The Potential Effects of Quinoa Seeds on the Biochemical, Nutritional and Histological Parameters on Obese Rats

التأثيرات المحتملة لبذور الكينوا على المقاييس الحيوية والغذائية والهستولوجية لدى الفئران البدينة

Prof. Dr. Rasha Mahmoud Arafa

Department of Home Economics, Faculty of Specific Education Damietta University, Damietta, Egypt.

Noha Khaled Mohamed Taher Mahmoud

Department of Home Economics, Faculty of Specific Education Damietta University, Damietta, Egypt.

Dr .Heba Mostafa El Kholey.

Department of Home Economics, Faculty of Specific Education Damietta University, Damietta, Egypt.

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Rasha M. Arafa *, Noha Khaled Mohamed Taher and Heba, M. El Kholey

Department of Home Economics, Faculty of Specific Education, Damietta University, Damietta, Egypt.

*Corresponding author: rarafa@du.edu.eg

ABSTRACT

Quinoa (Chenopodium quinoa Wild.) is a pseudo-grain that belongs to the amaranth family and has gained attention due to its nutritional properties and many health benefits to the human body. Therefore, this study was conducted to evaluate the effect of different proportions of cooked quinoa seeds on feed intake, body weight gain%, organ weights/body weight%, liver enzyme activity, lipid profile, kidney functions, serum glucose, leptin hormone, and antioxidant enzymes of rats suffering from obesity. Thirty male albino rats weighing (120 \pm 10 grams) 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 highfat diet for 8 weeks, and then this group was divided into four subgroups. One of these groups was fed a high-fat diet only, and obese rats were used as a positive control group. The rest of the groups were fed a high-fat diet containing 10, 15, and 20 % of cooked quinoa seeds (CQS) for 30 days. Results showed that the proximate chemical composition of CQS contained 8.57, 14.61, 4.76, 3.34, 5.12, and 63.60 g/100g for moisture, total protein, fat, ash, crude fiber, and carbohydrate, respectively. The biological results indicated that feeding rats diets containing CQS resulted in significant improvements in percentage gain in body weight, organ weight, average feed intake, blood glucose, kidney functions, liver enzymes, blood fats, the hormone leptin, and the percentage of oxidative stress. In addition, histological examination of the liver and kidneys of rats in groups treated with different levels of CQS showed a noticeable improvement in the tissue structure of these organs. Wholesome bread was produced with proportions of 10, 15, and 20% of CQS. Sensory evaluation indicated that all wholesome bread samples had an acceptance greater than 75%. In conclusion, the current study indicates that CQS has improved biomarkers in obese rats, and we recommend using cooked quinoa seeds as an addition in the field of functional foods. Key words: Functional Food - Glucose - Kidney Functions - Liver Enzymes - Lipid Profile quinoa- Leptin- oxidation.

Introduction

Obesity is a condition of excessive fat accumulation, which affects the health of the individual. It is a complex multifactorial disease and is associated with an increased risk of many diseases. Obesity has become increasingly prevalent worldwide not only as a major driver of non-communicable diseases but also as a disease itself (Zeng Q et al.,2021). Obesity occurs as a result of the interaction of many factors such as diet, environment, and genetics, which is often exacerbated by the modern lifestyle and lack of movement (Reilly, 2017). Numerous studies have shown that obesity is an important factor contributing to the development of chronic and serious diseases, such as type 2 diabetes, cardiovascular disease, stroke, heart failure, high blood pressure, dyslipidemia, uric acidosis, cancer and sleep apnea that causes Sudden death. Many studies indicate that weight loss is the primary path to treating obesity, and taking 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).

Egypt ranks 18th with the highest prevalence of obesity worldwide according to the World Health Organization (WHO). The estimated annual deaths due to obesity was 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). The consumption of functional foods with desirable properties has become a subject of increasing interest as they contain bioactive nutritional components that help reduce diseases complications associated with poor diet and physical inactivity due to obesity prevalence (Little *et al.*, 2021).

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 grown recently successfully in Egypt, providing seeds rich in nutrients and bioactive compounds (**Barakat** *et al.*, **2017**). 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**). Considering the above, the present study investigated the possibility of using quinoa seeds in the strategy of obesity disease treatment and its complications, as well as the effect of quinoa seeds on the biochemical, nutritional, and histological parameters in experimentally obese rats. Also, evaluating the sensory characteristics of wholesome bread supplemented with CQS.

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.
- Bread manufacturing components: Wheat flour, dry yeast, salt, sugar, skim milk powder, corn oil obtained from the local market of Damietta; Damietta Governorate, Egypt.
- Quinoa seeds were obtained from the Agricultural Seeds Company and the Natural Plants Company in Cairo, Egypt.

