The effects of Chia and Quinoa Seed mixes on the Nutritional, Biochemical and Histological Parameters on Obese Rats

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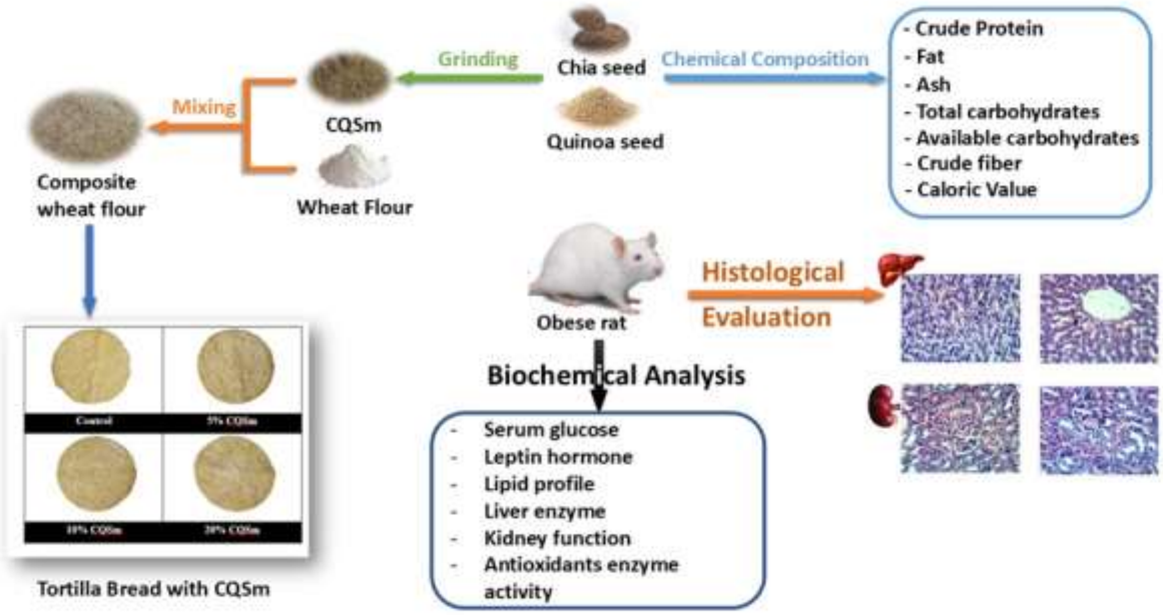
ABSTRACT

In the past few years, obesity and its complications have become more common. As a result, consumers are seeking to incorporate more functional foods into their diets. Chia) Salvia *hispanica*) and quinoa (*Chenopodium quinoa Willd*) are essential traditional crops with excellent nutritional properties. Therefore, the present study investigated the potential effect of chia and quinoa seeds mixes (CQSm) in an animal model to alleviate symptoms of obesity and other related complications. Thirty male albino rats weighing (130 ± 10 g) were used in this study. The rats were divided into two main groups: the first main group (6 rats) fed on a basal diet (as a control negative group). The second main group (24 rats) was fed on a high-fat diet (HFD) for eight weeks to induce obesity. This group was divided into four subgroups as follows: subgroup (1) fed on (HFD) as a positive control group; subgroup (2) fed on (HFD) diet containing 5% CQSm; subgroup (3): fed on (HFD) diet containing 10% CQSm; subgroup (4): fed on (HFD) diet containing 20% CQSm. Sensory evaluation for tortilla bread fortified with CQSm was also done. Results indicated that feeding obese rats with diets containing different levels of the mixture of both seeds induced a significant decrease in feed intake, body weight gain%, and peritoneal fat thickness. On the other hand, biochemical analysis showed a significant improvement in values of serum lipid profile, liver enzyme activity, kidney functions, blood glucose, leptin hormone, and antioxidant enzyme activities. In addition, histological examination of the liver and kidneys of rats in groups treated with different levels of the mixture of both seeds showed a noticeable improvement in the tissue structure of these organs. Healthy tortilla bread was produced with proportions of 5, 10, and 20% of CQSm. Sensory evaluation indicated that all tortilla bread samples had an acceptance greater than 75%. In conclusion, the current study indicates that the mixture of chia and quinoa seeds has improved biomarkers, and can be used as a functional food in the diets of obese rats. Based on these results, it is suggested that incorporating foods rich in soluble and insoluble fiber, such as chia and quinoa seeds, into our consumption habits through healthy and safe products may have a protective role against obesity and its associated complications and potentially prevent its progression.

Key words: Obesity- Functional Foods – Chia - Quinoa - Antioxidant

Graphical Abstract:



الملخص:

في السنوات القليلة الماضية، أصبحت السمنة ومضاعفاتها أكثر شيوعًا. ونتيجة لذلك، يسعى المستهلكون إلى دمج المزيد من الأطعمة الوظيفية في أنظمتهم الغذائية. تعد بذور الشيا والكينوا من المحاصيل التقليدية الأساسية ذات الخصائص الغذائية الممتازة. هدفت الدراسة الحالية إلى دراسة التأثير المحتمل لخليط من بذور الشيا والكينوا في تخفيف أعراض السمنة ومضاعفاتها لدي الفئران البدينة. استخدم في هذه الدراسة ثلاثون فأراً من ذكور الألبينو وزنها (١٣٠ ± ١٠ جرام)، تم تقسيمهم إلى مجموعتين رئيسيتين: المجموعة الرئيسية الأولى (٦ فئران تم تغذيتها على الغذاء الأساسي كمجموعة ضابطه سالبه (غير مصابة). المجموعة الرئيسية الثانية (٢٤ فأرًا) تغذت على نظام غذائي عالي الدهون لمدة ٨ أسابيع لتحفيز السمنة، تم تقسيم هذه المجموعة إلى أربع مجموعات فرعية على النحو التالي: المجموعة الفرعية (١) تغذت على نظام غذائي عالي الدهون (HFD) فقط، كمجموعة ضابطة إيجابية، المجموعة الفرعية (٢) تغذت على نظام غذائي (HFD) يحتوي على ٥٪ خليط بذور الشيا والكينوا (CQSm)؛ المجموعة الفرعية (٣): تغذت على نظام غذائي (HFD) يحتوي على ١٠٪ CQSm؛ المجموعة الفرعية (٤): تغذت على نظام غذائي (HFD) يحتوي على ٢٠٪ CQSm. أظهرت

