Potential hepato-protective effect of *Salvia hispanica* (chia) and *Chenopodium quinoa* (quinoa) in diabetic male albino rats

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ABSTRACT

Chia and quinoa seeds are novel sources of bioactive compounds that may improve lipid profile, blood glucose homeostasis, and body weight in diabetic rats. This work aims to study the effect of different percentages of chia and quinoa consumption, alone or in a mixture, compared to Metformin drug on regulating lipid profile, blood glucose level, and weight of male diabetic rats. The consumption of quinoa and chia seeds showed a significant decline in fasting blood glucose (FBG), fasting insulin level, Hemoglobin A1C (HbA1c), and Homeostatic Model Assessment for Insulin (HOMA-IR), while the best results were obtained with Metformin and chia seeds. A decrease in mean body weight, triglycerides, total cholesterol, low density lipoprotein (LDL-C), and very low-density lipoprotein (VLDL-C), and an increase in high density lipoprotein (HDL-C) occurred, which was significant when consuming chia and quinoa seeds rather than treatment with metformin. Chia and quinoa seeds could improve blood glucose homeostasis and lipid profile in diabetic rats. These research findings could promote the daily consumption of quinoa and chia as great functional foods that could improve blood glucose homeostasis and lipid profile.

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1. INTRODUCTION

Disturbances in blood glucose homeostasis and elevated lipid profile are considered serious worldwide disorders affecting health and life quality. One of the golden standards of public health goals is to improve the population's health status and prevent complications from such chronic diseases with minimal side effects. According to the International Diabetes Federation, diabetes prevalence worldwide in 2021 was 10.5% (537 million) and is expected to spring up to 783 million by the year 2045 [1]. According to the World Health Organization (WHO), obesity, and overweight have tripled since the year 1975. In 2016, 39% of the worldwide population aged 18 years old and above were found to be overweight while 13% were considered obese [2]. Although diabetes and obesity could be controlled, they are associated with changes in lipid profile and cardiovascular complications [3]. Currently, both are controlled by increasing physical activity and consumption of healthier food items. [4].

Chia and quinoa are well-known, highly nutritious seeds, especially in dietary fibers and fatty acids including; omega-3. They are rich in protein, minerals, vitamins, and polyphenols including; phenolic acids and flavonoids [5], [6]. Bioactive compounds include polyphenols, vitamins, dietary fibers, and fatty acids; they have shown positive effects on hypertension, diabetes, and lipid profile [7]. Male rats fed on a high-fat and fructose diet and received different concentrations of chia and quinoa seeds as well as their extracts showed a significant rise in high density lipoprotein (HDL) cholesterol level as well as a decline in total cholesterol, triglycerides, low density lipoprotein (LDL), and very low density lipoprotein (VLDL) cholesterol levels. In addition, there was a significant decrease in lipids peroxidation levels. Both seeds may have potentially beneficial effects in the management of fat accumulation in non-alcoholic fatty liver conditions [8].

Both quinoa and chia showed a lower tendency to elevate blood glucose levels compared to wheat bread, thus having a three-fold lower value in the homeostasis model assessment [9]. This indicates the improvement of insulin sensitivity therefore the improvement of controlling glucose homeostasis and preventing metabolic disorders that caused by elevated glucose levels. Recently the usage of herbal medicine in the prevention and as a cofactor in assessing treatment in parallel with medicine by reducing doses recommended by the physician is considered one of the greatest solutions to reduce the side effects associated with the use of medicines in addition to improving the quality of health. Thus, the aim of our work is to investigate the effect of quinoa and chia seeds consumption on lipid profile, blood glucose homeostasis, and body weight on male diabetic rats in comparison to a gold standard diabetic drug (Metformin). In addition to chemical composition of quinoa and chia seeds were determined.

2. METHOD

2.1. Chemical composition

Two kilograms of each of *Salvia hispanica* (chia) and *Chenopodium quinoa* seeds were purchased from the main hypermarket in Alexandria Governorate. The following parameters were measured in environmental pollutants analysis laboratory (EPA), High Institute of Public Health, Alexandria University; proximate analysis (protein, fat, fiber, and carbohydrate) according to Association of Official Agriculture Chemist AOAC International [10], mineral analysis (iron, zinc, calcium, phosphorus, and magnesium) according to AOAC [10], calories content calculation according to Food and Drug Admisteration (FDA) regulation [11], antioxidant activity by 2,2-diphenyl-1-picrylhdrazyl (DPPH) assay [12], folic acid content [10] and fatty acid profile using gas chromatography [13].

2.2. Experimental animals

Sixty healthy albino male adult (*Rattus norvegrous*) rats of Wistar strain (6-8 weeks), which were determined using Biomath sample size calculator, weighing from 110-130 grams, were purchased from animal house at Medical Research Institute, Alexandria University. The animals were housed in polyvinyl chloride (PVC) cages of convenient size with a stainless-steel wire cover and chopped bedding. Rats were housed in a well-ventilated animal facility (15% air circulation/hour). A friendly environment with a 12h/12h light and dark cycle at (22-27 ºC) temperature and relative humidity (45-65) was sustained. Rats were adapted to the laboratory conditions for 14 days before the start of the experiment. Animals' cages were enriched by shredded papers and tubes during research implementation [14]. Animals received standard compressed pellets (14% protein, 3.65% fiber, and 3.55% fat) and water ad-libitum during the two weeks quarantine and acclimatization periods prior to start the study for adaptation.