Animals: rats used in this study, adult male albino rats (Sprague Dawley strain) weighing (120±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 washed before heat treatment to reduce the saponin content. In washing, grains were exposed to manual rubbing in running water for 15 minutes. The grains were soaked in water for 30 minutes, filtered to get rid of the water, and then dried in a hot air oven at 55 °C for 14. After processing, grains were ground and stored at 20°C until analysis. This method was described according to **Nickel** *et al.*, (2016).

Chemical analysis

Quinoa seeds were analyzed for moisture, ash, crude fiber, fat, and crude protein, as determined as described in **A.O.A.C.** (2005), while total carbohydrates were calculated by the differences: CHO% = 100 - (moisture + fat + protein + crude fiber + ash). The energy values were calculated theoretically according to the method described by **James** (1995).

Experimental design

All biological experiments performed a complied with the rulings of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council (NRC, 2011). Thirty male albino rats weighing (120 \pm 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 8 weeks to induce obesity. This group was divided into four subgroups as follows: subgroups (1) fed on (HFD) as a positive control group; subgroups (2) fed on (HFD) diet containing 10% CQS; subgroups (3): fed on (HFD) diet containing 15% CQS; subgroups (4): fed on (HFD) diet containing 20% CQS.

During the experiment period (30 days), the quantities of diet, which were consumed and / or wasted, were recorded every day. In addition, rat's weight was recorded weekly, to determine feed intake and body weight gain %, 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. Total Cholesterol and Triglycerides were determined in the serum according to the method 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 creatinin ewere determined in the serum according to the method 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 method described by Reitman and Frankel (1957); Bergmeyer et al., (1974), respectively. Serum glucose was determined by Trinder (1969). Catalase, Glutathione Peroxidase (GPX) and Superoxide dismutase (SOD) were determined in the serum according to the method described by Aebi (1974); Mc Cord and Fridovich (1969), respectively. leptin was determined in the serum according to the method 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 weighted to calculate organs to body weight percentage. The kidneys and liver in each group were examined histopathologically, according to Sheehan and Hrapchak (1980).

Formulation and preparation of wholesome bread

Wholesome quinoa bread was prepared by using the **Isik and Topkaya** (2016) method with a little modification. The control and the other formulations

are presented in Table (1). In a mixing bowl, the dry and liquid ingredients were mixed for 3–4 minutes to form the dough, then left to rest for 10 minutes. The dough was rolled out as thin as possible, no thicker than 1/8 inch, and cut out as square. Wholesome quinoa bread was baked in an electric oven at 200 °C for 10 minutes. After baking, wholesome quinoa bread was left in the oven for an additional 2 minutes with the heat off but with forced air circulation. This process simulated the drying and cooling stages of a commercial baking oven. Baked, wholesome quinoa bread was then removed from the oven and allowed to cool down to room temperature. Wholesome quinoa bread samples were stored in airtight containers before sensory evaluation. The other experimental formulations were prepared by adding powder from CQS at 10, 15, and 20 %.

Ingredients (g)	Con.	10% CQS	15% CQS	20% CQS
wheat flour	500	450	425	400
CQS	-	50	75	100
corn oil	75	75	75	75
Sugar	5	5	5	5
Salt	5.5	5.5	5.5	5.5
baking powder	5	5	5	5
Water	200	200	200	200

 Table (1): Formulation of wholesome quinoa bread

Sensory evaluation

Sensory evaluation was performed by invited ten panelists of staff members from 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 cooked quinoa seeds

The content analysis of cooked quinoa seeds (CQS) is presented in table (2). The moisture, total protein, fat, ash, crude fibre and carbohydrate contents in percentage terms for CQS were 8.57, 14.61, 4.76, 3.34, 5.12, and 63.60, respectively. Quinoa has a comparable total energy content of 355.68 kcal/100g, as shown in the same table.

Components (g/100g)	Ingredients
Moisture	8.57±0.09
Crude Protein	14.61±0.46
Fat	4.76±0.08
Ash	3.34±0.18
Crude fiber	5.12±0.25
Carbohydrate*	63.60
Caloric Value (Kcal/100g)	355.68

Fable (2):	Gross	chemical	com	position	and	nutritive	values	of	CQ)S
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Each value represents the mean \pm SD.

* Total carbohydrates were calculated by differences

From these data, it was revealed that quinoa is a rich source of crude protein, crude fibre and carbohydrate. In similar previous studies, **Arafa and Elseedy (2016), Sezgin and Sanlier (2019) and Villacrés** *et al.* (2022) found that quinoa is characterized by its high protein content, which varies between 13.8% and 16.5%, carbohydrates (52%–69%) and 10% fibre. Quinoa protein can supply human with essential amino acids and the sulfur-containing amino acids. The soluble fibre in quinoa helps lower cholesterol and promotes the growth of healthy bacteria in the gut, while its insoluble fibre prevents constipation. Besides that, quinoa has a high percent of carbohydrate; most of these carbs come from complex carbohydrates, like starch. These digest slowly, enter the bloodstream gradually, and don't cause harmful blood sugar spikes.