النتائج أن تغذية الفئران على وجبات تحتوي على خليط من بذورالشيا والكينوا أدى إلى انخفاض كبير في متوسط تناول الطعام ونسبة الزيادة في وزن الجسم وسمك طبقة الدهن البريتوني. كما أظهر التحليل الكيميائي تحسنًا كبيرًا في قيم دهون الدم، نشاط إنزيمات الكبد، وظائف الكلى، سكر الدم، هرمون اللبتين ونشاط الإنزيمات المضادة للأكسدة. بالإضافة إلى ذلك، أظهر الفحص النسيجي للكبد والكلى لدى الفئران في المجموعات المعالجة بمستويات مختلفة من خليط بذور الشيا والكينوا تحسنًا ملحوظًا في بنية أنسجة هذه الأعضاء. تم إنتاج خبز التورتيلا الصحي المدعم بخليط بذور الشيا والكينوا CQSm واشارت نتائج التقييم الحسي إلى أن جميع عينات خبز التورتيلا المنتجة بالنسب ٥ و ١٠ و ٢٠٪ من CQSm كان لها قبول أكبر من ٧٥٪. خلصت الدراسة الحالية إلى أن خليط بذور الشيا والكينوا قد حسن المؤشرات الحيوية، ويمكن استخدامه كغذاء وظيفي في النظام الغذائي للفئران البدينة. وتوصي الدراسة بضرورة دمج الأطعمة الغنية بالألياف القابلة للذوبان وغير القابلة للذوبان، مثل بذور الشيا والكينوا في عاداتنا الاستهلاكية من خلال منتجات صحية وآمنة قد يكون له دور في الوقاية من السمنة ومضاعفاتها.

الكلمات المفتاحية: السمنة – الأطعمة الوظيفية – الشيا – الكينوا – مضادات الأكسدة

INTRODUCTION

Obesity is a chronic metabolic disease characterized by an excess of fat stores in the body. It is a major cause of disability and death because it does not only affect adults but also children and adolescents internationally **(Aktar *et al.*, 2017).** Obesity is associated with chronic inflammation, which alters the function of adipose tissue, the liver, the pancreas, and the nervous system **(Rubio-Tomás *et al.*, 2022).** Besides that, the chronic diseases resulting from obesity (metabolic syndrome, type 2 diabetes mellitus (T2D), dyslipidaemia, hypertension, osteoarthritis, sleep apnea, and various cancers) and the associated disabilities can lead to decreased productivity, life quality, and expectancy **(Simsek *et al.*, 2022; Masood & Moorthy, 2023).** Obesity is also linked to an increased risk of developing mental health problems, including depression and, potentially, addiction **(Ouakinin *et al.*, 2018).** According to a **(WHO,2019)** evaluation, there are more than 650 million obese people worldwide; Egypt ranks 18th with the highest prevalence of obesity worldwide. The estimated annual deaths due to obesity were about 115 thousand (19.08% of the total estimated deaths in 2020). Diseases attributable to obesity create a huge economic, humanistic, and clinical burden in Egypt. The economic burden of treating obesity-related diseases is around 62 billion Egyptian pounds annually **(Aboulghate *et al.,* 2021).** Numerous studies suggested that health organizations prioritize weight management and obesity prevention to reduce the prevalence of various metabolic disorders **(Konstantinidi & Koutelidakis, 2019).** However, there is currently no pharmaceutical medication that can effectively reduce obesity with minimal adverse effects. So, using medication as an option to treat obesity should be the final treatment tool because it is associated with many side effects **(Csige *et al.,* 2018).** Functional foods containing bioactive compounds may help prevent chronic conditions, including obesity, and promote health beyond basic nutrition **(Little *et al.,* 2021; Omran *et al.,* 2023; Sharma *et al.,* 2024).**

In recent years, chia seeds have become one of the world's most recognizable functional foods based on their nutritional properties and medicinal values. Chia (Salvia hispanica L.) is a small seed that comes from an annual herbaceous plant, Salvia hispanica L. Chia seeds contain a high fat content, carbohydrates, dietary fiber, proteins, vitamins (A, B1, B2, and B3), minerals, and antioxidants **(Knez *et al.,* 2019).** Besides this, chia seeds contain the flavonoids quercetin, chlorogenic acid, caffeic acid, rosmarinic acid, myricetin, quercetin, and others that are proven to have anti-cancerogenic, anti-hypertensive, and neuroprotective effects. Furthermore, chia seeds are a rich source of nutrients such as polyunsaturated omega-3 fatty acids that protect from inflammation, improve cognitive performance, and lower the level of cholesterol. Chia seeds contain antioxidant compounds that reduce the risk of chronic diseases (cancer and heart attack) and offer protection against some disorders such as diabetes, Alzheimer's, and Parkinson's disease. Chia seeds are also a rich source of soluble and insoluble dietary fibers, contributing to weight loss and reducing obesity complications, heart disease, and the risk for diabetes type 2, via their potential antioxidant and anti-inflammatory properties **(Oliveira-Alves *et al.,* 2017; Kulczyński *et al.* 2019; Mudgil *et al.,* 2023).** The seeds are also considered to be free from toxins, and they do not contain gluten **(Ullah *et al.,* 2016).** In the food industry, chia seeds can be used in different shapes: whole, ground, in the form of flour, oil, and gel. Chia seeds can be added or mixed into biscuits, pasta, cereals, snacks, and cakes as supplements **(Das, 2018)**.