2.3. Study design

The experimental study as illustrated in Figure 1 was divided into two phases, the 1st phase included the induction period which lasts for four weeks in which intervention groups (group 2 to group 6) fed on high fat diet followed by streptozocin (STZ) induction, while the 2nd phase is the intervention period that lasts for eight weeks. Rats were classified into a control group with ten rats as blank continue receiving standard compressed pellets along experimental period, while the remaining fifty rats fed on a high-fat diet consisting of the standard rodent pellets with 66.5% commercial feed, 13.5% lard, and 20% sugar (sucrose) for four weeks with free access to high-fat diet, based on lard. The standard pellets were supplied from the College of Veterinary Medicine. Rats were given a single dose of streptozocin subcutaneously at a concentration of 35 mg/kg body weight after the four weeks [15]. Animals had free access to water and food after the injection. Glycosuria test was checked daily for the rats. If glycosuria gives positive results for three consecutive days, the animals are considered diabetic.

2.4. Formulation of *Salvia hispanica* (chia) and *Chenopodium quinoa* diet

Chia and *Chenopodium quinoa* seeds and standard pellet were crushed to form fine particles then make the suitable substitutions mentioned in Figure 1 from the standard commercial diet, then compressed the formulated pellets mechanically to be ready for rats' consumption. The 2nd phase started after rats became diabetic. During this phase all groups have free access to standard compressed pellets (22% protein, 10% water, 5% fat, 52% carbohydrate, and 11% ash) and newly formulated diets with different concentrations. Group III ingested pellets with 60% chia, group IV ingested pellets with 60% quinoa and group V ingested pellets with a mixture of 30% chia and 30% quinoa. Rats have free access to formulated pellets.

Blood samples were collected from anesthetized rats from retro-orbital venous plexus by isoflurane inhalation <5% [16]-[19] at the baseline and after four weeks of ingestion of a high-fat diet. At the end of the experimental period (12 weeks), rats were fasted for 8-10 hours before euthanization by isoflurane inhalation >5% then blood samples collected, left for 10 minutes to clot, then it was centrifuged at 3,000 rpm to separate the serum [20]. The separated serum was used for biological analysis of the following parameters: fasting blood glucose [21], serum insulin [22], (Roche diagnostics GmbH), insulin resistance by homeostasis model assessment (HOMA) according to Hill *et al.* [23] using (1):

$$\mathrm{HOMA-IR} = \frac{(\mathrm{Glucose\ x\ Insulin})}{22.5} \quad (1)$$

As well as haemoglobin A1c (HbA1c) [24], and lipid profile including (LDL, HDL, VLDL, Total cholesterol and triglycerides) [21], [25] were analysed.