Effect of CQS on nutritional parameters of obese rats

Data presented in Table (3) revealed that, Rats with diet-induced obesity DIO had a significantly higher the mean of feed intake by about 32.8% than the negative control group. Treating DIO rats with different levels of cooked quinoa seeds resulted in a decrease in feed intake when compared to the positive control group. The highest reduction in feed intake was observed in the group fed with a diet containing 20% CQS.

The results show that the positive control group had the highest body weight gain (BWG%) and feed efficiency ratio (FER) compared to the negative control group. On the other hand, the lowest BWG% and FER were recorded for the groups that were fed with 20% CQS, and there were significant differences compared to the positive group. In the case of the peritoneal fat thickness (PFT), it could be noticed that the PFT of the positive control group recorded the highest value when compared with the negative control group. The lowest PFT was recorded for the group that was fed with 20% CQS, with a significant difference (P \leq 0.05) compared with a positive group.

Parameters	FI (g/day/	BWG%	FFD	PFT	Organs body w	Organs weight / body weight %	
Groups	(g/uay/ rat)	DWG70	TEK	(mm)	Liver	Kidney	
Control (-)	18.85	37.31±2.51 ^d	1.33±0.22 ^b	0.63±0.05 ^c	4.35±0.28 ^c	1.08±0.09 ^b	
Control (+)	25.03	53.73±4.27 ^a	1.80±0.21 ^a	1.76±0.15 ^a	6.50±0.27 ^a	1.26±0.08 ^a	
10% CQS	23.04	48.38±7.64 ^b	1.60±0.36 ^{ab}	1.70±0.06 ^a	6.38±0.38 ^a	1.20±0.06 ^a	
15% CQS	21.39	43.97±2.33 ^{bc}	1.55±0.13 ^b	1.71±0.17 ^a	5.66±0.45 ^b	1.21±0.04 ^a	
20%CQS	20.63	42.48±1.79 ^c	1.40±0.15 ^b	0.90 ± 0.08^{b}	4.50±0.26 ^c	1.10±0.00 ^b	

Table (3): Effect of CQS on FI, BWG%, FER, PFT and Organs weight/body weight% on obese rats

FI: feed intake, BWG: body weight gain, FER: feed efficiency ratio, PFT: peritoneal fat thickness, CQS: Cooked Quinoa seeds. Values in each column which have different litters are significant different ($p \le 0.05$).

Furthermore, the mean value \pm 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 rats which treated with 20% CQS recorded the highest decrease in the mean values of the liver and kidney weight/body weight% as compared to the positive control group, at the same time showed no significant difference as compared to the negative control group.

Quinoa has an adequate content of dietary fiber, which decreases grain digestibility, these fibers contributes to granting satiety. Results are consistent with the study of Ge et al. (2024) who showed that the dietary fiber of quinoa showed a significant reduction in body weight compared to those in the model group of obese mice. Fotschki et al. (2020) found that the body weight of rats fed with diets containing quinoa protein-rich flour was lower than that of the control group. Moreover, El_Dashlouty et al. (2019) involved that eating quinoa seeds led to a decrease in weight gain, feed intake, feed efficiency ratio. Ali (2019) 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. Also, Halaby et al. (2017) showed that, consumption of quinoa seeds powder (QSP) plays a role in regulating energy homeostasis and maintain body weight balance. Moreover, they indicated that the Quinoa is an excellent example of functional food that aims to improved nutrient intakes and lower body weight and possibly reducing the risk of various diseases. Graf et al. (2014) found that administration of 20HE-enriched quinoa extract to animals fed a high fat diet for 3 weeks resulted in the reduction of the development of adipose tissue in mice.

Effect of CQS on fasting plasma glucose and leptin hormone of obese rates

Data presented in Table (4) showed that serum glucose increased in the positive control group by about 141.5% 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 indicated that addition of CQS to the DIO of rats resulted in a significant reduction ($p \le 0.05$) in values of serum glucose than that of the positive control group, except that group fed on DIO containing the lowest level of CQS 10% which recorded non-significant difference

Parameters Group	Serum Glucose mg/dl	Serum Leptin ng/ml
Control (-)	$87.85{\pm}1.25^{d}$	4.03±0.314 ^d
Control (+)	212.23±7.46 ^a	$7.38{\pm}0.598^{a}$
10% CQS	204.85±7.94 ^a	7.13±0.631 ^a
15% CQS	177.11 ± 7.00^{b}	6.05 ± 0.926^{b}
20% CQS	$115.21 \pm 6.36^{\circ}$	4.81±0.515 ^c

 Table (4): Effect of cooked quinoa seeds CQS on fasting plasma glucose and leptin

 hormone of obese rates

CQS: Cooked Quinoa seeds

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

Data in the same table (4) observed that, the mean value \pm SD of serum leptin hormone in the positive control group increased significantly (p \leq 0.05) as compared to the negative control group by increasing about 83.12%. Rats fed with HFD (group II) and receiving the two different levels of cooked quinoa seeds (15% 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% CQS by about 34.82% as compared to the positive control group.