On the other hand, quinoa is a candidate food crop that has received significant attention in the past decade for its nutritional and bioactive potential. It is a plant recently grown successfully in Egypt, providing seeds rich in nutrients and bioactive compounds **(Barakat *et al.,* 2017; Alamri *et al.,* 2023).** Quinoa is a good source of dietary fiber and high-quality protein. It is not only rich in protein, fatty acids and minerals but is also rich in many phytochemicals including saponins, phytosterols, plant desteroids, phenols and biologically active peptides. These compounds have beneficial effects on metabolic, cardiovascular, and gastrointestinal health, playing a very important role in both disease prevention and treatment and human health **(Xu *et al.*, 2019). Ren *et al.* (2023)** reported that quinoa and its bioactive components have a variety of health-promoting effects. Quinoa seeds is an excellent example of a 'functional food' that aims at lowering the risk of various diseases and has health benefits including antioxidant, antidiabetic, antihypertensive, anti-inflammatory, and anti-obesity properties. Also, **Alasalvar *et al.* (2021)** added that quinoa is also reducing body mass index (BMI) and fasting triglyceride in post-menopausal women, lowering postprandial glucose responses, antioxidant activities in high fructose-fed rats, and alleviating the inflammatory cytokines level. Regarding the effect of quinoa on hepatic steatosis, **Noratto *et al.* (2019)** showed that quinoa intake reduces cholesterol in the plasma and liver, lessens obesity-associated chronic inflammation, and prevents hepatic steatosis in obese mice. For all the above reasons, adding chia and quinoa seeds to food products will be of great importance to users of those products. Therefore, the present study was carried out to investigate the potential effects of mixing chia and quinoa seeds on the biochemical, nutritional, and histological parameters of obese rats.

Materials and Methods

Materials

- Casein, all vitamins and minerals, cellulose, choline chloride and salt mixture were obtained from El-Gomhoriya Company for Trading Drugs, Chemicals and Medical instruments, Cairo, Egypt.
- Chia and quinoa seeds were obtained from the Haraz Company for Agricultural Seeds and Medicinal Plants, Egypt.
- All ingredients used in tortela bread formulation (Wheat flour, salt, skim milk powder, corn oil and water) were obtained from the local market of Damietta Governorate, Egypt.
- **Animals:** rats used in this study, adult male albino rats (Sprague Dawley strain) weighing (130±10g) were obtained from the Medical Experimental Research Center, Faculty of Medicine, Mansoura University

Chemicals: All chemicals, reagents and solvents were of analytical grade and purchased from Al-Gomhoryia Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt.

Methods

Preparation of quinoa seeds

The quinoa seeds were prepared according to **Nickel et al. (2016)** with some slight modifications in removing saponins. Whole seeds were washed twice with cold water, seeds were soaked in the alkaline solution (0.2 M sodium bicarbonate) for 10–20 min, and then rinsed with a 1% citric acid solution for 10 min. The cleaned seeds were washed with manual rubbing in running water for 10 minutes until there was no foam, which indicates saponin removal from the seed hull. Later, saponin-free seeds were overnight oven-dried at 55 ± 1°C. The seeds were spread thinly throughout the drying process in order to prevent germination and any further contamination. Finally, treated whole seeds were ground into flour using Moulinex (LM2411EG) and kept at 5 °C for further analysis.

Preparation of chia seeds

Chia seeds were cleansed of dust using air pressure and then ground using the same ground machine, kept in polyethylene bags, and stored at 5°C until analysis.

Chemical analysis

Chia and Quinoa seeds were analyzed for ash, crude fiber, fat, and crude protein, as determined as described in **A.O.A.C. (2005)**, while available carbohydrates were calculated by difference.

- Total carbohydrates% = 100 – (protein + fat + ash)

- Available carbohydrates % = 100 – (protein + fat + ash + crude fibers)

- The energy values were calculated theoretically according to the method described by **James (1995).**

Experimental design

All biological experiment carried out complied with National Research Council, Commission on Life Sciences, and Institute of Laboratory Animal Resources rules **(NRC, 2011).** Thirty male albino rats weighing (130 ± 10 gm) will be keep in individual stainless-steel cages under hygienic conditions and fed one week on basal diet for adaptation according to **Reeves *et al.* (1993)**. Meanwhile, salt and vitamin mixtures followed that of **Hegested *et al.* (1941) and Campbell, (1963)** respectively. After a period of adaptation to the basal diet (one week), the rats were divided into two main groups. The first main group (6 rats) was fed a basal diet as a control negative group. The second main group (24 rats) was fed on a high-fat diet (HFD) for eight weeks to induce obesity. This group was divided into four subgroups as follows: subgroup (1) fed on (HFD) as a positive control group; subgroup (2) fed on (HFD) diet containing 5% CQSm; subgroup (3): fed on (HFD) diet containing 10% CQSm; subgroup (4): fed on (HFD) diet containing 20% CQSm.

During the experiment period (6 weeks), the quantities of diet that were consumed and wasted, were recorded every day. The weight of rats was also recorded weekly in order to calculate feed consumption and body weight gain percentage according to **Chapman *et al.* (1959).** The peritoneal fat layer thickness was measured on dead animals using a caliper. Measurements were expressed in mm according to the method described by **Tekus *et al.* (2018)**. At the end of the experiment, the animals were fasted overnight, then the rats were anaesthetized and sacrificed, and blood samples were collected from the aorta. The blood samples were centrifuged, and serum was separated to estimate some biochemical parameters, i.e. Serum glucose was determined by **Trinder (1969).** Total cholesterol and triglycerides were determined in the serum according to the methods described by **Allain *et al.* (1974)** and **Fossati and principe (1982)**, respectively. Serum HDL-C, LDL-C and VLDL-C were determined in the serum according to the method described by **Lopes – Virella *et al.* (1977)** and **Friedewald *et al.* (1972)** respectively. Uric acid, urea nitrogen and creatinine were determined in the serum according to the methods described by **Fossati *et al.* (1980), Patton and Crouch (1977); Bohmer (1971)**, respectively. Aspartate amine transaminase (AST), alanine amine transaminases (ALT) and alkaline phosphatase (ALP) activities were measured according to the methods described by **Reitman and Frankel (1957); Bergmeyer *et al*., (1974)**, respectively. Catalase, glutathione peroxidase (GPX) and superoxide dismutase (SOD) were determined in the serum according to the methods described by **Aebi (1974); Mc Cord and Fridovich (1969)**, respectively. Leptin was determined in the serum according to the methods described by **Prolo *et al.* (1998)**.