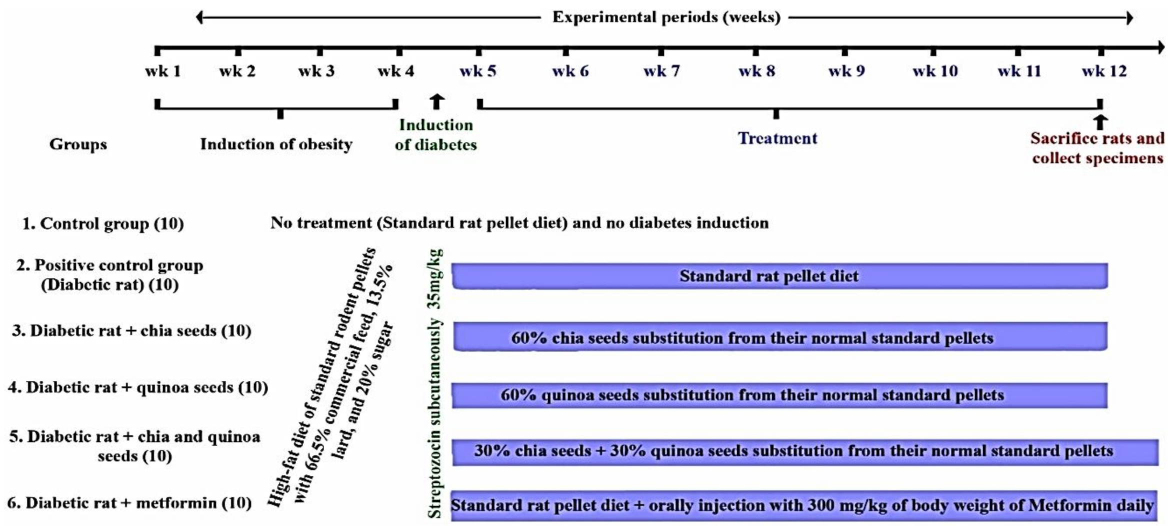

Figure 1. Experimental design

2.5. Histological examination

According to sampling and trimming procedures, the pancreatic tissue was dehydrated and paraffinized [26]. In an automatic procedure, a section of about 4µm was stained by conventional hematoxylin and eosin (H&E) stains [27]. Semi-quantitative grading (scoring) system for pancreatic histopathological alterations was done: In brief, from each animal pancreatic histopathological sections, five random fields were examined (×100), the percentage of the affected area/entire section was the method used to assess the grade of the detected lesion severity and recorded as follow (-): absence of lesion, (+): for mild degree of lesions (5–25%), (++): for moderate lesions degree (26–50%), and (+++): for severe degree of lesions (≥50%). The scoring system for the different groups extensively illustrated the effectiveness of the treatment protocols.

2.6. Statistical analysis

Data were entered into the computer and analysed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). To verify the normality of distribution quantitative data, the Kolmogorov-Smirnov test was used and was expressed using mean and standard deviation range. The obtained results significance was judged at the 5% level using the following tests; F-test (ANOVA) for quantitative variables normally distributed, to compare between more than two groups, post-hoc test (Turkey) for pairwise comparisons, and ANOVA normally distributed quantitative variables with repeated measures, to contrast between more than two stages or periods, and post hoc test (Bonferroni adjusted) for pairwise comparisons.

2.7. Ethical approvals

Ethical approval was obtained with serial (AU0920040714) from The ethics committee "Institutional Animal Care and Use Committee (IACUC)" OF High Institute of Public Health, Alexandria Univerity. The study was carried out in conformity with the International Guidelines for Research Ethics. The authors declared no conflict of interest.

3. RESULTS AND DISCUSSION
3.1. Laboratory findings

Data presented in Table 1 showed that chia seeds are rich in protein, fiber, and fat compared to quinoa seeds. Carbohydrates are higher in quinoa seeds compared to chia seeds. The caloric content of both seeds is nearly the same and as regard to minerals content, iron, calcium, zinc, and phosphorous are present in high concentrations in chia seeds, while quinoa seeds contained more magnesium and folic acid. The antioxidant activity of chia seeds is higher than quinoa seeds. The chemical composition of chia seeds and quinoa seeds varies from one research to another since the environment they are cultivated in and genotype affects their chemical composition [5], [6].

It was also observed the presence of saturated and unsaturated fatty acids in chia and quinoa seeds. Chia seeds were richer in saturated fatty acid as it contains lauric acid C12, palmitic acid C16, and stearic acid C18. Palmitic acid and stearic acid were found in quinoa seeds but in lower amounts. Arachidic acid C20 was also found in quinoa seeds. Vaccenic acid (omega 7) was found in both quinoa and chia seeds but the amount was higher in quinoa seeds. Omega 6 was also found in both seeds but quinoa had a higher percentage. Omega 3 was also found in quinoa seeds by a small amount. Overall, Chia seeds show to be richer in saturated fatty acids while quinoa seeds are richer in unsaturated fatty acids.

Since obesity and diabetes prevalence are increasing, several studies on chia and quinoa were done to find their effect on body weight, glucose, and lipid profile [28]. Although it was insignificant when comparing each group with the positive control group Figure 2, there was an increase in the mean body weight in all treated groups with chia and quinoa which was in line with [8]. The group receiving 60% chia and the group on a mixture of chia and quinoa had a significant increase in bodyweight along the timeline of the experiment. Chia contains more fiber than quinoa presented in Table 1 but quinoa has higher insoluble fiber content [28] and thus caused bulkiness and increased satiety in the rats leading to the end weight results being lower than the positive group.

Chia and quinoa proved to positively affect diabetes blood parameters [9]. A significant decline in fasting blood glucose level appeared in diabetic rats treated with metformin, rat's fed on 30% chia and 30% quinoa as well as those fed 60% chia, while rats fed on quinoa had a stabilized effect as seen in Table 2. When the rats were fed on the mixture of chia and quinoa or chia alone, the results were similar. Chia and quinoa also showed a significant decline in insulin levels, HbA1c, and HOMA-IR levels. Although metformin showed the best results on lowering insulin levels, the mixture of chia and quinoa obtained similar results but metformin was the only group that showed a significant decrease in HbA1c when compared to other groups. It also had the best results on HOMA-IR but groups treated with 60% chia or a mixture of chia and quinoa had similar significant results to each other. Chia is richer in soluble fiber than quinoa and has higher protein content thus causing a significant reduction in FBS, insulin level, insulin resistance, and improved HbA1c control. The soluble fiber form (mucilage) hinders the absorption of glucose thus stabilizing the blood glucose level and overall HbA1c which is in line with other studies [9], [29].

