EFFECT OF CHIA AND SORGHUM SEEDS ON BIOLOGICAL, BIOCHEMICAL, AND HISTOPATHOLOGICAL CHANGES IN DIABETIC RATS

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Article History: Received: 28.05.2023 **Revised:** 02.07.2023 **Accepted:** 05.07.2023

Abstract

This study evaluated the effect of chia, sorghum, and their combination on diabetic rats. eight groups of six adult male albino rats, each weighing (150±10g), were formed from the 48 rats used in this investigation. for 28 days, 2.5 and 5%, respectively, of chia and sorghum seeds and their powdered mixes were given to the main diet. subcutaneous injections of alloxan (150 mg/kg body weight) were given to induce diabetic rats. samples were examined for biochemical markers 28 days after the experiment ended. diabetic groups resulted in a considerable drop in hdl whereas a large rise in tc, tg, vldl, ldl, u.a, creatinine, urea, got, gpt, alp, and glucose was observed. the results showed that diabetic rats fed with various experimental diets had improved in all previous parameters. a diet with 2.5% chia seeds outperformed one with sorghum seeds, while the best diet included a 5% mixture, indicating synergistic effect. this suggested that the first case's therapeutic outcomes were superior.

keywords: chia, sorghum, diabetes, biochemical parameters, histopathological changes

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INTRODUCTION

Diabetes mellitus is a worldwide burden; it affects approximately 1 in 11 people, globally. some researchers have revealed that almost a third of the population globally will develop the disease by the year 2050 **(Dyer *et al.*, 2020)**. diabetes is a typical chronic condition that needs ongoing monitoring for both glucose management and other risk factors that may coexist with diabetes **(Gariani *et al.*, 2020)**. Diabetes is a chronic metabolic illness and is defined by chronic hyperglycemia. it could be brought on by poor insulin secretion, peripheral insulin action resistance, or both **(Zheng *et al.*, 2018)**. Egypt is the eighth leading country regarding the prevalence of dm **(Safdar and khan, 2017)**. frequent urine, increased thirst, and increased hunger are signs of elevated blood sugar. The three types of diabetes are: the pancreas' inability to produce adequate insulin as a result of beta cell loss causes type 1 diabetes. Previously, this type was referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". The cause isn't known. If left un-treated, diabetes can cause many complications (**WHO, 2013.a)**. insulin resistance, in which cells do not respond to insulin as it should, and it is the first symptom of type 2 diabetes. maintaining a nutritious diet, routine exercise, a normal body weight, and abstaining from smoking are all necessary for prevention and treatment. for those who have the condition, keeping good foot hygiene and controlling blood pressure are crucial. Insulin injections are necessary to treat type 1 diabetes. Insulin may or may not be used in the treatment of type2 (dm) **(WHO, 2013.b)**. low blood sugar can be caused by oral medicines and insulin **(Rabail *et al.*, 2021)**.

The chia plant has become more well-known all across the world, especially for its seeds. all of the seeds, leaves, and flowers of this annual herbaceous plant, which belongs to the *lamiales* order, *lamiaceae* family, and *nepetoideae* subfamily, can be used. chia seeds have been produced as plant-based nutraceuticals. the richness of chia seeds is attributed to their high content of protein (15–25%), lipids (15–35%), ash (4-6%), and minerals, phytochemicals, vitamins, and antioxidants **(Khalid *et al.*, 2023**). Chia seeds are a good source of vegetable protein, unsaturated fat, carbs, and ash. They also contain fiber, polyphenols, antioxidants, omega-3 fatty acids, vitamins, and minerals **(Ashura *et al.*, 2021)**. Antioxidants including chlorogenic acid, myricetin, caffeic acid, quercetin, and kaempferol are abundant in chia seeds and are thought to have protective effects against cardiac, hepatic, aging, and carcinogenic traits. while its healthy -3 pufa, gluten-free protein, vitamin, phenolic compounds, and minerals also play a role in controlling hypertension, diabetes, inflammation, dyslipidemia, oxidative stress, immunity, blood clotting, laxative, depression, anxiety, and vision improvement **(Ullah *et al.*, 2016)**. It is beneficial for regulating diabetes and aiding the digestive system. linolenic acid (ala), which makes up around 60% of all fatty acids and is found in high concentrations in chia seeds, is one of these polyunsaturated fatty acids. lower concentrations of linoleic, oleic, and palmitic acids can be observed **(Villanueva-bermejo *et al.*, 2019)**. Chia is hypoglycemic, anti-inflammatory, antioxidant, anti-hypersensitive, and cardio protective, according to studies. due to chia's advantageous benefits on diabetes, obesity, cardiovascular disease, and various types of cancer, consumption has increased. these advantages are principally attributable to the seed's high content of important antioxidants, fatty acids, dietary fiber, anthocyanins, flavonoids, vitamins, minerals and carotenoids **(Prathyusha *et al.*, 2019)**.

