Current Science International
ISSN: 2077-4435

Volume : 06 | Issue : 01 | Jan.- Mar. |2017
Pages**:** 259-270

Protective Influence of Quinoa on Hypercholesterolemia in Male Rats

Mona S. Halaby, Manal K. Abdel-Rahman and Rehab A. Hassan

Nutrition and Food Sci. Dept., Faculty of Home Economics, Helwan Univ., Cairo-Egypt.

Received: 25 February 2017 / Accepted: 27 March 2017 / Publication Date: 28 March 2017

ABSTRACT

The main target of this research was to conducted effect of dietary supplementation with Quinoa seeds powder (QSP) under two various concentrations (30% and 40%) to give more protection against hypercholesterolemia disease. Twenty eight male albino rats were used in this experiment. These rats were put on ideal diet for two weeks before then divided into four groups (seven rats of each). The first group was fed on basal diet as a (negative control group). The second group (positive control group) was fed on basal diet + 2% cholesterol to induce hypercholesterolemia. Third group was fed as the second group +30% QSP and the fourth group was fed as the second group + 40 % QSP. At the end of the experimental period (60 days) rats were fasted over night and sacrificed; blood samples were collected from the aorta to determine lipids profiles, also for liver and kidney functions. Besides, nutritional and biological parameters were recorded. From the obtained results we concluded that group of rats fed on diet with 2% cholesterol were considered as a major risk factor for hypercholesterolemia disease. Our results could be summarized that diet fortified at 30% and 40% QSP can improved the body weight gain, feed consumption, feed efficiency ratio, reduced blood cholesterol and other lipids as well as reducing hazards on liver and kidney functions compared with positive control group. Diet with 40% QSP reduced the adverse effect of hypercholesterolemia. Histopathological liver observation proved that the last group is considered as a negative control group.

Key words: Hypercholesterolemia, Ouinoa, liver function, kidney function, histopathology.

Introduction

Hypercholesterolemia provokes several human diseases or induces tissue damage, it was reported that hypertriglyceridemia and hypercholesterolemia may be responsible for oxidative modification of LDL, protein glycation, gluco-seautooxidation with excess production of free radicals and lipid peroxidation products, which represent major risk factors for ischemic heart diseases (Olorunnisola *et al*., 2012). Nowadays, the treatment of hypercholesterolemia with medicinal plants has increased in recent years, consumer demand for healthy, safe, natural, and fresh foods that require only a minimum effort and time for their preparation has increased (Ramos, 2012; Halaby *et al*., 2013 and Yang & Ludewig (2014).

Some cereals and pseudocereals have been playing an increasingly vital role in modern lifestyles, to meet consumer demand due to their physiological and metabolic properties, since the consumption of cereals has expanded from the breakfast table to any time of the day, these products have become an excellent vehicle for the inclusion of functional ingredients in the consumers diet Machado *et al*., (2012) and Ruini *et al*., (2015). Quinoa has a high biological value (73%), similar to that of beef (74%), and higher than those of white rice (56%), wheat (49%) and corn (36%). Quinoa also contains all ten essential amino acids, and its protein content ranges from 12.9 to 16.5%. Quinoa has been cultivated for the last 5,000 - 7,000 years in the Andean region of Bolivia and Peru. 2013 was declared by the United Nations as the International Year of Quinoa as recognition of its significant potential published by Tang *et al*., (2015b) and Gordillo *et al*., (2016).

Despite all these health benefits, Quinoa is not widely consumed due to several reasons, such as high import costs of the grain and lack of knowledge regarding its benefits among consumers. The aim of the present investigation was to evaluate the biochemical, and biological changes that may

Corresponding Author: Mona S. Halaby, Nutrition and Food Sci. Dept, Faculty of Home Economics, Helwan Univ., Cairo-Egypt. E-mail: monahalaby03@yahoo.com

occur in rats fed on diet with high cholesterol to induce hypercholesterolemia, and to evaluate the potential effect of dietary supplementation with quinoa seeds powder in adult male albino rats.

Materials and Methods

Materials:

Dry Quinoa variety (Chenopodium quinoa W.) was obtained from Agricultural Research Center. Cholesterol, cellulose, casein, vitamins and minerals were obtained from El-Gomhoria Pharmaceutical Company, Cairo, Egypt. Corn oil and starch were obtained from the local market. Twenty eight normal adult male albino rats (Sprague-Dawley) were obtained from the laboratory animal colony. Ministry of Health & population, Helwan, Cairo- Egypt. Weighting were approximately between (130 ± 10g). Kits used to determine serum cholesterol, triglycerides, LDL, uric acid, urea nitrogen, creatinine, and transaminases supplied by Alkan Company. Chemicals for histopathological examinations: saturated formaldehyde solution 40%, sodium monohydrate, and ethylene diamine tetra-acetic acid (EDTA) were obtained from faculty of veterinary medicine, Cairo University.