Table 1. Chemical composition of chia and quinoa seeds

Micro and macronutrients content	Quinoa seeds	Chia seeds
Carbohydrates%	72.06	61.72
Fiber%	1.89	6.23
Protein%	14.78	27.09
Fat%	0.69	1.53
Caloric content /100 gm (Cal.)		
Calories	353.57	369.01
Minerals content in µg/g (ppm)		
Fe	53.06	132.31
Ca	124.71	412.57
Zn	49.9	54.48
Mg	52.07	49.85
Phosphorous	306.25	662.42
Vitamin content in µg/g (ppm)		
Folic acid	541.8559	279.8834
Antioxidant activity DPPH activity %		
Scavenging activity	31.22	65.61
Fatty acid %		
C12:0		2.438359961
C16:0	21.95350231	31.01762681
C18:0	26.9963915	40.41270594
C18:1c	35.40541779	24.14096539
C18:2c	12.15721957	1.990341897
C18:3ω3	2.458175916	
C20:0	1.029292916	

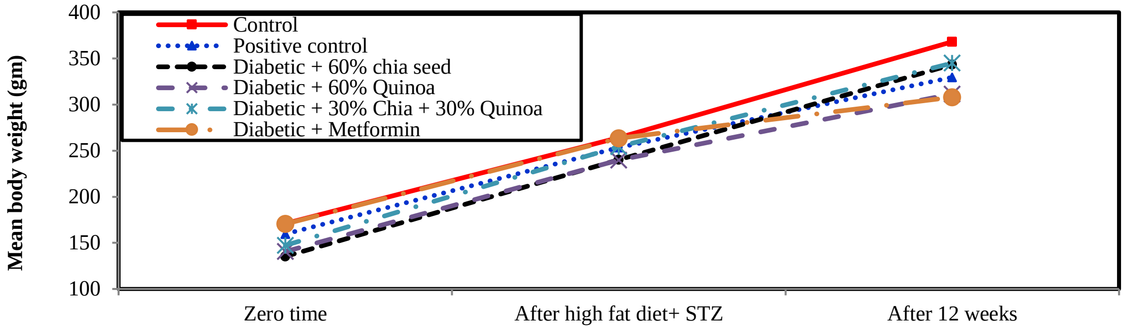

Figure 2. Mean body weight of rats of different experiment

Table 2. Blood glucose hemostasis parameters of different studied experimental groups

Parameters		Mean FBS (mg/dl)	p1	Mean Insulin (μIU/ml)	p1	Mean HbA1c (%)	p1	Insulin resistance HOMA-IR	p1
Control	Zero time	73.75 ± 14.34	0.999	10.10 ± 0.55	0.048*	5.0 ± 0.77		1.57 ± 0.55	0.156
	After high fat Diet	98.50 ± 9.29		14.48 ± 1.60		5.78 ± 0.17		2.48 ± 0.22	
	After 12 week	98.22 ± 8.42		8.50 ± 0.63		4.71 ± 0.98		2.06 ± 0.28	
Positive control	Zero time	98.75 ± 8.06*a	<0.001*	9.68 ± 0.63	0.013*	5.48 ± 0.24		2.36 ± 0.27	0.065
	After high fat Diet	139.75 ± 10.47**a		13.05 ± 1.10**a		6.58 ± 0.68		4.50 ± 0.45**a	
	After 12 week	180.80 ± 5.89***a		11.32 ± 0.33***a		6.02 ± 1.56		5.05 ± 0.22***a	
Diabetic+60% chia seed	Zero time	73.50 ± 9.33*b	<0.001*	9.48 ± 0.53	<0.001*	5.13 ± 0.40	0.004*	1.73 ± 0.31	<0.001*
	After high fat Diet	141.0 ± 20.45**a		14.20 ± 1.01***a		5.95 ± 0.24		4.98 ± 1.04***a	
	After 12 week	73.38 ± 7.67***ab		9.35 ± 0.48*a		4.56 ± 0.71		1.70 ± 0.24***b	
Diabetic+60% Quinoa	Zero time	66.75 ± 6.50**b	0.994	9.30 ± 1.28	<0.001*	5.18 ± 0.34		1.55 ± 0.33	0.004*
	After high fat Diet	156.25 ± 18.95***a		14.70 ± 0.65***a		5.73 ± 0.40		5.68 ± 0.82***a	
	After 12 week	155.50 ± 9.64***ab		11.20 ± 0.85***ab		5.21 ± 1.0		4.30 ± 0.50***ab	
Diabetic+30% Chia+30% Quinoa	Zero time	68.25 ± 8.18**b	<0.001*	10.23 ± 0.53	<0.001*	5.35 ± 0.44	0.021*	1.73 ± 0.28	<0.001*
	After high fat Diet	142.25 ± 11.67**b		14.78 ± 0.84***a		5.80 ± 0.29		5.18 ± 0.33***a	
	After 12 week	78.87 ± 7.95***ab		9.01 ± 0.38***b		4.49 ± 0.87		1.76 ± 0.24***b	
Diabetic+ Metformin	Zero time	93.25 ± 12.58	<0.001*	10.10 ± 0.55	<0.001*	5.95 ± 0.49	0.001*	2.34 ± 0.43	<0.001*
	After high fat Diet	133.25 ± 6.70*a		14.48 ± 1.60***a		5.45 ± 0.45*b		4.77 ± 0.70***a	
	After 12 week	61.0 ± 4.86***ab		8.50 ± 0.63***b		3.72 ± 0.52**b		1.28 ± 0.13***ab	