Reported a decreased postprandial glycemia in people who had bread daily with up to 24 g of chia seeds **(Khalid *et al.*, 2022)**.

By enhancing beta-cell function with antioxidants and polyphenols and lowering blood glucose with fiber, chia seed components prevent type 2 diabetes **(Ashura *et al.*, 2021)**.

In terms of cereal crops, sorghum (*sorghum bicolor*, l.) comes in at number five. It originally belonged to food made from plants and is a member of the poaceae family. By lowering the risk of chronic diseases including diabetes and cvds, sorghum's functional composition has a considerable positive impact on human health **(Khalid *et al.*, 2022)**. The grains, rice bran, and stalks of sorghum are the primary components used. sorghum grains have the following nutritional values: 4.4-21.1% protein, 2.1–7.6% fat, 1.0–3.4% crude fiber, 57.0–80.6% total carbs, 55.6-75.2% starch, and 1.3–3.5% total minerals (ash). Along with providing 350 kcal of energy, calcium, phosphorus, potassium, carotene, and thiamin, sorghum also contains phenolics and various kinds of tannins, which act as antioxidants **(Ratnavathi and Komala, 2016)**. Sorghum includes a number of compounds that have been shown to be highly effective in preventing several major chronic human diseases, including inflammation, atherosclerosis, diabetes, obesity, and cancer **(Amarakoon *et al.*, 2021)**. In terms of cereal crops, sorghum (sorghum bicolor, l.) comes in at number five. the phenolic substances in sorghum include tannins, flavonoids, stilbenes, polycosanols, and phytosterols) **(de Morais Cardoso *et al.*, 2017)**. The ingestion of sorghum grain is safe for those with celiac disease because it lacks proteins that are similar to gluten and gliadin **(Pontieri *et al.*, 2013)**. diets containing sorghum lower blood glucose levels, control how quickly carbohydrates are digested and absorbed, and increase insulin sensitivity **(de Morais Cardoso *et al.*, 2017)**. The phenolic compounds in sorghum have antidiabetic benefits via a number of methods. first, they slow down the pace of carbohydrate digestion by suppressing the activity of digestive enzymes **(Park *et al.*, 2012)**.

thus, in this study, we are investigating the impact to find out how chia seeds and their combination on biochemical, biological and anti-diabetic alterations of male albino rats.

MATERIAL AND METHODS

Materials

Chia and sorghum seeds sources:

Chia and sorghum seeds (zea mays, l.) powders were purchased in a local market in Shibin El kom city, Menoufia governorate, Egypt.

Alloxan

Alloxan, or 5, 5-dihydroxyl pyrimidine-2, 4, 6-trione, is a urea derivative organic carcinogen and cytotoxic glucose derivative was obtained from the El-gomhoria company in Cairo, Egypt.

Experimental animals

The vaccine and immunity organization, ministry of health, helwan farm, Cairo, Egypt, gave 48 adult normal male albino rats of the "sprague dawley" strain weighing 150±10 g.

The chemicals

sigma chemical co., Egypt, provided saline solutions and pure cholesterol powder. morgan co. egypt provided cellulose, casein, choline chloride and dl methionine powder. Al-gomhoria company for trading drugs, chemical and medical instruments, Egypt, provided the chemical kits of liver enzymes, alp, urea, creatinine, tc, tg, and hdl-c.

Methods

Chia and sorghum seed powder preparation

grinding of chia and sorghum seeds in an air mill was performed to obtain a fine powder, then added to a high-speed mixer (Molunix, Al-araby firm, Egypt) and served as powder seize, then put in bags until they were.