Foods that contain carbohydrates are an important part of a healthy diet. Carbohydrates provide the body with glucose, which is converted into energy used to support bodily functions and physical activity. But carbohydrate quality is important; some types of carbohydrate-rich foods are better than others. Carbohydrates 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. Arafa and Elseedy (2016) reported that white bread has a high glycemic index because it's made from refined grains that are rapidly absorbed during digestion, causing sharp spikes in blood sugar and insulin levels. A diet that includes a lot

of white bread and other high-glycemic foods increases the risk of weight gain, type 2 diabetes, and heart disease. When blood sugar increases, it causes many problems. This is a pro-inflammatory process that plays a role in a host of inflammatory diseases, including cataracts and heart disease. So, carbohydrates from quinoa can be considered a nutraceutical food because they have beneficial hypoglycemic effects and induce the lowering of free fatty acids.

In similar studies, **Ge** *et al.* (2024) revealed that the dietary fiber of quinoa showed a significant reduction in blood glucose compared to those in the model group of obese mice. Also, **Zeng** *et al.* (2023) showed that adding quinoa to the staple diet can reduce postprandial blood glucose, improve fat metabolism and insulin resistance, delaying the development of diabetes in people with disabled glucose tolerance. Alamri *et al.* (2023) stated that, eating quinoa seeds had a hypoglycemic effect and caused the best changes regarding cholesterol, HDL and LDL levels among diabetic rats.

Also, Marques *et al.* (2016) showed that rats fed with high-fat diet consumed higher amounts of food and, therefore, higher amounts of energy compared to control rats fed with the same diet. Consequently, weight gain was larger in these animals and was mainly due to an expansion of adipose tissue mass. On another study; **de Lartigue** *et al.* (2014) showed that plasma leptin concentration increases in proportion to body fat mass. As a result, rats fed a high-fat diet displayed higher leptin plasma levels than control rats on the same diet. In addition, the amount of leptin released by each gram of body fat mass (plasma leptin to body fat mass ratio) was also more elevated in control rats.

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. When the animals lose weight, the mass of adipose tissue is diminished, resulting in a decrease in the leptin concentration in the blood (Ahmadi *et al.*, 2016 and Barrios-Correa *et al.*, 2018).

On the other hand, **Zieba** *et al.*, (2020) showed that in obesity, an excess of leptin is secreted, which eventually leads to leptin resistance. When cells in the hypothalamus become resistant to leptin, the signal for satiety is not received, and the person remains hungry. Leptin levels decrease with food deprivation and increase after eating (fasting for a short time). Blood leptin is also correlated with other characteristics, such as stress, physical activity, sleep duration, insulin concentration, obesity, and diabetes. Also, the current study's findings are consistent with **Omran** *et al.* (2023) who showed that treatment with the quinoa seed extracts reduced leptin levels by 67.2%.

Effect of CQS on serum lipids profile concentration of obese rats

Data presented in Table (5) revealed that rats with diet-induced obesity (DIO) had a significant increase (P \leq 0.05) in serum TC, TG, LDL-c, and VLD-c as compared to the negative control group. However, all treated groups that fed on a basal diet containing 10%, 15%, and 20% CQS showed a significant decrease (p \leq 0.05) in serum TC, TG, LDL-c, and VLDL-c as compared to the positive control group. The highest decrease was recorded for the groups treated with 20% cooked quinoa seeds. On the other hand, the mean value of total serum HDL-c reduced significantly (p \leq 0.05) in the positive control group compared to the negative control group. While, the data showed a significant increase in serum HDL-c levels for all DIO groups that were fed on CQS, except for the group that consumed 10% of CQS, which showed a non-significant difference when compared to the control positive group.

Table (5): 7	The effect of o	ooked quino	a seeds on lipo	oproteins in	the serum of
obese rats.					

Parameters	Cholesterol	Triglycerides	LDL-c	HDL-c	VLDL-c		
Groups	mg /dl						
Control (-)	75.11±2.88 ^e	59.71±2.86 ^e	17.23±1.18 ^e	54.48±4.12 ^a	11.95±58 ^e		
Control (+)	213.41±17.06 ^a	$183.00{\pm}8.50^{a}$	56.18±2.59 ^a	18.88±91 ^d	36.61±1.69 ^a		
10% CQS	$193.81{\pm}8.45^{b}$	173.16±6.57 ^b	41.28±2.99 ^b	22.20±2.41 ^d	34.63±1.31 ^b		
15% CQS	172.11±12.23 ^c	$144.33 \pm 7.60^{\circ}$	33.70±3.64 °	35.83±3.60 [°]	28.86±1.53 ^c		
20% CQS	119.88±6.95 ^d	113.96±6.41 ^d	29.50±2.31 ^d	40.38±3.43 ^b	22.80 ± 1.28^{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, CQS: Cooked Quinoa seeds. Values in each column which have different litters are significant different ($p \le 0.05$).