ZT: Zero time (n=10); AD: After high fat diet+STZ (n=10); A12: After 12 weeks (n=10); ANOVA test, pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey); 'a' Control vs Positive control, Diabetic+ 60%chia seed, Diabetic +60% Quinoa, Diabetic +30% Chia +30% Quinoa and Diabetic + Metformin; 'b' Positive control vs Diabetic+ 60%chia seed, Diabetic +60% Quinoa, Diabetic +30% Chia +30% Quinoa and Diabetic + Metformin; *p<0.05, **p<0.01 and ***p<0.001 *(n=10, Mean±S.D.); p1: p-value for comparing between after high fat diet and After 12 weeks in same group

Obesity and diabetes may disrupt lipid profile leading to cardiovascular occurrence [8]. Chia contains β-sitosterol, stigmasterol, and campesterol [5] and soluble fibers causing mucilage formation that reduces the dietary lipids and cholesterol absorption [29]. All groups showed a significant reduction in triglycerides (TG), LDL, and VLDL except the metformin-treated group. The best decline in TG and VLDL was obtained through 60% chia followed by rats fed on 60% quinoa, while those fed on a mixture of chia and quinoa had almost the same results as rats fed on chia seeds. Concerning cholesterol level, diabetic rats fed on 60% chia had the highest decline as well as the rats fed on mixture of chia and quinoa. The level of HDL increased significantly with treatment with 60% chia but those treated with 60% quinoa and control positive groups had a significant increase as illustrated in Table 3. Another explanation to support the results is that the DPPH scavenging activity of chia was found higher than quinoa as presented in Table 1 as well as being richer in omega 3, chia contained 31% and 40% of polyunsaturated fatty acids versus 21% and 26% found in quinoa respectively, so overall, chia seeds improved lipid profile significantly than quinoa.

Table 3. Lipid profiles parameters of rats in different studied groups

Parameters	Time	Mean TG (mg/dl)	p1	Mean total choles. (mg/dl)	p1	Mean HDL-choles. (mg/dl)	p1	Mean LDL-choles. (mg/dl)	p1	Mean VLDL (mg/dl)	p1
Control	ZT	63.75 ± 8.10	0.023*	105.25 ± 7.04	<0.001*	38.75 ± 3.30		53.75 ± 9.86	0.001*	12.75 ± 1.62	0.023*
	AD	97.50 ± 9.15		187.50 ± 10.79		35.50 ± 2.65		132.50 ± 12.28		19.50 ± 1.83	
	A12	110.78 ± 6.06		75.56 ± 1.67		36.78 ± 1.92		16.62 ± 2.29		22.16 ± 1.21	
Positive control	ZT	65.0 ± 12.08	<0.001*	105.0 ± 6.27	<0.001*	41.75 ± 2.22	0.306	50.25 ± 5.19	<0.001*	13.0 ± 2.42	<0.001*
	AD	111.25 ± 9.64		194.25 ± 7.09		32.25 ± 1.71		139.75 ± 6.92		22.25 ± 1.93	
	A12	164.20 ± 6.50***a		95.60 ± 5.18***a		30.20 ± 1.92***a		32.56 ± 4.08***a		32.84 ± 1.30***a	
Diabetic+ 60% chia seed	ZT	63.0 ± 5.03	<0.001*	97.25 ± 8.30	<0.001*	37.75 ± 2.06	<0.001*	46.90 ± 5.37	<0.001*	12.60 ± 1.01	<0.001*
	AD	121.75 ± 9.50**a		183.50 ± 19.05		30.25 ± 1.71**a		128.90 ± 17.48		24.35 ± 1.90**a	
	A12	54.75 ± 3.73***ab		66.13 ± 3.76***ab		37.38 ± 1.69***b		17.80 ± 3.14***b		10.95 ± 0.75***ab	
Diabetic+ 60% Quinoa	ZT	66.0 ± 5.10	<0.001*	96.0 ± 11.46	<0.001*	42.75 ± 1.50	0.988	40.05 ± 11.57	<0.001*	13.20 ± 1.02	<0.001*
	AD	131.50 ± 10.47***a **b		176.25 ± 10.75		33.75 ± 0.96		116.20 ± 11.78		26.30 ± 2.09***a*b	
	A12	87.38 ± 5.63***ab		101.0 ± 5.83***a		33.88 ± 1.46*a**b		49.65 ± 4.95***ab		17.48 ± 1.13***ab	
Diabetic+ 30% Chia+30% Quinoa	ZT	63.25 ± 6.65	0.275	91.25 ± 10.31	<0.001*	39.75 ± 3.30	0.944	38.85 ± 8.95	<0.001*	12.65 ± 1.33	0.275
	AD	147.25 ± 3.59***ab		183.50 ± 6.24		31.75 ± 2.50		122.30 ± 6.40		29.45 ± 0.72***ab	
	A12	153.25 ± 6.63***a* *b		78.88 ± 4.97***b		31.25 ± 2.12***a		16.98 ± 3.62***b		30.65 ± 1.33***a*b	
Diabetic+ Metformin	ZT	78.50 ± 7.94	<0.001*	97.25 ± 13.89	<0.001*	40.50 ± 3.42	0.059	41.05 ± 10.12	<0.001*	15.70 ± 1.59	<0.001*
	AD	126.75 ± 5.06***a		180.0 ± 12.99		29.25 ± 0.96**a		125.40 ± 13.73		25.35 ± 1.01***a	
	A12	168.33 ± 5.85***a		124.50 ± 4.42***ab		32.50 ± 0.84***a		58.67 ± 2.50***ab		33.67 ± 1.17***a	