Methods:

Preparation of Quinoa seeds: was washed, dried and crushed using electric blender. These seeds powder were mixed with basal diet daily.

Chemical analysis of Quinoa:

Moisture, fat, protein, ash and crude fiber were determined according to the method outlined in AOAC. (2000). Total carbohydrates were determined by difference as mentioned by Abd El-Latif, (1990). Total antioxidant activity was determined according to the methods described by Politeo *et al*., (2006). Vitamin contents including (A, E, C, thiamine, riboflavin, niacin and folic acid) and minerals including (Cu, Fe, Mn, Zn, Ca, P, Mg, Na and K) were determined according to the method described by J. Chrom. (1999 & 2006). Total fatty acids (saturated and unsaturated FA) were determined by "Hydrolytic Extraction Gas Chromatographic" according to the method described by ISO (1990 &2000). Phenolic and Flavonoid compounds were determined by HPLC according to the methods of J.Sci.Food Agric. (1999) and J.og.Agric. & Food Chem. (2000).

Induction of hypercholesterolemia:

It was induced by 2% cholesterol powder in the diet according to Hassarajani *et al*., (2007).

Experimental design:

Rats were adapted for two weeks prior to commencement of the experiment. Water was introduced ad-libitum. Rats were divided into four groups and fed on diets for sixty days as follows:

Group 1:

Negative control group consisted of Casein 14%, Corn oil 4%, Choline choloride 0.25%, Vitamin mixture 1%, Salt mixture 3.5%, Cellulose 5%, Sucrose 10% and the remained is corn starch 62.25% according to Reeves *et al*., (1993).

Group 2:

Positive control group, as the same of first group fed on basal diet +2% cholesterol.

Group 3:

As the same of group 2 + 30% Quinoa seeds powder.

Group 4:

As the same of group 2 + 40% Quinoa seeds powder.

Feed Intake, Body Weight Gain % and Feed Efficiency Ratio were determined according to (Chapman *et al*., 1959).

Blood Sampling:

At the end of the experiment period, the rats were fasted overnight then the rats were anaesthetized and sacrificed and blood samples were collected from the aorta. The blood samples were centrifuged for 15 minutes at 3000 rpm to separate the serum. The serum was carefully separated into dry clean Wassermann tubes by using a Pasteur pipette and kept frozen at -20°C till analysis.

Biochemical analysis of serum:

Serum samples were used for the determination of total cholesterol (Allain *et al*., 1974), triglycerides (Fassati & Prencipe 1982), HDL-C (Lopes, 1977), LDL-C and VLDL-C were calculated by using the method of Friedewald *et al*., (1972). Uric acid, urea nitrogen and creatinine were determined according to the methods described by Fossati *et al*., (1980), Patton & Crouch (1977) and Bartels *et al*., (1972), respectively. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to Retiman & Frankel (1957).

Histopathological Examination:

Tissues from heart of the sacrificed rats were examined as described by Bancroft *et al*., (1996).

Statistical analysis:

Results are expressed as mean ± SD. Data were statistically analyzed for variance using one-way analysis of variance "ANOVA" according to (Armitage & Berry, 1987).Computer software system SPSS (version 15) was used for these calculations.

Results and Discussion

Chemical composition of raw materials:

Quinoa seed powder (QSP) was investigated on dry weight basis. The following parameters in Tables (1) were pointed out for moisture, fat, protein, ash, total carbohydrates, crude fiber (g/100g DW), and total antioxidant activity by DPPH (µg/mL) as follows (9.5; 6.9; 14.2; 3.2; 63.5; 2.7 and 5.16% DW), respectively. In fact, antioxidants are an important part of the defense system of the human body and help to cope with oxidative stress caused by reactive oxygen species. From the tabulated results it could be noticed that QSP are rich source of macronutrients.

The present results are in agreement with those Vega-Galvez *et al*., (2010), they showed that Quinoa, which provides a protein value similar to casein in milk. Quinoa is considered one of the best vegetal protein sources, as its protein levels are higher than those present in wheat, rice, maize, barley, corn, rye, and sorghum. Quinoa also has been used by the National Aeronautics and Space Administration (NASA) due to its versatility in meeting the needs of humans during space missions González *et al*., (2014); Cooper (2015) and USDA, (2015). In addition, Quinoa is an excellent source of dietary fiber, comprising about 2.6% - 10% of the total weight of the grain; about 78% of its fiber content is insoluble and 22% soluble Lamothe *et al*., (2015). According to Gordillo *et al*., (2016) evaluated

antioxidant potential extracted from Quinoa flour and from whole cereals (wheat, barley, millet, rice, buckwheat), published that the Quinoa presented higher antioxidant potential comparison with the other whole cereals, these results suggested that Quinoa possesses potent free radical-scavenging compounds. In fact, chemical constituents and antioxidant of plants depend on several factors such as different genotype, growing condition, agronomic practices employed, season, maturity, post-harvest and storage conditions confirmed by Navruz & Sanlier (2016).