Histological examination

Liver and kidneys were removed from each rat by careful dissection, washed with saline solution, dried with filter paper, and weighed to calculate organ-to-body weight percentage. The kidneys and liver in each group were examined histopathologically, according to **Sheehan and Hrapchak (1980).**

Formulation and preparation of tortilla bread

Tortilla bread was prepared by using the method described by **Rendón-Villalobos *et al.* (2009).** The control and the other formulations are presented in Table (1). The wheat flour (72% for all purposes), corn flour, and oil have been mixed for 2 minutes before adding water. After that, the salt and baking powder were added. Once water was added, the batter was further mixed to form dough and cut into pieces of 35 grams, then left to rest for 10 minutes. These pieces were rolled out and extruded into thin circles to make tortillas that were 2 mm thick. Tortillas were baked on a hot griddle for 1 min per side at an approximate temperature of 250 ± 5 ◦C. Baked, the tortilla was then removed from the oven and allowed to cool down to room temperature. The samples were stored in airtight containers before sensory evaluation. The other experimental formulations were prepared by adding powder from chia and Quinoa seeds mixture by the equally proportion from each other 5% , 10%, 20%.

Table (1): Formulation of tortilla bread

Ingredients (g)	Con.	CQSm		
		5%	10%	20%
Wheat Flour	100	100	100	100
Corn Flour	20	20	20	20
CQSm	-	5	10	20
Corn Oil	5	5	5	5
Salt	0.5	0.5	0.5	0.5
Baking Powder	1	1	1	1
Water (ml)	120	120	120	120

Sensory evaluation

Sensory evaluation was performed by inviting ten panelists of staff members from the Home Economics Department, Specific Education Faculty of Damietta University. Each panelist was asked to evaluate unfortified and fortified bread samples with quinoa seeds, according to color, odor, taste, texture and overall acceptability **(Abd El-Latif, 2018).**

Statistical Analysis

The obtained data was analyzed statistically for standard deviation and one way ANOVA test according to **Armitage and Berry (1987).**

Results and Discussion

Proximate chemical composition of chia and quinoa seeds

The chemical composition of chia and quinoa seed powder was presented in Table 2. Chia seed powder comprised 17.35% protein, 28.62% fat, 4.21% ash, 30.56% crude fiber, and 49.80% total carbohydrates. These results were similar to those of **El-Dreny *et al.* (2023)** who indicated that chia seed contained 30.06% fat, 19.78% protein, 31.88% crude fiber and 45.34% total carbohydrates. The same table also showed the chemical composition of quinoa seed powder, which comprised 13.82% protein, 4.12% fat, 4.82% ash, 3.37% crude fiber, and 78.69% total carbohydrates. These results were in harmony with those of **El-Kholy *et al.* (2022) & Arafa *et al.* (2024)** who revealed that quinoa seeds are distinguished by their high protein level, which ranges from 13.8% to 16.5%, their high carbohydrate content (52% to 69%), and their 10% fiber content. Thus, the findings demonstrated that quinoa and chia seeds are excellent providers of fat, crude protein, and crude fiber.

Table (2): Chemical composition of chia and quinoa seeds (on dry weight basis)

Components (g/100g)	Chia	Quinoa
Crude Protein	17.35±0.55	13.82±1.04
Fat	28.64±0.60	4.12±0.46
Ash	4.21±0.32	3.37±0.57
Total carbohydrates*	49.80±0.45	78.69±0.61
Available carbohydrates*	19.24±0.21	73.41±0.32
Crude fiber	30.56±0.58	5.28±0.35
Caloric Value (Kcal/100g)	497.72	407.12

Each value represents the mean ± SD.
* Total carbohydrates and available carbohydrates were calculated by differences

Effect of chia and quinoa seed mixes on nutritional parameters

Data presented in Table (3) revealed that, obese rats had a mean feed intake (FI) that was approximately 39.56% greater than that of the negative control group. Treating obese rats with different levels of chia and quinoa seed mixes CQSm resulted in a decrease in FI compared to the positive control group. Such data indicated that obesity induced significant ($p \leq 0.05$) increases in the mean value±SD of BWG% and PFT of the obese rats, which recorded 56.94±2.80 and 1.81±0.13, respectively, as compared to the normal control rats. The intervention with CQSm by 5, 10, and 20% led to a significant ($p \leq 0.05$) decrease on the same parameters of the obese rats when compared to the positive control group. From the previous results it could be observed that the rats fed a high-fat diet consumed more food. Consequently, they gained more weight, primarily as a result of an increase in adipose tissue mass in comparison to control rats. Further, a negative relationship was noticed between the rate of body weight gain and the increase in the concentration of CQSm intervention. The similar behavior was reported by **Ge *et al.* (2024)** who demonstrated that quinoa's dietary fiber significantly reduced body weight as compared to rats in the model group that were obese. **Omran *et al.* (2023)** who reported that use of chia and quinoa extracts as nutraceutical supplements could promote weight wellness, alleviate its related metabolic disorders, and decrease the atherogenic. **El_Dashlouty *et al.* (2019)** who involved that eating quinoa seeds led to a decrease in weight gain and feed intake. **Ali (2019)** who reported that rats fed on quinoa powder at a ratio 5 and 10% showed significant reduction in body weight gain and feed efficiency ratio compared with other research groups. The current data with the others proved that positive roles of chia and quinoa seeds in the control of obesity could be attributed to their high-level content of soluble and insoluble dietary fibers, which reduces grain digestion and helps promote fullness; beside several classes of bioactive compounds in both chia and quinoa seeds including polyphenols, flavonoids, saponins, phytosterols, phenols and biologically active peptides.

Additionally, the mean value±SD of liver and kidney weight/body weight% of obesity rats increased significantly ($p<0.05$), as compared to healthy rats fed on basal diet. While, the group of obese rats which treated with 20% CQSm recorded the highest decrease in the mean values of the liver and kidney weight/body weight% as compared to the positive control group. Nevertheless, there was no significant difference between them and the negative control group.

Table (3): Effect of CQSm on FI, BWG%, PFT and Organs weight relative on obese rats

Parameters Groups	FI (g/day)	BWG %	PFT (mm)	Organs weight/body weight %	
				Kidney	Liver
CN (-)	17.06±0.77[e]	28.50±1.50[e]	0.60±0.05[e]	1.34±0.10[b]	5.13±0.24[c]
CP (+)	28.23±1.96[a]	56.94±2.80[a]	1.81±0.13[a]	2.16±0.07[a]	7.50±0.28[a]
5% CQSm	25.58±0.76[b]	50.34±1.64[b]	1.63±0.07[b]	2.07±0.06[a]	7.32±0.37[a]
10% CQSm	22.12±0.67[c]	43.11±2.44[c]	1.34±0.05[c]	1.85±0.10[a]	6.31±0.48[b]
20% CQSm	19.43±0.75[d]	37.58±2.20[d]	0.93±0.09[d]	1.5±0.16[b]	5.46±0.30[c]

FI: feed intake, BWG: body weight gain, PFT: peritoneal fat thickness, CQSm: Chia and Quinoa seed mixes.