ZT: Zero time (n=10); AD: After high fat diet+STZ (n=10); A12: After 12 weeks (n=10); ANOVA test, pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey); 'a' Control vs Positive control, Diabetic+ 60%chia seed, Diabetic +60% Quinoa, Diabetic +30% Chia +30% Quinoa and Diabetic + Metformin; 'b' Positive control vs Diabetic+ 60%chia seed, Diabetic +60% Quinoa, Diabetic +30% Chia +30% Quinoa and Diabetic + Metformin; *p<0.05, **p<0.01 and ***p<0.001 *(n=10, Mean±S.D.); p1: p-value for comparing between after high fat diet and After 12 weeks in the same group

Feeding rats with a high-fat diet and diabetes induction by STZ, don't affect haemoglobin (Hb) content in rats in all studied groups as illustrated in Figure 3, Hb increased gradually in all groups. The group treated with metformin had a time-dependent increase but there isn't significance difference. Diabetic rats fed with 60% chia, 60% quinoa, and 30% chia and 30% quinoa had the highest Hb level (16.04±0.38, 16.14±0.29, and 16.26±0.13 gm/dl respectively). Their effect was astonishing on the Hb level which needs further investigation as they are an easily and non-costly source of iron to treat anemia.

3.2. Histopathological findings

Light microscopy examination of pancreatic tissue sections illustrated that the control group showed normal architecture of pancreatic tissues and islets of Langerhans. While the positive control group showed diffuse haemorrhage, necrosis, congestion of blood vessels with interstitial edema, and mononuclear inflammatory cells infiltration, besides vacuolation of β-cells which were infiltrated with mononuclear inflammatory cells. On the other hand, the diabetic group on 60% chia showed minimal vacuolation of β-cells with congestion of blood vessels, interstitial edema, and hemorrhage.

The diabetic group on 60% Quinoa showed congestion of blood vessels with perivascular inflammatory cells infiltration and moderate hemorrhage, while the diabetic group on a mixture of 30% chia and 30% Quinoa showed only congestion of blood vessels and presence of apparently normal islets of Langerhans. Finally, the diabetic group on metformin showed perivascular edema and mononuclear inflammatory cells infiltration. The results supported the lab parameters data. There were significant histopathological changes within all groups, especially the untreated diabetic group as presented in Table 4. These results recommend that a long-term study is required to determine the effect of chia and quinoa in reversing the damage that occurred to the pancreas.

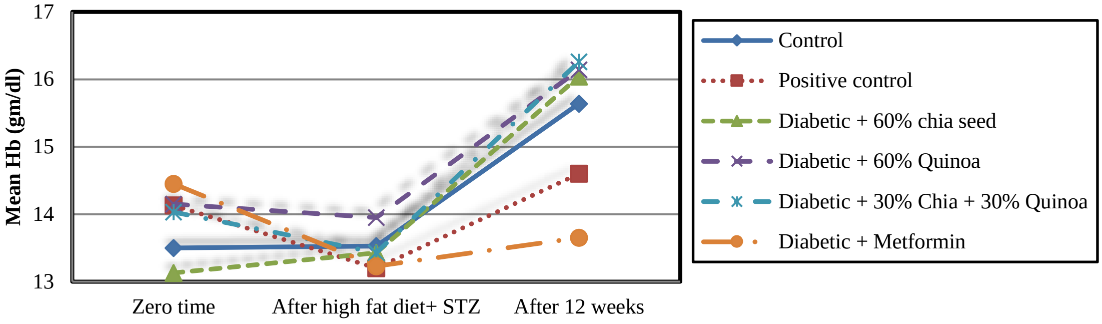

Figure 3. Hb (gm/dl) of rats of different experiments

Table 4. Incidence1 and Severity2 of histopathological lesions

Scored pancreatic lesions	Positive control				Diabetic+60% chia seed				Diabetic+60% Quinoa				Diabetic+30% chia +30% Quinoa				Diabetic+Metformin			
	(-)	(+)	(++)	(+++)	(-)	(+)	(++)	(+++)	(-)	(+)	(++)	(+++)	(-)	(+)	(++)	(+++)	(-)	(+)	(++)	(+++)
Congestion of blood vessels	0	2	2	3	4	0	3	0	4	3	0	0	4	2	1	0	3	3	1	0
vacuolar degeneration of β-cells	0	1	2	4	3	3	1	0	5	1	1	0	5	2	0	0	5	1	0	0
Perivascular Inflammatory cells infiltration	0	0	4	3	5	1	1	0	2	5	0	0	6	1	0	0	4	3	0	0
Pancreatic necrosis	0	2	0	5	7	0	0	0	6	1	0	0	7	0	0	0	7	0	0	0
Interstitial edema	0	1	5	1	2	4	1	0	3	3	1	0	5	1	1	0	6	1	0	0
Hemorrhage	0	3	3	1	3	2	2	0	3	4	0	0	7	0	0	0	7	0	0	0