Determination of phenolic compounds using hplc:

Hplc analysis of extracts an agilent 1200 chromatograph was used, which was outfitted with a pda model g1315b, a bin pump type g1312a, an auto-sampler model g1313a, and an rr zorbax eclipse plus c18 column (1.8 m, 150 mm 4.6 mm). the mobile phases a and b were 0.2% formic acid in water and acetonitrile, respectively. Elution was carried out at 0.95 ml min-1 using the solvent b gradient programme: 0-20 minutes, 5-16%; 20-28 minutes, 16-40%; 28-32 minutes, 40-70%; 32-36 minutes, 70-99%; 36-45 minutes, 99%; and 45-46 minutes, 99-5%.30. The injection volume was ten liters. for detection, wavelengths of 280 nm (for flavan-3-ols and benzoic acid derivatives) and 360 nm (for flavonols and cinnamic acid derivatives) were chosen. The components were quantified using hplc calibration curves of pure standards: gallic acid, caffeic acid, (+)-catechin, (-)-epicatechin, and ellagic acid. as an internal standard, rutin was used. some compounds were quantified as chemical equivalents of the most similar chemical structures: gallic acid for gallic acid glucoside, p-hydroxybenzoic acid, and methyl gallate; caftaric acid for caffeic acid; (+)-catechin for proanthocyanidins dimers, trimers, and monogallates; (-)-epicatechin for epicatechingallate; ellagic acid for ellagic acid pentoside the hplc method was used according to **Radovanović *et al*., (2010)** with some modification (elution gradient and flow rate).

EXPERIMENTAL DIABETES INDUCTION:

The induction of experimental diabetes rats, according to method described by **oh *et al*., (2020),** diabetes was initiated in healthy male rats by intraperitoneal injection of allaxon of 150 mg/kg per body weight. samples of fasting blood were taken one week following the injection to assess fasting serum glucose 200 mg/dl in diabetes animals **(Desai and Bhide, 1985)**.

DESIGN OF THE EXPERIMENT:

The research was once conducted in animal house at the university of Menoufia in Egypt, which has been authorized, department of nutrition and food science, faculty of home economics according to ethical approval of the science research ethics committee of faculty of home economics cleared the study protocol 26-srec-03-2022.

Adaptation of the enrolled rats involved feeding rats casein as a basal diet prepared according to**, (Nddg, 1979)** for one week. After this period, rats were categorized into eight groups, six rats in each: group (1): animals fed on casein only as negative control. group (2): diabetic animals fed on casein only as a positive control group. group (3): diabetic models fed on casein and chia seeds (2.5%) of diet. group (4): diabetic models fed on combination of casein and chia powder (5%) of diet group (5): diabetic animals fed on casein and sorghum seeds as powder by 2.5% of diet. group (6): diabetic rats fed on basal diet and sorghum seeds as powder by 5% of diet. group (7): diabetic rats fed on basal diet and mixture (1:1) of chia and sorghum seeds as powder by 2.5% of diet. group (8): diabetic rats fed on basal diet and mixture (1:1) of chia and sorghum seeds as powder by 5% of diet. The experiment lasted for 28 days; each rat was weighed separately at the end of the experiment, then blood samples were obtained after slaughtering.

BLOOD SAMPLING

The fasting of rats for 12-h was done at the end of the experiment period, then they were scarified. portal vein was the source of blood samples which were drained in dry clean tubes centrifuged at 4000rpm for 10 minutes for serum separation based on **(Reeves *et al*., 1993)**. serum was frozen at -18 $^{\circ}$c and kept for analysis.

BIOCHEMICAL ANALYSIS

Serum glucose was evaluated using the modified kinetic approach in accordance with **(Schermer, 1967)** by using kit supplied by spin react. Spain.

The liver enzymes, and serum alkaline phosphatase (alp) were tested using the approaches described by **(Kaplan, 1984), (Hafkenscheid, 1979) and (Henary, 1974)**, respectively.

Serum total cholesterol was assessed by colorimetric approach illustrated by **(Moss, 1982)**. serum triglycerides were measured by enzymatic method in agreement with the **(Thomas, 1992) and (Young, 1975)**. Hdl-c was assessed in line with the method described by **(Fossati, 1982) and (Friedwaid, 1972)**. Vldl-c was calculated in mg/dl according to **(Grodon, 1977)** using the following formula: vldl-c (mg/dl) = triglycerides / 5. ldl-c was calculated in mg/dl according to**, (Lee and Nieman, 1996)** as follows: ldl-c (mg/dl) = total cholesterol – (hdl-c + vldl-c).