In general, coronary heart disease (CHD) is a major health problem in both industrial and developing countries including Egypt. Many studies have now shown that blood elevated concentrations of LDL and TC in the blood are powerful risk factors for CHD. The composition of the human diet has an important effect on the management of lipid and lipoprotein concentrations in the blood (Arafa and Elmaadawy, 2015; Arafa and Elseedy, 2016 and Elhassaneen *et al.*, 2019). Dietary fiber plays an essential role in bodily health, it is contributes to colonic health, coronary artery health, cholesterol reduction, glucose metabolism, insulin response, blood lipids, cancer etc. (Arafa and Mahran, 2018). In the current study, quinoa can be used to enhance the fiber content of foods such as bread and give it excellent organoleptic properties. Also, quinoa seeds contain phytochemicals, i.e., saponins and squalene, which may contribute to their hypocholesterolemic impact. In similar studies, Ge *et al.* (2024) found that the dietary fiber of quinoa showed a significant reduction in total cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol levels compared to those in the model group of obese mice. Moreover, **Cao** *et al.* (2020) 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. Atefi *et al.* (2022) indicated that, quinoa supplementation in doses higher than 50 g/day and the duration more than six weeks significantly reduced serum triglyceride (TG) levels. Also, Fotschki *et al.* (2020) observed that different quinoa-supplemented diets reduced plasma total cholesterol and LDL cholesterol. Overall, the results of previous studies demonstrated that the presence of sterols in plants inhibits the body's absorption of cholesterol.

Effect of CQS on liver enzyme activities of obese rats

From presented data in Table (6) revealed that, rats fed on a diet to induced obesity led to increased AST, ALT, and ALP enzymes activity by about 221.25%, 190%, and 35.5% in the positive control group than in 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 (**Elhassaneen** *et al.*, **2024**). However, the two treated groups of obesity with 15% and 20% CQS showed a significant decrease p<0.05 in serum AST, ALT and ALP enzymes, as compared to the positive control group. On the other hand, the obesity rats fed on cooked quinoa seeds containing 20% recorded the lowest (P \leq 0.05) values of AST, ALT and ALP enzymes, as compared with obesity rat groups fed on other levels.

	Parameters	AST	ALT	ALP
Group			u/l	
Control (-)		26.35 ± 1.18^{d}	33.01 ± 2.52^{e}	$120.43 \pm 1.69^{\circ}$
Control (+)		84.65 ± 9.83^{a}	95.70 ± 5.40^{a}	163.15 ± 4.44^{a}
10% CQS		75.01 ± 5.30^{a}	87.23 ± 5.02^{b}	159.88 ± 5.18^{a}
15% CQS		56.45±2.36 ^b	$81.30 \pm 4.53^{\circ}$	145.63 ± 2.65^{b}
20% CQS		$45.31 \pm 3.11^{\circ}$	45.21 ± 3.59^{d}	142.10±4.66 ^b

Table (6): Effect of CQS on liver enzyme activities in obese rats

ALT: alanine amino transferase, AST: aspartate amino transferase, ALP: alkaline phosphatase, CQS: Cooked Quinoa seeds. Values in each column which have different litters are significant different ($p \le 0.05$).

Aminotransferases (ALT and AST) and alkaline phosphatase (ALP) are normally intracellular enzymes, and the presence of elevated levels of such enzymes in plasma indicates damage to cells rich in these enzymes. The data of the present study indicated that quinoa improves liver functions in obese rats induced by obesity through the activity of hepatic aminotransferases and ALP. Such data are in accordance with those observed by Arafa and Elseedy (2016) who have used quinoa seed powder by 5 to 20% in the intervention process. Also, Abdel-Wahhab et al. (2021) reported that betacyanins, rutin, quercetin, and other flavonoids are present in quinoa; these compounds have been proven to have an anti-inflammatory effect, perform as an antioxidant, and have been established to decrease lipid peroxidation, hepatic lipid accumulation, inflammation, and oxidative stress. Additionally, Cao et al. (2020) showed that feeding quinoa for 8 weeks improved liver tissue and the level of transaminases such as ALT and AST in rats fed a high-fat diet. Perhaps due to the abundance of proteins, minerals, dietary fibers, essential amino acids, and bioactive compounds in quinoa seeds, which protect the liver from oxidative stress. El-Kholie et al. (2023) found that guinoa has improved serum lipid profiles and reduced hyperglycemia, liver enzymes, and renal functions. Omran et al. (2023) revealed that quinoa showed liver-protective effects, anti-inflammatory and antioxidant activities, and modulated blood lipids and blood sugar. Furthermore, Zhong et al. (2023) showed that a long-term high-fat diet causes hepatic steatosis, which further leads to oxidative stress and inflammation. The findings demonstrated that quinoa consumption considerably reduced splenomegaly and hepatomegaly and improved the pathological state of hepatic steatosis.