1 Number of rats with lesions per total examined (7 rats); 2 Severity of lesions was graded by estimating the percent; (-) Absent (+) Mild (++) Moderate (+++) Severe

4. CONCLUSION

Chia and quinoa were found to affect weight stabilization due to their fiber content. Eventually, they didn't cause a decrease in body weight. Chia improves lipid profile parameters as well as blood glucose homeostasis, while quinoa has shown no improvement in cholesterol and LDL levels alone but only when present with chia. Chia seems to have the best results in both areas to an extent that if further investigations are made in comparison to drugs available in the market it might have an equivalent effect. Metformin gave the best results in improving blood homeostasis and chia alone or in a mixture had similar effects. On the other hand, metformin effect on lipid profile and Hb level was the lowest or no effect was obtained and chia or a mixture of chia and quinoa had the best results. Overall, chia alone or in a mixture achieved better results than quinoa on most of the tested parameters. A natural source of treatment with unsatisfactory side effects is a very important ground-breaking discovery. Further research is required to prove their efficiency in raw form or extracts of them in comparison to other metabolic syndrome traditional drug treatments.

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REFERENCES

[1] International Diabetes Federation (IDF), "IDF diabetes atlas 2021," 2021. [Online]. Available: https://diabetesatlas.org/atlas/tenth-edition/.
[2] WHO, "Obesity and overweight," *Geneva: Switzerland*, 2021. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.
[3] D. Tomic, J. E. Shaw, and D. J. Magliano, "The burden and risks of emerging complications of diabetes mellitus," *Nature Reviews Endocrinology*, vol. 18, no. 9, pp. 525–539, Sep. 2022, doi: 10.1038/s41574-022-00690-7.
[4] M. Madmoli, Z. M. Dehcheshmeh, A. Rafi, Z. Kord, F. Mobarez, and P. Darabiyan, "The rate of some complications and risk factors of diabetes in diabetic patients: Study on cases of 3218 diabetic patients," *Medical Science*, vol. 23, no. 95, pp. 63–4, 2019.
[5] B. Kulczyński, J. Kobus-Cisowska, M. Taczanowski, D. Kmiecik, and A. Gramza-Michałowska, "The chemical composition and nutritional value of chia seeds—current state of knowledge," *Nutrients*, vol. 11, no. 6, p. 1242, May 2019, doi: 10.3390/nu11061242.
[6] R. Vilcacundo and B. Hernández-Ledesma, "Nutritional and biological value of quinoa (Chenopodium quinoa Willd.)," *Current Opinion in Food Science*, vol. 14, pp. 1–6, Apr. 2017, doi: 10.1016/j.cofs.2016.11.007.