Serum urea and creatinine were estimated using an enzymatic technique **(Patton and Crouch, 1977) (henary, 1974)**. While serum uric acid was evaluated using a calorimeter method of **(Barham and Trinder, 1972)**.

STATISTICAL ANALYSIS

SAS which is a completely randomized factorial design was used for data analysis **(sas, 1988)**; the student-newman-keuls test was used for the determination of mean. variations between groups of ($p \leq 0.05$) were considered significant using costat program. one-way ANOVA was used to analyze biological results.

RESULTS AND DISCUSSION

Identification of phenolics compounds of chia and sorghum seeds

Data tabulated in table (1) confirmed the identification of phenolic compounds of chia and sorghum seeds by way of hplc technique. The results showed that the best phenolic components found in chia seeds were caffeic acid and flavonol glycosides. the readings were 31.05 mg/100g dw and 16.21 mg/100g dw, respectively. On the other hand, the least phenolic chemicals found in chia seeds were quercetin and myricetin, with values of 0.17 and 3.51 mg/100g dw, respectively.

In terms of sorghum seeds, records showed that the best phenolic compounds found in sorghum seeds were tannic acid and catechin, with values of 53.57 and 30.37 mg/100g dw, respectively. in contrast, the least phenolic chemicals identified in sorghum seeds were caffeic acid and vanillic acid. the figures were 0.40 and 0.75 mg/100g, respectively. these outcomes support the research results found by **Reyes *et al.*, (2008)**, they reported that chia seeds contain natural antioxidants such as phenolic glycoside-q and k, chlorogenic acid, caffeic acid, quercetin, and kaempferol, which protect consumers against some adverse health conditions such as protection against some cardiovascular diseases and some types of cancer, as well as vitamins and minerals. In addition, a diverse range of biologically active phenolic compounds have been identified in sorghum such as flavonoids (flavanones, flavonols, flavanonols and flavan-3-olderivatives), ρ-coumaric, ferulic, caffeic, hydroxycinnamic, hydroxybenzoic and synaptic acid **(Kang *et al.*, 2016)**.

Table (1): phenolics compounds of chia and sorghum seeds

Phenolics Compounds	Concentration mg/100g dw	
	Chia Seeds	Sorghum Seeds
Gallic Acid	ND	0.97
Caffeic Acid	31.05	0.40
Quercetin	0.17	6.11
Kaempferol	4.76	9.28
Naringin	ND	6.87
4- Hydroxybenzoic Acid	ND	1.68
Vanillic Acid	ND	0.75
Synergic Acid	ND	1.60
Tannic Acid	ND	53.57
Catechin	ND	30.37
Epicatechin	ND	25.88
Chlorogenic Acid	4.27	ND
Myricetin	3.51	ND
Flavonol Glycosides	16.21	ND

ND = not detectable

Effect of Different Concentrations of Chia, Sorghum Seeds and Their Mixture on Glucose of Diabetic Rats:

The impact of chia, sorghum seeds and their combination on diabetic rats' glucose level was shown in table (2). The control (+) had a higher glucose, on the other hand, the control (-) displayed a lower level, with a significant variation ($p \leq 0.05$), which were 269 and 101.16 mg/dl, respectively. diabetic rats fed a 5% mixed powder had the lowest level of glucose, being 129 mg/dl ($p \leq 0.05$). in diabetic rats, the greatest glucose level was found in 2.5% chia seeds powder ($p \leq 0.05$), which was 171.16 mg/dl. the findings were in line with **(da Silva *et al.*, 2016)**, who reported that treated animals presented that the chia seed reduced in the blood glucose level compared to the positive control. Furthermore, the group rats fed on chia seeds had lower fasting blood glucose levels, with significant differences compared with positive control group **(Alamri, 2019)**. Also, studies on rats and mice have revealed reduction in blood glucose due to administration of sorghum extract **(Park *et al.*, 2012)**.