Effect of cooked quinoa seeds CQS on kidney function of obese rats

Data presented in Table (7) showed that rats fed on a diet to induce obesity led to increased U. acid, BUN, and creatinine by about 138.41%, 143.87%, and 388.33% in the positive control group compared to the negative control group. On the other hand, all treated groups that were fed diets containing three levels of CQS showed a significant reduction in the mean value of serum U. acid, BUN, and creatinine ($p \le 0.05$) as compared to the positive control group (the untreated group). It could be observed that serum U. acid, BUN, and creatinine decreased gradually with increasing the level of CQS, the best results were recorded for the groups that were fed with 20% of CQS.

Pa	rameters	Uric acid	BUN	Creatinine	
Groups		mg/dl			
Control (-)		1.51 ± 0.17^{d}	16.66 ± 1.6^{e}	$0.60{\pm}0.00^{e}$	
Control (+)		3.60±0.64 ^a	40.63±3.06 ^a	2.93±0.31 ^a	
10% CQS		2.70 ± 0.30^{b}	37.10±3.33 ^b	2.41 ± 0.30^{b}	
15% CQS		2.00 ± 0.20^{c}	$26.66 \pm 2.32^{\circ}$	1.53±0.20 ^c	
20% CQS		1.71±0.15 ^{cd}	22.01±1.73 ^d	1.11 ± 0.14^{d}	

Table (7): Effect of cooked quinoa seeds CQS on kidney fu	unction of obese rats
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CQS: Cooked Quinoa seeds. Values in each column which have different litters are significant different ($p \le 0.05$).

Kidneys remove metabolic wastes such as urea nitrogen, uric acid and creatinine, so optimum chemical composition of body fluids is maintained. Urea is often produced in the liver as a byproduct of the metabolism of proteins. Amino acids are produced when protein is consumed, these amino acids undergo catbolization in the liver where free ammonia is produced. One of the main products of protein catabolism, urea, is created when ammonia is mixed with it. The most widely used laboratory test for evaluating renal function is urea measurement (Mohiuddin and Khattar, 2019). The current study's findings are consistent with Halaby et al. (2017) who reported that there was improved in the serum uric acid, urea nitrogen and creatinine levels, in all groups fed quinoa seeds powder at 40% compared with the other groups of rats. Arafa and Elseedy (2016) who reported that non-significant changes in kidney functions among groups fed quinoa verses control negative group. Also, Pasko et al. (2010) showed that quinoa can act as a protective agent against potential by reducing lipid peroxidation and by enhancing the antioxidant capacity of blood (plasma) in heart, kidney, testis, lung and pancreas.

Effect of cooked quinoa seeds on Antioxidant enzymes activity in obese rats

From the data in table (8), it could be noticed that rats fed with HFD (group II) had a significant reduction ($p \le 0.05$) 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 DIO groups receiving diets supplemented with 10, 15, and 20% of CQS had a significant increase in antioxidant enzyme concentration as compared to the positive control group.

Parameters Group	SOD (%)	GPX (U/ml)	CAT (nm/ml/min)
Control (-)	64.66 ± 3.40^{a}	$3.30{\pm}0.08^{a}$	$1.58{\pm}0.09^{a}$
Control (+)	22.31±2.35 ^e	0.70 ± 0.08^{e}	0.40 ± 0.06^{e}
10% CQS	26.03 ± 0.97^{d}	$1.10{\pm}0.20^{d}$	$0.80{\pm}0.08^{d}$
15% CQS	$35.53 \pm 3.23^{\circ}$	$2.23 \pm 0.34^{\circ}$	1.00 ± 0.08^{c}
20% CQS	49.43 ± 2.48^{b}	2.85 ± 0.33^{b}	1.21 ± 0.07^{b}

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

SOD: Super Oxide dismutase, GPX: Glutathione Peroxidase, CAT: Catalase, CQS: Cooked Quinoa seeds, Values in each column which have different litters are significant different ($p \le 0.05$).

Generally, systemic oxidative stress results from an imbalance between oxidants derivatives production and antioxidants defenses. Excess circulating lipids induce ROS formation pathways, which contribute to the increase in lipid oxidation and protein carbonylation. Leptin and angiotensin, secreted at high levels by adipocytes, are inducers of ROS generation and might therefore promote inflammation and lipid peroxidation. Altogether, dysregulation of metabolic parameters occurring with fat mass expansion will contribute to inducing oxidative stress damage, notably at the vascular level (Elmaadawy *et al.*, 2016).