[7] S. N. Jimenez-Garcia *et al.*, "Role of natural bio-active compounds as antidiabetic agents," in *Advanced Structured Materials*, Springer International Publishing, 2021, pp. 535–561.
[8] M. A. El-Hay, "Down regulation of lipid accumulation in rats by antioxidant rich seed plants supplementation," *Scientific Bulletin*, vol. XXIV, no. 2, pp. 161–169, 2020.
[9] R. Selma-Gracia, C. M. Haros, and J. M. Laparra, "Potential beneficial effects of Chenopodium quinoa and salvia Hispanica l. in glucose homeostasis in Hyperglycemic mice model," in *The 2nd International Conference of Ia ValSe-Food Network*, Aug. 2020, p. 2, doi: 10.3390/proceedings2020053002.
[10] G. W. Latimer, *Official methods of analysis of AOAC international*, 20th ed. Association of Official Analytical Chemists (AOAC) international, 2019.
[11] L. K. Mahan and J. L. Raymond, *Krause's food & the nutrition care process*, 16th ed. Saunders, 2020.
[12] B. M. Moukette, C. A. Pieme, J. R. Njimou, C. P. N. Biapa, B. Marco, and J. Y. Ngogang, "In vitro antioxidant properties, free radicals scavenging activities of extracts and polyphenol composition of a non-timber forest product used as spice: Monodora myristica," *Biological Research*, vol. 48, no. 1, p. 15, Dec. 2015, doi: 10.1186/s40659-015-0003-1.
[13] S. S. Nielsen, *Food analysis*. Boston, MA: Springer US, 2017.
[14] National Academy of Sciences, *Guide for the care and use of laboratory animals*, 8th ed. Washington, DC.: National Academies Press, 2011.
[15] N. Subhasree, A. Kamella, I. Kaliappan, A. Agrawal, and G. Dubey, "Antidiabetic and antihyperlipidemic activities of a novel polyherbal formulation in high fat diet/streptozotocin induced diabetic rat model," *Indian Journal of Pharmacology*, vol. 47, no. 5, p. 509, 2015, doi: 10.4103/0253-7613.165200.
[16] D. Turck *et al.*, "Safety of chia seeds (Salvia hispanica L.) as a novel food for extended uses pursuant to Regulation (EU) 2015/2283," *EFSA Journal*, vol. 17, no. 4, pp. e05657–e05657, Apr. 2019, doi: 10.2903/j.efsa.2019.5657.
[17] M. Castillo-Ruiz, H. Cañon-Jones, T. Schlotterbeck, M. A. Lopez, Á. Tomas, and R. San Martín, "Safety and efficacy of quinoa (Chenopodium quinoa) saponins derived molluscicide to control of Pomacea maculata in rice fields in the Ebro Delta, Spain," *Crop Protection*, vol. 111, pp. 42–49, Sep. 2018, doi: 10.1016/j.cropro.2018.04.016.
[18] G. Arun, R. Rajaram, K. Kaleshkumar, N. Gayathri, T. Sivasudha, and S. Kandasamy, "Synergistic effect of novel chitosan combined metformin drug on streptozotocin-induced diabetes mellitus rat," *International Journal of Biological Macromolecules*, vol. 153, pp. 1335–1349, Jun. 2020, doi: 10.1016/j.ijbiomac.2019.10.270.
[19] M. P. Quaile *et al.*, "Toxicity and toxicokinetics of metformin in rats," *Toxicology and Applied Pharmacology*, vol. 243, no. 3, pp. 340–347, Mar. 2010, doi: 10.1016/j.taap.2009.11.026.
[20] G. M. Constantinescu and N. E. Duffee, "Comparison of submental blood collection with the retroorbital and submandibular methods in mice (mus musculus)," *Journal of the American Association for Laboratory Animal Science*, vol. 56, no. 6, pp. 711–712, 2017.
[21] N. Rifai, "Copyright," in *Tietz Textbook of Laboratory Medicine*, Elsevier, 2020, p. iv.
[22] L. C. Antunes, J. L. Elkfury, M. N. Jornada, K. C. Foletto, and M. C. Bertoluci, "Validation of HOMA-IR in a model of insulin-resistance induced by a high-fat diet in Wistar rats," *Archives of Endocrinology and Metabolism*, vol. 60, no. 2, pp. 138–142, Apr. 2016, doi: 10.1590/2359-3997000000169.
[23] N. R. Hill, J. C. Levy, and D. R. Matthews, "Expansion of the homeostasis model assessment of β-cell function and insulin resistance to enable clinical trial outcome modeling through the interactive adjustment of physiology and treatment effects: iHOMA2," *Diabetes Care*, vol. 36, no. 8, pp. 2324–2330, Aug. 2013, doi: 10.2337/dc12-0607.
[24] R. R. Little, C. Rohlfing, and D. B. Sacks, "The national glycohemoglobin standardization program: over 20 years of improving hemoglobin A1c measurement," *Clinical Chemistry*, vol. 65, no. 7, pp. 839–848, Jul. 2019, doi: 10.1373/clinchem.2018.296962.
[25] M. Sampson *et al.*, "A new equation for calculation of low-density lipoprotein cholesterol in patients with normolipidemia and/or hypertriglyceridemia," *JAMA Cardiology*, vol. 5, no. 5, p. 540, May 2020, doi: 10.1001/jamacardio.2020.0013.
[26] K. I, M. N, Y. Y-J, and L. J-Y, "Induce hyperlipidemia in rats using high fat diet investigating blood lipid and histopathology," *Journal of Hematology and Blood Disorders*, vol. 4, no. 1, Aug. 2018, doi: 10.15744/2455-7641.4.104.
[27] J. D. Bancroft, S. K. Suvarna, C. Layton, J. D. Bancroft, S. K. Suvarna, and C. Layton, "Some morphological structures and histological features of the gills of Binni (Mesopotamichthys sharpeyi)," in *Bancroft's Theory and Practice of Histological Techniques*, Elsevier, 2019, pp. ix–x.
[28] A. Agarwal, Rizwana, A. D. Tripathi, T. Kumar, K. P. Sharma, and S. K. S. Patel, "Nutritional and functional new perspectives and potential health benefits of quinoa and chia seeds," *Antioxidants*, vol. 12, no. 7, p. 1413, Jul. 2023, doi: 10.3390/antiox12071413.
[29] A. Tamargo, D. Martin, J. Navarro del Hierro, M. V. Moreno-Arribas, and L. A. Muñoz, "Intake of soluble fibre from chia seed reduces bioaccessibility of lipids, cholesterol and glucose in the dynamic gastrointestinal model simgi®," *Food Research International*, vol. 137, p. 109364, Nov. 2020, doi: 10.1016/j.foodres.2020.109364.

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