Values in each column which have different litters are significant different ($p\leq0.05$).

Effect of chia and quinoa seed mixes on serum glucose and leptin hormone.

Data presented in Table (4) showed that, the mean value±SD of serum leptin hormone in the positive control group increased significantly ($p\leq0.05$) as compared to the negative control group by increasing about 102.4%. Rats fed with HFD and receiving the two different levels of CQSm (10% and 20%) had a significant reduction in serum leptin hormone when compared to the positive control group. The highest reduction was observed in the group fed with a high-fat diet containing 20% CQSm by about 42.29% as compared to the positive control group. On the other hand, the same table observed that, serum glucose increased in the positive control group by about 138.7% compared to the negative control group. The increase in serum glucose may suggest disrupted carbohydrate metabolism due to enhanced breakdown of liver glycogen. Results also showed that addition of CQSm with different ratios to the DIO of rats resulted in a significant reduction ($p \leq 0.05$) in values of serum glucose than that of the positive control group.

Table (4): Effect of CQSm on fasting plasma glucose and leptin hormone of obese rats

Parameters / Group	Serum Leptin ng/ml	Serum Glucose mg/dl
Control (-)	3.75±0.49[d]	89.27±1.48[e]
Control (+)	7.59±0.64[a]	213.07±7.90[a]
5% CQSm	7.17±0.53[a]	176.05±7.08[b]
10% CQSm	5.94±0.72[b]	158.66±3.06[c]
20% CQSm	4.38±0.57[c]	112.73±14.53[d]

CQSm: chia and quinoa seed mixes

Values in each column which have different litters are significant different ($p \leq 0.05$).

Carbohydrate-containing foods are an essential component of a balanced diet. Glucose, produced by the body from carbohydrates, is required to support both physical activity and internal processes. However, the quality of the carbohydrates is important; some types of carbohydrate-rich foods are better than others. **Espinosa-Salas & Gonzalez-Arias (2023)** indicated that carbs that contain abundant amounts of fiber, such as whole grains, brown rice, oats, and quinoa seeds, are the healthiest sources of carbohydrates. While the intake of white flour and white sugar causes the secretion of large amounts of insulin, which causes the body to become resistant to leptin. According to **Arafa *et al.* (2024)** white bread has a high glycemic index because it contains refined grains that are quickly absorbed during digestion, leading to sudden rises in insulin and blood sugar levels. This raises the risk of heart disease, type 2 diabetes, and weight gain. Concerning leptin, **Zieba *et al.* (2020)** indicated that leptin is an appetite-regulating hormone secreted by fat cells. The quantity of leptin produced in an organism is correlated with the size and number of adipocytes and, of course, by the lipid tissue mass. The action of leptin is in accordance with the neuropeptide Y, which signals the brain to increase appetite and make the animal eat. then, obesity causes an overabundance of leptin to be released, ultimately resulting in leptin resistance.

In the present study, when the animals lost weight, the mass of adipose tissue was diminished, resulting in a decrease in the leptin concentration in the blood. Therefore, it could be observed that obese rats consuming chia and quinoa seed mixes may have a hypoglycemic effect and produce the greatest decreases in cholesterol, HDL, and LDL levels. In similar studies, **Akkuş *et al.* (2023)** demonstrated that the efficacy of chia seeds in glycemic control, increased satiety, and reduced hunger, primarily attributed to their soluble fiber content. The effect of consuming soluble fiber on the feeling of satiety is attributed to its ability to form a gel-like structure in the stomach during digestion, leading to increased gastric distension and triggering satiety signals. **Omran *et al.* (2023)** revealed that treatment with chia and quinoa seed extracts reduced leptin levels by 67.2% (quinoa) and 54.26% (chia), and they showed hepatoprotective effects and anti-inflammatory and antioxidant activities and modulated leptin, adiponectin, serum lipid, and glycemic profiles. **Sosa-Crespo *et al.* (2021)** indicated that healthy subjects and subjects with prediabetes or T2DM had lower plasma glucose values after 30–60 min of ingestion of chia seeds. Also, **(Karakas *et al.*, 2016)** explained that fibers content of chia seeds increases stomach and small intestine viscosity, delaying gastric emptying and prolonged gastrointestinal transit time. Consequently, the absorption of carbohydrates is delayed, resulting in reduced glycemic response, delayed insulin release, and increased satiety. Moreover, lignan, one of the three primary phytoestrogens found in chia seeds, aids in glycemic control by suppressing the expression of the phosphoenolpyruvate carboxykinase (PEPCK) gene responsible for glucose production through gluconeogenesis, thus inhibiting glucose production. Furthermore, the alpha-linolenic acid (ALA) content of chia seeds contributes to glycemic control through improved insulin sensitivity.

Effect of chia and quinoa seed mixes on serum lipids profile concentration.

The effect of chia and quinoa seed mixes CQSm on the serum lipid profile of obese rats was shown in Table (5). From such data, it could be noticed that obesity induced an increase in the mean values of TC (213.81±16.93), TG (183.17±8.60), LDL (158.32±17.33), and VLDL (36.64±1.72) while it caused a decrease in the mean value of HDL (18.85±0.85) compared to negative controls. Replacement of diet with different ratios of CQSm induced improvements on blood lipid profile through decreasing the TC, TG, LDL and VLDL, while the opposite direction was observed for the HDL levels. The higher effects in improving the serum lipid profile disorders induced by obesity in rats were recorded for the 20% of CQSm.

On important risk factors such as obesity and diabetes mellitus, the atherogenic index and coronary risk index are often calculated to estimate CHD risk. As presented in the same table (5), LDL-C/HDL-C and TC/HDL-C ratios were calculated. These ratios significantly increased for the positive control group in comparison with the negative control group. Treating obese rats with a basal diet containing various levels of CQSm had lower mean values on LDL-C/HDL-C and TC/HDL-C than that of the PC group. Especially the mixture of chia and quinoa seed (with high level), which showed a significant decrease (P<0.05) in the mean values of LDL-C/HDL-C and TC/HDL-C as compared to other treated groups.