The present study observed that, the mean values of serum antioxidant enzymes activity increased gradually with increasing levels of CQS. All of these improvements could be principally attributed to the strong antioxidant activities of quinoa seeds. These outcomes could be attributed to the high nutritional content of quinoa seeds, which are a great source of minerals (magnesium, iron, potassium, and calcium) and vitamins (C, A, K, and E). Also, quinoa seeds are rich source of flavonoids which improve the biological functions for its antioxidant properties. The tocopherols content of quinoa is essential and acts as an antioxidant at the level of the cell membrane, defending the membranes' fatty acids against oxidative stress. Values of phytochemical content in quinoa seeds indicate that quinoa can serve as a natural antioxidant and necessary to avoid the chronic and degenerative diseases. Such as mentioned by **Vilcacundo** *et al.* (2017); liu *et al.* (2021) and Zhong *et al.* (2023).

Histopathological Examination

While significant changes were observed in the liver and kidneys of obese rats from the positive control group, like pronounced hepatic steatosis, ballooning degeneration, infiltration of inflammatory cells, piecemeal necrosis, degeneration of some renal areas, and shrinking of glomelular structures (Photos 1B & 2B). Meanwhile, obese rats that received a basal diet containing different ratios of CQS showed gradual improvements in both liver tissue (Photos 1C, 1D

& 1E) and kidney tissue (Photos 2C, 2D & 2E). In this respect, Arafa and Elseedy (2016) showed that adding 20% of quinoa seeds powder QSP to white bread achieved the best results, followed by 10% compared to the control group. The liver and kidneys were examined microscopically in mice fed bread supplemented with 10 and 20% QSP and showed a normal condition in both hepatocytes and kidney cells.

Photo 1: Liver of rats





Photo (1A): Liver of an obese rats fed a basal diet (negative control group).

Photo (1B): Liver of an obese rats fed a basal diet (positive control group).

Photo (1C): Liver of an obese rats fed a diet 10%CQS.



Photo (1D): Liver of an obese rats fed a diet containing 15%CQS.

containing



Photo (IE): Liver of an obese rats fed a diet containing 20%CQS





Photo 2: Kidney of rats



Photo (2B): Kidney of an obese rats fed a basic diet (positive control group).

Photo (2C): Kidney of an obese rats fed a diet containing 10% CQS.

Photo (2D): Kidney of an obese rats fed a diet containing 15% CQS.

Photo (2E): Kidney of an obese rats fed a diet containing 20% CQS

Fig (1): Histological examination of liver and kidneys of obese rats

Sensory evaluation of wholesome quinoa bread fortified with different levels of quinoa.

Data showed that the mean (value \pm SD) of the color, odor, texture, taste, general acceptable, and total scores were 19.92 \pm 0.175, 19.73 \pm 0.394, 19.50 \pm 0.849, 19.58 \pm 0.373, 19.69 \pm 0.425, and 98.42 \pm 1.775, respectively, in the control (unfortified bread). Wholesome bread supplemented with 10%, 15%, and 20% of CQS resulted in slightly significant changes in all sensory characteristics (color, odor, texture, taste, and general acceptability) as compared to unsupplemented bread (control). In general, results from the total score indicated that all samples obtained a score higher than 75%.

White bread has a high glycemic index because it's made from refined grains that are rapidly absorbed during digestion, causing sharp spikes in blood sugar and insulin levels. A diet that includes a lot of white bread and other high-glycemic foods increases the risk of weight gain, obesity, type 2 diabetes, and heart disease (Queiroz *et al.*, 2012). Such data are in accordance with those observed by Arafa and Elseedy (2016) who concluded that adding quinoa seed powder to white bread has achieved acceptable results in organoleptic evaluation.

Treatment	Color (20)	Odor (20)	Texture (20)	Taste (20)	General acceptable (20)	Total Score (100)
Control	19.92 ^a ±∙.175	19.73 ^a ±•.394	19.50 ^a ±∙.849	19.58 ^a ±•.373	19.69 ^a ±•.425	$98.42^{a} \pm 1.775$
10% CQS	19.35 ^b	18.52 ^b	17.65 ^b	18.89 ^b	19.21 ^{ab}	93.65 ^b
	±∙.479	±∙.739	±1.310	±•.490	±•.624	±1.936
15% CQS	19.19b ^c	18.27 ^{bc}	16.45 ^c	18.51 ^c	18.32 ^b	92.08 ^b
	±∙.582	±•.316	±1.202	±∙.409	±•.557	±1.472
20% CQS	18.84 ^c	17.85 ^c	14.63 ^d	17.24 ^d	16.40 ^c	87.31 ^c
	±•.712	±∙.395	±•.603	±•.620	±1.859	±3.442

 Table (9): Sensory evaluation of wholesome quinoa bread fortified with different levels

 of quinoa

CQS: Cooked Quinoa seeds, Values in each column which have different litters are significant different ($p \le 0.05$).