Table (2): Impact of the chia, sorghum, and their mixture on glucose levels of diabetic rats

Treatment/Parameter	Glucose Level (Mg/Dl)
g1 control –ve	$101.16^{g}\pm 1.75$
g2control +ve	$269.00^{a}\pm 6.00$
g3 rats + chia seeds (2.5%)	$171.16^{c}\pm 4.25$
g4 rats + chia seeds (5%)	$146.16^{c}\pm 1.75$
g5 rats + sorghum (2.5%)	$155.76^{b}\pm 3.25$
g6 rats + sorghum (5%)	$155.00^{c}\pm 5.00$
g7 rats+ mix (2.5%)	$139.33^{e}\pm 0.28$
g8 rats +mix (5%)	$129.00^{f}\pm 2.00$

lsd (p≤0.05)	**6.116**

Values are given as mean ± sd. the mean values in each column with the same letters are not different significantly. lsd: least significant differences (p≤0.05).

2-Effect of Different Levels of Chia, Sorghum Seeds and Combination Powder on Liver Enzymes of Diabetic Rats

Table (3) indicates the effects of chia, sorghum seeds and their combination powder on diabetic rats' liver functions (alt, ast, and alp). The findings showed that the ast in the control (+) had a p≤0.05 than the control (-) group, with mean values of 77.16 and 22.88 u/l, respectively. With a p≤0.05, the highest ast of diabetes group was recorded for rats fed on 2.5% chia seeds, but the lowest level was noted for rats fed on 5%mixed powder, which were 54.26 and 29.23 u/l, respectively.

As for alt, the control (+) recorded a higher level when compared with control (-) with (p≤0.05). the mean levels were 95.30 and 33.40 u/l, respectively. while the highest alt of diabetic rats was found for those fed on 2.5% chia seeds, the lowest level noted for rats fed on 5% mixture powder with a p≤0.05. The mean values were 71.70 and 57.5 ul, respectively. regarding alp, the control (+) group had a greater level than the (-) control, with a p≤0.05, with mean levels of 72.3 and 33.43 u/l, respectively. Alp of diabetics was found in the group fed 2.5% sorghum seeds, the lowest level was detected in those fed 5% combination powder, (p≤0.05). The means were 75.4 and 41.1 u/l, respectively. our result is consistent with studies of **Alamri, (2019)** who found that consumption of both white and black chia is effective in decreasing the levels of liver enzymes in rats .our result is consistent with studies that the reduce in the activities of these transaminases (alt, ast& alp) in the plasma and liver due to treatment such as sorghum diet is a pointer to improved hepatic function **(Fernández Martínez *et al.*, 2019)**.

Table (2): Influence of Varied Levels of Chia, Sorghum Seeds Combination Powder on Liver Functions of Diabetic Animals

Treatment/Parameter	Liver Functions (U/L)		
	Ast	Alt	Alp
G1 Control –Ve	22.88e±2.80	33.40f±3.87	33.43e±3.10
G2control +Ve	77.16a±4.95	95.30a±4.75	72.30a±1.75
G3 Rats + Chia Seeds (2.5%)	54.26b±4.30	71.70b±3.30	42.46d±4.15
G4 Rats + Chia Seeds (5%)	45.00c±3.26	67.63bc±3.80	43.63d±2.90
G5 Rats + Sorghum (2.5%)	34.03d±3.03	57.60e±5.96	75.40b±2.60
G6 Rats + Sorghum (5%)	48.50bc±5.10	64.33bc±3.80	51.53c±4.40
G7 Rats+ Mixture (2.5%)	48.5bc±3.5	62.00cde±3.00	50.26c±2.95
G8 Rats+ Mixture (5%)	29.23d±1.04	57.50de±2.50	41.10d±3.30
Lsd (P≤0.05)	**6.469**	**6.719**	**5.611**

values are given as mean ± sd. the mean values in each column with the same letters are not different significantly. Lsd: least significant differences (p≤0.05)

3. Impact of Different Levels of Chia, Sorghum Seeds and Combination on Total Cholesterol and Triglycerides Level of Diabetic Animals:

Table (4) indicates the influence of chia, sorghum seeds and their combination on diabetic rats' blood total cholesterol and triglycerides. the total cholesterol of the control (+) was significantly higher than that of the control (-) (p≤0.05). The means were 172 and 88.66 mg/dl, respectively. The group provided 2.5% chia seeds displayed the highest cholesterol values, the group fed 5%combination powder displayed the lowest, with a considerable difference group fed (p≤0.05). the means were 123.2 and 84.73 mg/dl, respectively.