Table 1: Chemical composition of Quinoa seeds powder (g / 100g dry weight basis)

Component (g / 100g DW)	Quinoa seed powder	Vitamins %	Quinoa seed powder	Elements (mg/100g DW)	Quinoa seed powder
Moisture	9.5	Vitamin A (µ/100g)	0.39	Copper	17.00
Fat	6.9	Vitamin E (ppm)	5.37	Iron	68.00
protein	14.2	Vitamin C (mg/100g)	4.00	Manganese	31.00
Ash	3.2	Thiamine (mg/100g)	0.38	Zinc	42.00
Total carbohydrates	63.5	Riboflavin (mg/100g)	0.39	Calcium	1042.00
Crude fiber	2.7	Niacin (mg/100g)	1.06	Phosphorous	5519.00
Total antioxidant activity DPPH (µg/mL)	5.16	Folic acid (mg/100g)	78.32	Magnesium	2532.00
---	----	----	----	Sodium	46.00
				Potassium	885.00

Nutritional activity in QSP is usually related to the particular elements, it contained good amounts of vitamins including (A, E, C, thiamine, riboflavin, niacin and folic acid). Results given in Table (1) indicated that QSP contained the values of vitamins with an average of 0.39 (µ/100g); 5.37 (ppm); 4.00; 0.38; 0.39; 1.06 and 78.32 (mg/100g), respectively. Our results are in agreement with Alvarez *et al*., (2010a) and Hejazi (2016).

Micro and macro nutrient elements can play an important role in many metabolic processes and functions throughout the life cycle. The data given in Table (1) illustrated that the QSP contained the various values of micro and macro elements including copper; iron; manganese; zinc; calcium; phosphorous; magnesium; sodium and potassium. Results indicated that (QSP) contained the values of mineral with an average of 17.00; 68.00; 31.00; 42.00; 1042.00; 5519.00; 2532.00; 46.00 and 885.00 (mg/100g DW), respectively. These results were in agreement with those (Vega-Galvez *et al*., 2010 and Hejazi, 2016) they revealed that dietary minerals are essential chemical elements that play a role in regulating electrolyte balance, glucose homeostasis, the transmission of nerve impulses and enzyme cofactors in the body, moreover, calcium, magnesium and potassium in quinoa are found in sufficient quantities and bioavailable forms necessary for maintaining a balanced human diet.

Fatty acids composition of Quinoa seeds powder (dry weight basis):

Types and concentrations of fatty acids extracted from the QSP using gas chromatography (GC) give in Table (2). Five saturated fatty acids were recognized in QSP, besides two mono-unsaturated and two poly- unsaturated fatty acids were identified in the same variety.

Results in Table (2) revealed that the total saturated fatty acid, monounsaturated and polyunsaturated fatty acids in QSP were 13.86; 26.17 and 59.97 (g/ 100g), and the most abundant poly and mono-unsaturated fatty acids in QSP were Linoleic and Linolenic acids (52.84% & 07.13) and Oleic acids (25.60%). The results given in the same Table (2) revealed that the most abundant saturated fatty acid in QSP was Palmitic acid (11.40%). Results detected other quantities of fatty acids including Eicosenic acid (01.14%), Stearic acid (00.79%), Arachidic (00.29%) and Behenic acid

(00.24%) were among the minor FA found in QSP. Quinoa provides good-quality lipids rich in unsaturated fats. The obtained data were in the line with those of Vega-Galvez *et al*., (2010); Tang *et al*., (2015) and Hejazi, 2016) they showed that the total lipid content of Quinoa is 14.5%, with approximately 70%-89.4% being unsaturated (38.9%-57% of linoleic acid, 24.0%-27.7% of oleic acid and 4% of α-linolenic acid). The unsaturated fatty acid content is protected by vitamin E in this plant. The ratio between omega-6 and omega-3 in Quinoa is about 6:1, confirmed by Gordillo *et al*., (2016).