Conclusion

From the previous results, study can conclude that cooked quinoa seeds improve fat molecules, kidney function, liver enzyme activity, glucose, leptin hormone and antioxidant activity. Also, ameliorate body weight, nutritional efficiency ratio, organ weight relative to body weight, and the thickness of the peritoneal membrane in obese rats. These improvements increased with a high concentration of cooked quinoa seeds. Therefore, it is recommended for use in the field of functional foods. Promoting quinoa consumption is to advise consumers of the good properties of quinoa and let them incorporate it into their daily diet as a healthy, nutritious, good tasting, and versatile food. It is necessary to make them available on the market for the ordinary user and scale them up to the industrial level.

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التأثيرات المحتملة لبذور الكينوا على المقاييس الحيوية والغذائية والهستولوجية لدى الفئران البدينة

> رشا محمود عرفه ، نهى خالد محمد طاهر ، هبة مصطفى الخولي قسم الاقتصاد المنزلي - كلية التربية النوعية - جامعة دمياط - مصر

> > الملخص العربى

الكينوا هي حبوب زائفة وقد نالت الإهتمام بسبب خصائصها الغذائية وفوائدها الصحية العديدة لجسم الإنسان. لذا أجربت هذه الدراسة لتقييم تأثير نسب مختلفة من بذورالكينوا المطبوخة على كمية الطعام المستهلك ، النسبة المئوية للزيادة في الوزن ، أوزان الأعضاء منسوبة لوزن الجسم ، نشاط إنزيمات الكبد ، صورة الدهون ، وظائف الكلى ، مستوى سكر الدم ، هرمون اللبتين وقياس نشاط مضادات الاكسدة الإنزيمية لدى الفئران التي تعانى من السمنة. وقد استخدم في هذه الدراسة ثلاثون فأراً من ذكور الألبينو وزنها (١٢٠ ± ١٠ جرام) تم تقسيمهم إلى مجموعتين رئيسيتين: المجموعة الرئيسية الأولى (٦فئران) تم تغذيتها على الغذاء الأساسي كمجموعة ضابطه سالبه (غير مصابة). المجموعة الرئيسية الثانية (٢٤ فأرًا) تغذت على نظام غذائي عالى الدهون لمدة ٨ أسابيع، ثم تم تقسيم هذه المجموعة إلى أربع مجموعات فرعية. تم تغذية إحدى هذه المجموعات على نظام غذائي عالى الدهون فقط، واستخدامها كمجموعة ضابطة إيجابية. تم تغذية بقية المجموعات على نظام غذائي عالى الدهون يحتوي على ١٠٪ ، ١٥٪ ، ٢٠٪ من بذور الكينوا المطبوخة لمدة ٣٠ يومًا. احتوى التركيب الكيميائي التقريبي لبذور الكينوا المطبوخة (CQS) على ٨,٥٧ ، ١٤,٦١ ، ٤,٧٦ ، ٣,٣٤ ، ٥,١٢ و ٦٣,٦٠ جم / ١٠٠ جم لكلا من الرطوبة والبروتين الكلي والدهون والرماد والألياف الخام والكربوهيدرات على التوالي. أشارت النتائج البيولوجية إلى أن تغذية الفئران على وجبات تحتوي على بذورالكينوا المطبوخة أدى إلى تحسن كبير في نسبة الزيادة في وزن الجسم ، وزن الأعضاء ، متوسط تناول الطعام ، نسبة الجلوكوز في الدم ، وظائف الكلي ، وظائف الكبد ، دهون الدم ، هرمون اللبتين ونسبة الإجهاد التأكسدي. بالإضافة إلى ذلك أظهر الفحص المجهري للكبد والكلى للفئران في المجموعات المعالجة بمستويات مختلفة من بذور الكينوا المطبوخة تحسنًا ملحوظًا في بنية أنسجة هذه الأعضاء. تم إنتاج خبز صحى بنسبة ١٠، ١٠، ٢٠٪ من بذور الكينوا المطبوخة، وأشار التقييم الحسى إلى أن جميع عينات الخبز الصحى لها قبول أكبر من ٧٥٪. وفي الختام، تشير الدراسة الحالية إلى أن CQS قد حسنت المؤشرات الحيوية في الفئران البدينة ونوصبي باستخدام بذور الكينوا المطبوخة كإضافة في مجال الأغذية الوظيفية.

الكلمات المفتاحية: الأغذية الوظيفية – الجلوكوز – وظائف الكلي – انزيمات الكبد – دهنيات الدم – الكينوا– اللبتين – الأكسدة.