Regarding triglycerides, the control (+) group outperformed the control (-) with a p≤0.05. the means were 141.13 and 77 mg/dl, respectively. in terms of tg, the maximum value was observed for the 2.5 % chia seeds rats, while the lowest was reported for the 5%mixed powder group, with a p≤0.05. The means were 106.63 and 76.6 mg/dl, respectively. These results agree with that chia seeds reduced total cholesterol and triglycerides in treated compared to the control in diabetic animals **(Alamri, 2019)**. Sorghum lipids and phenolics control synthesis and metabolism of cholesterol, and showed antagonistic activities on initiation and progression of cvd initiation in animal models **(de Morais *et al.*, 2117)** .

Table (4): Influence of Chia, Sorghum Seeds and Their Combinations on Total Cholesterol and Triglycerides of Diabetic Animals

Treatment/Parameter	Total Cholesterol (Mf/Dl)	Triglycerides (Mf/Dl)

g1 control –ve	88.66 ^{e}±3.75	77.00 ^{e}±0.50
g2control +ve	172.00 ^{a}±3.60	141.13 ^{a}±7.96
g3 rats + chia seeds (2.5%)	123.20 ^{b}±6.80	106.63 ^{b}±0.76
g4 rats + chia seeds (5%)	102.83 ^{d}±2.25	86.06 ^{cd}±1.26
g5 rats + sorghum (2.5%)	111.50 ^{c}±2.50	100.16 ^{b}±0.76
g6 rats + sorghum (5%)	105.50 ^{cd}±2.00	92.80 ^{c}±1.70
g7 rats with mix (2.5%)	86.03 ^{e}±1.45	86.06 ^{de}±0.25
g8 rats with mix (5%)	84.73 ^{e}±0.25	76.60 ^{e}±1.50
lsd (p≤0.05)	**7.771**	**6.948**

values are given as mean ± sd. the mean values in each column with the same letters are not different significantly. lsd: least significant differences (p≤0.05)

4-Impact of Different Levels of Chia, Sorghum Seeds and Combinations Powder on Lipid Profile Level of Diabetic Models:

The influence of chia, sorghum seeds and their combination on diabetic rats' blood lipid profile is presented in table (5). the high-density lipoprotein (hdl-c) of the control (-) revealed a higher level when compared with the control (+), (p≤0.05). The means were 50.88 and 35 mg/dl, respectively. The highest (hdl-c) levels recorded for rats fed on 5% mixture powder but, the lowest value found for rats fed on 2.5 %chia seeds (p≤0.05), which were 48.3 and 38.36 mg/dl, respectively.

The data also revealed that the control (+) group's low-density lipoprotein (ldl-c) was considerably (p≤0.05) greater than those of the control (-). The means were 109.3 and 23.63 mg/dl, respectively. animals fed on 2.5 % chia seeds recorded the highest (ldl-c) (63.68mg/dl), whereas animals fed on 5 % mixture powder recorded the lowest values (21.46 mg/dl), (p≤0.05). Very low-density lipoprotein (vldl-c) in the control (+) group recorded a significant (p≤0.05) higher mean value (28.16 mg/dl) when compared with the control (-) (15.39 mg/dl). The greatest (vldl-c) mean level (21.18 mg/dl) was found in the 2.5% chia seeds group, whereas the lowest mean value was found in the 5%mixed powder group (15.3 mg/dl), (p≤0.05). Such findings are in line with **(de Morais Cardoso *et al.*, 2017)** who stated that feeding rats with chia seeds as powder caused a significant (p≤0.05) increase in hdl-c. Administration of sorghum reduced the concentration of ldl-cholesterol and improved lipid profile in rats' model **(Kim and Park , 2012)**.

Table (5): Influence of Chia, Sorghum Seeds and Their Combination on Lipid Profile of Diabetic Animals

Treatment/Parameter	Hdl-C (Mg/Dl)	Ldl-C (Mg/Dl)	Vldl-C (Mg/Dl)
g1 control –ve	50.88^{ab}±7.61	23.63^{e}±3.50	15.39^{e}±0.10
g2 control +ve	35.00^{d}±0.69	109.30^{a}±0.96	28.16^{a}±1.60
g3 rats + chia seeds (2.5%)	38.36^{cd}±0.80	63.68^{b}±1.08	21.18^{b}±1.53
g4 rats + chia seeds (5%)	42.90^{bc}±0.79	42.77^{d}±2.14	17.19^{cd}±0.25
g5 rats + sorghum (2.5%)	39.53^{cd}±0.55	52.10^{c}±1.90	19.86^{b}±0.35
g6 rats + sorghum (5%)	45.03$^{ab.}$±0.45	42.22^{d}±2.14	18.20^{c}±0.88
g7 rats + mix (2.5%)	46.73^{ab}±0.30	23.40^{e}±1.70	16.00^{de}±0.10
g8 rats + mix (5%)	48.30^{a}±0.26	21.46^{e}±0.75	15.30^{e}±0.30
lsd (p≤0.05)	**4.754**	**6.927**	**1.503**

values are given as mean ± sd. the mean values in each column least significant differences (p≤0.05)

