في كل من البروتين الكلي والألبيومين فى سيرم الدم لفئران التجارب في كل من المجموعة الضابطة المصابة و المجاميع تحت الدراسة عند مقارنتها بالمجموعة الضابطة العادية .

و أظهر الفحص الهسيتوباثولوجي لانسجة و خلايا كل من الكبد والكلي والبنكرياس ان تغذية فئران التجارب علي الكشك المدعم ب10% من بذور الشيا الخام و المنبتة والمخمرة ادت الي تحسن ملحوظ في انسجة الكبد و الكلي والبنكرياس وحمايتها من الالتهابات وذلك عند مقارنتها بالمجموعة الضابطة المصابة .