Table (5): The effect of CQSm on lipoproteins in the serum of obese rats

Parameters / Groups	TC	TG	HDL-c	LDL-c	VLDL-c	AI LDL-c/HDL-c	CRI TC/HDL-c
	mg/dl						
CN (-)	76.97±4.602[e]	59.54±3.07[d]	52.86±3.50[a]	12.20±5.33[e]	11.90±0.62[d]	0.23±0.10[e]	1.46±0.12[e]
CP (+)	213.81±16.93[a]	183.17±8.60[a]	18.85±0.85[d]	158.32±17.33[a]	36.64±1.72[a]	8.40±0.89[a]	11.35±0.89[a]
5% CQSm	193.45±7.96[b]	177.49±10.30[a]	22.30±2.30[d]	135.65±9.75[b]	35.50±2.06[a]	6.16±1.03[b]	8.76±1.12[b]
10% CQSm	172.09±8.96[c]	144.14±13.93[b]	35.92±3.77[c]	107.34±18.47[c]	28.83±2.78[b]	3.02±0.65[c]	4.84±0.76[c]
20% CQSm	117.50±10.54[d]	113.91±6.47[c]	40.50±3.42[b]	54.20±12.71[d]	22.79±1.29[c]	1.36±0.41[d]	2.93±0.44[d]

TC: Total Cholesterol, TG: Triglyceride, LDL-c: Low Density Lipoprotein Cholesterol, HDL-c: High Density Lipoprotein Cholesterol, VLDL-c: Very Low-Density Lipoprotein Cholesterol, CQSm: chia and quinoa seed mixes, AI: atherogenic index, CRI: coronary risk index. Values in each column which have different litters are significant different (p≤0.05).

Many studies have demonstrated that high blood levels of TC and LDL are great risk factors for CHD. The way that blood lipid and lipoprotein concentrations are managed is significantly impacted by the composition of the human diet. Dietary fiber plays a critical role in overall body health; it contributes to colonic health, coronary artery health, cholesterol reduction, glucose metabolism, insulin response, blood lipids, cancer etc. **(Arafa and Elmaadawy, 2015; Arafa and Mahran, 2018 and Elhassaneen *et al.,* 2019).** According to the present study, quinoa and chia may be added to meals like bread to increase their fiber content and provide them with superior organoleptic qualities. Being that the compounds found in quinoa seeds, including squalene and saponins, may help explain their hypocholesterolemic effects. On the other hand, the water-holding capabilities of chia seeds' dietary insoluble fiber enhance feelings of satiety **(Alfredo *et al.*, 2009)** Moreover, its viscous and soluble fibers have demonstrated a cholesterol-lowering effect by influencing hepatic lipid metabolism **(Riccioni *et al.*, 2012),** this effect is achieved by enhanced bile acid loss, diminishing the absorption of cholesterol and other circulating lipids, and impeding hepatic free fatty acid synthesis **(Ullah *et al.*, 2016).** In addition, functional proteins and bioactive peptides of chia inhibit 3-hydroxy-3-methylglutaryl coenzyme reductase, a regulatory enzyme in cholesterol synthesis, thus reducing cholesterol synthesis (**Coelho *et al.*, 2018).** Also, recent studies confirmed that chia seeds show cardioprotective, hypotensive, antidiabetic, antioxidant, anti-inflammatory, hypolipemic, neuroprotective, hepatoprotective and immunostimulatory properties **(Urrutia *et al.*, 2020; Rabail *et al.*, 2021; Motyka *et al.*, 2023);** their beneficial effect on improving lipid profiles may be due to their high content of α-linolenic acid (ALA), which is associated with beneficial changes in plasma lipids **(El-Dreny *et al.*, 2023)**. Moreover, **(Cao *et al.*, 2020; Fotschki *et al.*, 2020; Ge *et al.*, 2024)** found that anti-hyperlipidemia benefits in rats treated with a high-fat diet after eating quinoa for 8 weeks. Quinoa has a high fiber content, which binds to bile acid and increases cholesterol degradation. The fermentation of fiber in the colon produces short-chain fatty acids and reduces cholesterol synthesis in the liver. Overall, the results of previous studies demonstrated that the presence of sterols in plants inhibits the body's absorption of cholesterol.

Effect of chia and quinoa seed mixes on liver enzyme activity.

As indicated by data shown in Table (6), rats fed a diet that caused obesity raised the activity of the ALT, AST and ALP enzymes by approximately 143.1%, 102.5%, and 66.4% in the positive control group compared to the negative control group. According to data from this study and others, obesity in humans and experimental animals can result in considerably raised aminotransferases in addition to non-hepatic diseases. However, obesity groups treated with different ratios of CQSm recorded a significant decrease ($p \leq 0.05$) in serum ALT, AST, and ALP enzyme activity as compared to the positive control group.

Table (6): Effect of CQSm on liver enzyme activity in obese rats

Parameters / Groups	ALT	AST	ALP
	mg/dl		
CN (-)	22.93±2.36[e]	34.86±3.38[e]	86.34±4.20[e]
CP (+)	55.74±5.71[a]	70.60±4.066[a]	143.64±5.42[a]
5% CQSm	43.32±2.72[b]	58.73±3.71[b]	136.01±5.76[b]
10% CQSm	38.35±2.81[c]	53.42±2.68[c]	123.26±4.33[c]
20% CQSm	32.62±2.07[d]	44.04±4.11[d]	98.63±1.06[d]

ALT: alanine amino transferase, AST: aspartate amino transferase, ALP: alkaline phosphatase,

CQSm: chia and quinoa seed mixes. Values in each column which have different litters are significant different (p≤0.05).

When adipose tissue reaches the upper limit of lipid storage capacity in obese people, excessive lipids will be redirected to other organs, most notably the liver. Consequently, it causes dysfunction in the liver. On the other hand, elevated levels of alkaline phosphatase (ALP) and aminotransferases (ALT and AST), which are typically intracellular enzymes, in plasma are indicative of damage to cells that are abundant in these enzymes. According to the study's results, seeds of chia and quinoa enhance the activity of hepatic aminotransferases and ALP, which in turn improves liver functioning in obese rats. Such data are in accordance with those observed by **Alamri, (2019)** who observed that the groups fed with chia seeds had significantly lower levels of liver enzymes (AST and ALT) compared to the control groups; therefore, chia seeds were effective in improving liver function and public health. Also, **Abdel-Wahhab *et al*., 2021; Zhong *et al*., 2023** showed that a long-term high-fat diet causes hepatic steatosis, which further leads to oxidative stress and inflammation; quinoa contains betacyanins, rutin, quercetin, and other flavonoids that have been associated with anti-inflammatory and antioxidant properties, and its consumption considerably reduced splenomegaly and hepatomegaly and improved the pathological state of hepatic steatosis.