5. Impact of Different Levels of Chia, Sorghum Seeds and Combination Powder on Kidney Parameters of Diabetic Animals:

Data tabulated in table (6) show the influence of chia, sorghum seeds and combination powder on the kidney functions of diabetic rats. the urea levels of the control (+) demonstrated a significantly higher value (p≤0.05) when compared with the control (-), with mean values of 38.13 and 22.74 mg/dl, respectively. The highest mean of urea was found for group fed on 2.5% chia seeds powder (32.83mg/dl), whereas the lowest mean was noted among those for fed on 5% mixture, (p≤0.05).

Regarding uric acid levels, the data revealed that the control (+) group had a significantly greater value than the control (-) group (p≤0.05). The means were 10.96 and 7.46 mg/dl, respectively. While the group fed 2.5% chia seeds powder had the highest creatinine levels, the group fed 5%combination powder had the lowest, with a p≤0.05. The mean values were 9.33 and 7.80 mg/dl, respectively.

With regard to creatinine, the control (+) had a significant (p≤0.05) greater mean (1.8mg/dl) than the control (-) (1.26mg/dl) group. While the group fed 2.5% chia seeds powder had the highest creatinine levels, the group fed 5% combination powder had the lowest, with a p≤0.05. The mean values were 1.52 and 1.03 mg/dl, respectively. these results are in accordance with [51] who reported that all treated rat with chia seeds demonstrated a considerable reduction in urea p≤0.05 in compared with +ve control group (**Mahfouz,2020)**. Also, omega-3 fatty acids from sorghum seeds possess a protective role against renal impairment **(Fayez *et al.*, 2014)**.

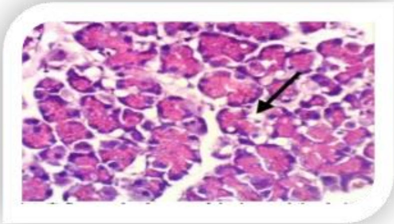
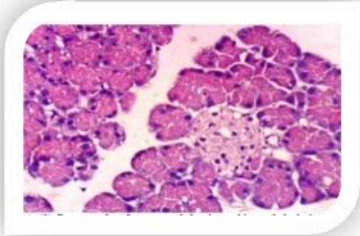
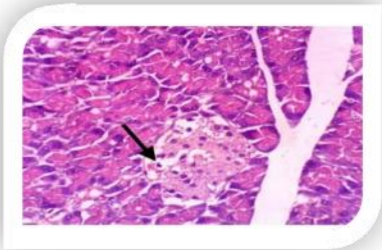
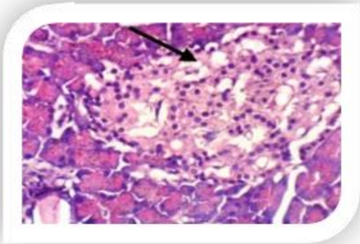
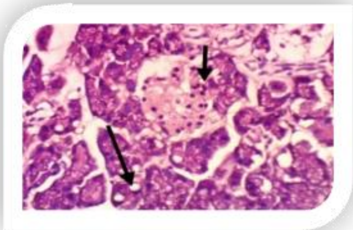
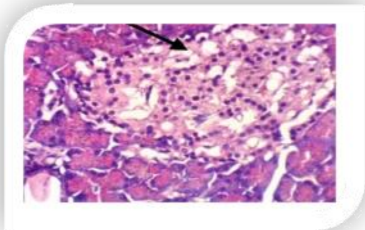
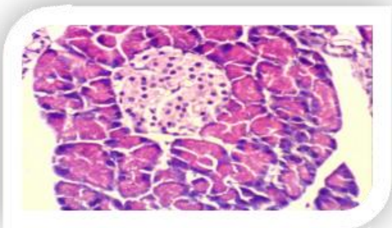
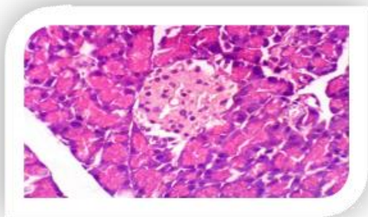
Table (6): Influence of Chia, Sorghum Seeds and Combination on Kidney Functions of Diabetic Animals:

Treatment/Parameter	Kidney Functions (Mg/Dl)		
	Urea	**Uric Acid**	**Creatinine**
g1 control –ve	$22.74^{d}\pm2.40$	$7.46^{b}\pm0.85$	$1.26^{b}\pm0.30$
g2control +ve	$38.13^{a}\pm1.95$	$10.96^{a}\pm0.40$	$1.80^{a}\pm0.12$
g3 rats + chia seeds (2.5%)	$32.83^{b}\pm2.91$	$9.33^{ab}\pm1.40$	$1.52^{a}\pm0.09$
g4 rats + chia seeds (5%)	$31.40^{bc}\pm2.10$	$8.63^{b}\pm0.60$	$1.45^{ab}\pm0.06$
g5 rats + sorghum (2.5%)	$26.70^{cd}\pm3.21$	$7.96^{b}\pm0.60$	$1.44^{ab}\pm0.17$
g6 rats + sorghum (5%)	$30.06^{bc}\pm2.60$	$8.83^{b}\pm0.41$	$1.40^{a}\pm0.07$
g7 rats with mixture (2.5%)	$31.8^{bc}\pm4.75$	$8.80^{b}\pm1.95$	$1.18^{b}\pm0.32$
g8 rats with mixture (5%)	$24.5^{d}\pm1.08$	$7.80^{b}\pm0.45$	$1.03^{b}\pm0.21$
lsd (p≤0.05)	**4.871**	**1.707**	**0.338**

values are given as mean ± sd. the mean values in each column with the same letters are not different significantly. lsd: least significant differences (p≤0.05)

Histopathological examination of pancreas:

Pancreas of rats from group 1 showed no histopathological changes (photo. 1). on the other hand, pancreas of rats from group 2 showed vacuolation of epithelial cells lining pancreatic acini (photo. 2). meanwhile, sections from groups 3 revealed vacuolation of cells of islets of langerhans's (photo. 3). pancreas rat's sections from groups 4 revealed vacuolation of some cells of islets of langerhans's in some sections (photo. 4). However, pancreas from group 5 showed vacuolation of epithelial cells lining pancreatic acini (photo. 5). sections from group 6 showed vacuolation of some cells of islets of langerhans's and epithelial lining pancreatic acini (photo. 6). meanwhile, pancreas from group 7 revealed no histopathological changes (photo. 7). pancreas of rats from group 8 revealed no histopathological changes (photo. 8).

 **photo (2): pancreas of rat from group 2 showing vacuolation of epithelial cells lining pancreatic acini (h & e x 400).**	 **photo (1): pancreas of rat from group 1 showing histopathological changes (h & e x 400).**
 **photo (4): pancreas of rat from group 4 showing vacuolation of some cells of islets of langerhan's (h & e x 400).**	 **photo (3): pancreas of rat from group 3 showing vacuolation of cells of islets of langerhan's (h & e x 400).**
 **photo (6): pancreas of rat from group 6 show vacuolation of some cells of islets of langerhan's and epithelial lining pancreatic acini (h&ex400).**	 **photo (5): pancreas of rat from group 5 showing vacuolation of epithelial cells lining pancreatic acini (h & e x 400)**
 **photo (8): pancreas of rat from group 8 showing n histopathological changes (h & e x 400).**	 **photo (7): pancreas of rat from group 7 showing histopathological changes (h & e x 400).**

CONCLUSION

Our findings revealed that feeding experimental rats with chia seeds as powder and sorghum as powder resulted in a remarkable (p≤0.05) elevation in hdl-c, with a remarkable (p≤0.05) reduction in serum lipid profile and blood glucose levels. and significant (p≤0.05) improvement in liver and kidney functions compared to the group control (+ve), reflecting the strong therapeutic effect of feeding on chia seeds as powder and sorghum as powder for the treatment of diabetes rats and the best diet was that contained 5%, indicating synergistic action .and chia seeds diet 2.5% caused better results than sorghum seeds diet.

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