Effect of chia and quinoa seed mixes on kidney function.

Data in Table (7) indicate that obesity resulted in elevated mean values of U. acid (3.84±0.63), BUN (41.18±3.45), and creatinine (2.96±0.37) when compared to the negative control group. All treated groups receiving diets with three levels of CQSm exhibited a significant reduction in the mean serum levels of uric acid, BUN, and creatinine (p≤0.05) compared to the positive control group (untreated group). It could be observed that the group of obese rats that received 20% of chia and quinoa seed mixes had no significant difference in serum U. acid compared to the negative control group.

Table (7): Effect of CQSm on kidney function of obese rats

Parameters / Groups	U.acid	BUN	Creatinine
	mg/dl		
CN (-)	1.57±0.15[d]	17.21±1.34[e]	0.65±0.04[e]
CP (+)	3.84±0.63[a]	41.18±3.45[a]	2.96±0.37[a]
5% CQSm	2.73±0.29[b]	37.19±3.37[b]	2.50±0.34[b]
10% CQSm	2.19±0.37[c]	26.80±2.45[c]	1.56±0.21[c]
20% CQSm	1.80±0.26[cd]	21.58±1.71[d]	1.22±0.19[d]

CQSm: chia and quinoa seed mixes

Values in each column which have different litters are significant different ($p \leq 0.05$).

In vitro studies showed that dry chia seed extract prevented the formation of calcium oxalate stones by inhibiting the process at the initial stages, including nucleation, aggregation, and growth phases. Research showed that flavonoids, mainly quercetin, may be responsible for this activity, which mainly prevents renal stone precipitation through its strong antioxidant potential. These findings are consistent with **Saleem *et al.* (2020),** who indicated that the experimental group receiving dry chia seed extract showed reduced serum levels of total protein, creatinine, urea, and uric acid as compared to the control group. **Arafa and Elseedy (2016)** reported that non-significant changes in kidney functions among groups fed quinoa compared to the control negative group.

Effect of chia and quinoa seed mixes on antioxidant enzymes activity.

From the data in table (8), it could be noticed that rats fed with a high-fat diet had a significant reduction ($p \leq 0.05$) in the mean values of SOD, GPX, and CAT activities; these parameters decreased by about 65.49%, 78.78%, and 74.68%, respectively, in the positive group as compared to the negative control group. All obese groups receiving diets supplemented with 5, 10, and 20% of CQSm had a significant increase in antioxidant enzyme concentration as compared to the positive control group.

Table (8): The effect of CQSm on antioxidants enzymes activity of obese rats

Parameters / Groups	SOD %	Catalase (nm/ml/min)	GPX (U/ml)
CN (-)	63.57±2.29[a]	1.68±0.15[a]	3.49±0.22[a]
CP (+)	33.00±2.93[d]	0.43±0.07[e]	0.84±0.08[e]
5% CQSm	43.62±5.84[c]	0.82±0.07[d]	1.19±0.27[d]
10% CQSm	47.54±4.55[c]	1.12±0.17[c]	2.29±0.36[c]
20% CQSm	55.78±4.41[b]	1.38±0.14[b]	2.81±0.43[b]

SOD: Super Oxide dismutase, GPX: Glutathione Peroxidase, CAT: Catalase, CQSm: chia and quinoa seed mixes

Values in each column which have different litters are significant different ($p \leq 0.05$).

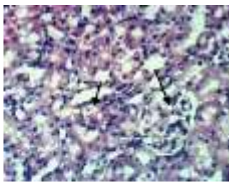
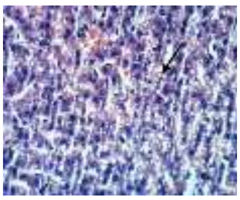
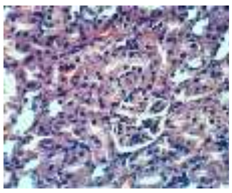
Adipocytes are not only lipid storage but also secretory cells that produce proinflammatory cytokines and adipokines that drive a chronic low-grade inflammatory state **(Alarcon *et al*.,2020).** In addition, obesity can increase the systemic oxidative stress through biochemical mechanisms, such as superoxide generation from NADPH oxidases (nicotinamide adenine dinucleotide phosphate oxidases), oxidative phosphorylation, and protein kinase C activation. Moreover, hyperleptinemia, low antioxidant defense, chronic inflammation, and postprandial reactive oxygen species generation may contribute to obesity-oxidative stress. In turn, oxidative stress could trigger obesity by stimulating the deposition of white adipose tissue and increasing preadipocyte proliferation (**Segura-Campos *et al*.,2013). Creus *et al.* (2020)** demonstrated that the addition of chia seeds in the diet decreased the SOD activity, improved the CAT activity, and increased the level of manganese superoxide dismutase (MnSOD), which is an enzyme that neutralizes oxygen-free radicals.

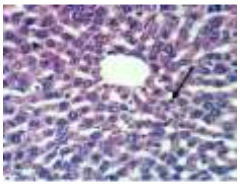
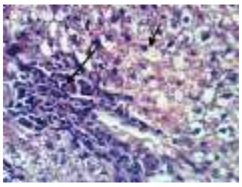
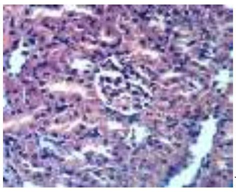
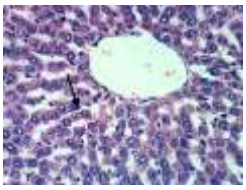
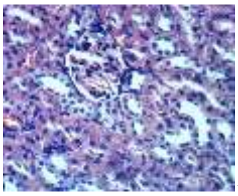
The present study observed that the mean values of serum antioxidant enzyme activity increased gradually with increasing levels of chia and quinoa seeds. All of these improvements could be principally attributed to the strong antioxidant activities of chia and quinoa seeds. These outcomes could be attributed to the high nutritional content of quinoa, which is a great source of bioactive compounds such as flavonoids, which improve the biological functions for their antioxidant properties. The tocopherol content of quinoa is essential and acts as an antioxidant at the level of the cell membrane, defending the membrane's fatty acids against oxidative stress. Such as mentioned by **Vilcacundo *et***

al. **(2017); liu *et al.* (2021) and Zhong *et al.* (2023).** The antioxidant activity of chia seeds is related to their significant content of EFAs, polyphenolic compounds, and proteins **(Sargi *et al.*, 2013).** Polyphenols responsible for the antioxidant activity of chia seeds are mainly flavonoids and cinnamic acid derivatives. The total content of phenolic compounds in chia is about 97.7 mg total phenols (GAE)/100 g **(Beltrán-Orozco *et al.*, 2020).**

Histopathological Examination

Photo (1) & (2) showed the histopathological examination of liver and kidneys tissue. Microscopically, the liver and kidneys of rats from the control negative group which fed on basal diet showed the normal histological structure of both hepatic lobule and renal parenchyma in (Photo 1A & 2A, respectively). While significant changes were observed in the liver and kidneys of obese rats from the positive control group, like pronounced hepatic steatosis, infiltration of inflammatory cells, degeneration of some renal areas, and shrinking of glomelular structures (Photos 1B & 2B respectively), as compared to the negative group. Meanwhile, obese rats that received a basal diet containing different ratios of CQSm showed gradual improvements in both liver tissue (Photos 1C, 1D & 1E) and kidney tissue (Photos 2C, 2D & 2E).

Photo (1): Effect of CQSm on histological examination of liver tissue	**Photo (2): Effect of CQSm on histological examination of kidneys tissue**
 **Photo (1A):Control-** Normal histological structure of hepatic lobule (H & E X 400).	 **Photo (2A):Control +** Normal histological structure renal parenchyma (H & E X 400).
 **Photo (1B):Control+** Pronounced hepatic steatosis and infiltration of inflammatory cells (H & E X 400).	 **Photo (2B):Control +** Degeneration of some renal areas, and shrinking of glomelular structures (H & E X 400).

	**Photo (1C): Group1** Some inflammatory cells (H & E X 400).		**Photo (2C): Group1** Congestion of renal blood vessels (H & E X 400)
	**Photo (1D): Group2** No changes except slight activation of Kupffer cells (H & E X 400).		**Photo (2D): Group2** No histopathologica l alternation (H & E X 400).
	**Photo (1E): Group3** No histopathologica l alternation (H & E X 400).		**Photo (2E): Group3** no histopathologica l alternation (H & E X 400).

Histopathological examination of liver and Kidney

Sensory evaluation of tortela bread fortified with different levels of mixes chia and quinoa seed

The bread may be prepared in a variety of thicknesses, from a few millimeters like a tortilla to a few centimeters like an Arabic flat bread, and it is quite simple to prepare because it only requires a few basic components. Data in table (9) showed that the mean (value ± SD) of the color, taste, odor, texture, general acceptability, and total scores were 19.82±0.42, 19.22±0.83, 19.31±0.31, 19.87±0.30 and 19.53±0.28, respectively, in the control (unfortified tortilla bread). Tortilla bread supplemented with 5%, 10%, and 20% of CQSm resulted in slightly significant changes in all sensory characteristics (color, odor, texture, taste, and general acceptability) as compared to unsupplemented tortilla bread (control). In general, results from the total score indicated that all samples obtained a score higher than 75%.

Table (9): Sensory evaluation of tortilla bread supplemented with CQSm

Sensory Characteristics	Control 0%	Tortela bread with CQSm %		
		5	10	20
Color (20)	19.82[a] ±0.42	19.20[a] ±0.76	18.73[b] ±0.37	18.41[b] ±0.54
Taste (20)	19.22[a] ±0.83	18.65[ab] ±0.67	18.21[b] ±0.60	16.19[c] ±0.42
Odor (20)	19.31[a] ±0.31	18.47[a] ±0.62	17.82[b] ±0.36	16.57[c] ±0.47
Texture (20)	19.87[a] ±0.30	19.25[a] ±0.35	18.27[a] ±0.78	18.35[a] ±0.85
General Acceptance (20)	19.53[a] ±0.28	19.36[a] ±0.28	18.35[b] ±0.52	18.00[c] ±0.32
Total Score (100)	97.75[a] ±0.81	94.73[b] ±0.47	91.08[c] ±1.55	87.82[d] ±1.58

CQSm: Chia and quinoa seeds mixes, Values in each column which have different litters are significant different ($p \leq 0.05$).

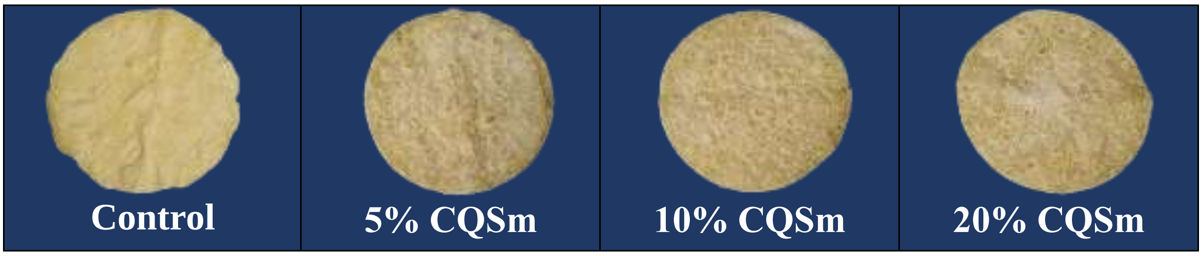


Photo (3): Tortilla bread supplemented with CQSm

Conclusion

The present study demonstrated that chia and quinoa seeds serve as an important source of many therapeutically efficient chemicals. In accordance with biological investigations, the model obese rats' body weight significantly decreased after receiving chia and quinoa seed mixes, and their lipid profile and glucose homeostasis improved as well. Additionally, they reduce oxidative stress in the body and preserve the safety of liver and kidney functions. Therefore, chia and quinoa seed mixes can be integrated up to 20 percent into functional foods with high nutritional and health benefits for obese people.

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