Table 2: Fatty acids composition of Quinoa seeds powder

Component of fatty acids (g/100 g)		Quinoa seeds powder
Saturated Fatty acid	Palmitic acid	11.40
	Eicosenic acid	01.14
	Stearic acid	00.79
	Arachidic acid	00.29
	Behenic acid	00.24
Mono-unsaturated Fatty acid	Oleic acid	25.60
	Erucic acid	00.57
Poly-unsaturated Fatty acid	Linoleic acid	52.84
	Linolenic acid	07.13
Total saturated Fatty acid		13.86
Total Monounsaturated Fatty acid		26.17
Total polyunsaturated Fatty acid		59.97

Types and concentrations of Flavonoids and Phenolic compounds of QSP:

The results given in Table (3) indicated that Hisperdin, Naringin, Quercetrin and Rutin were the abundant flavonoid compounds, which were at concentration of (265.32; 72.32; 48.34 & 30.32 µg/100g) respectively. While, Hespertin, 7-OH flavones and Apegenin 14.28; 7.44 & 5.79 (µg/100g) were the moderate abundant flavonoid compounds in QSP and the lowest abundant were Quercetin, Kampherol, Rosmarinic & Narengenin 2.481, 2.003, 1.572 & 0.580 (µg/100g) respectively.

Table 3: Types and Concentrations of Flavonoids Compounds of QSP

Flavonoids	Flavonoids of Quinoa (g/100µg)By HPLC analysis
Naringin	72.320
Rutin	30.327
Hisperdin	265.323
Rosmarinic	1.572
Quercetrin	48.349
Quercetin	2.481
Narengenin	0.580
Kampherol	2.003
Hespertin	14.288
Apegenin	5.792
7-OH flavone	7.444

According to previous studies (Zhang *et al*., 2011 and Mbikay 2012) they revealed that, flavonoid compounds is a potent antioxidant with multiple therapeutic properties, anti-dyslipidemic, hypotensive, and anti-diabetic effects in the obese rat model of metabolic syndrome. These findings are in agreement with Gordillo *et al*., (2016) they published that Quinoa is a more effective functional food, in terms of a source of bioactive flavonoids, than conventional cereal and pseudocereal grains.

Results given in Table (4) indicated that QSP contained considerable amount of Phenolic compounds with an average from 28481.032 to 69.487 *(µg/100g)* respectively. It is evident from the data that Salicylic, Benzoic, e-vanillic and Pyrogallol were the predominant Phenolic compounds present in of QSP, which were at concentration of 28481.032; 11052.665; 7544.670 and 5989.741*(µg/100g)* respectively. Comparing with other Phenolic compounds present in moderates concentrations such as Chlorogenic acid; P-coumaric; P-OH-benzoic; Cinnamic and Gallic acid *(µg/100g)*. While data in the same table revealed that Vanillic; Ellagic; Coumarin; Catechol; 3-oh-

Tyrosol; Ferulic and Caffeic *(µg/100g)* were present in the lowest abundant levels. Previous studies noticed that, these essential nutrients including (polyphenols and flavonoids) can help decrease the nutritional deficit and combat many chronic diseases (Lako *et al*., 2007). According to Mbikay, (2012) showed that, Phenolic compounds anti-dyslipidemic properties are more evident as its dietary supplementation has been shown also, to significantly reduce plasma TC and TG (cardiovascular prevention), anti-allergic, anti-inflammatory, antiviral and anti-carcinogenic. Moreover, Tang *et al*., (2015b) revealed that Quinoa presents at least 23 phenolic compounds. The total phenolic content (mg/kg quinoa) is 466.99, 634.66 and 682.05 for white, red and black Quinoa, respectively. Confirmed by Asao & Watanabe, (2010) and Gordillo *et al*., (2016) that Quinoa contains more phenols than whole cereals.

Table 4: Types and Concentrations of Phenolics Compounds of *QSP*

Phenolics	Phenolic compounds (µ g/100g) of Quinoa	Phenolics	Phenolic compounds (µ g/100g) of Quinoa
Gallic	1771.864	Ferulic	563.101
Pyrogallol	5989.741	Iso-ferulic	105.406
4-Amino-benzoic	101.494	Reversetrol	69.487
3-oh-Tyrosol	595.786	Ellagic	875.389
Protocatchuic	363.721	e-vanillic	7544.670
Chlorogenic	3677.153	Alpha-coumaric	184.190
Catechol	753.191	Benzoic	11052.665
Epicatechein	362.190	3,4,5-methoxy-cinnamic	256.905
Caffeine	118.341	Coumarin	787.637
P-OH-benzoic	2890.779	Salycilic	28481.032
Caffeic	533.506	P-coumaric	2967.012
Vanillic	962.511	Cinnamic	2649.760

Biological evaluation:

The mean values of body weight gain (BWG %); feed intake (g/day for each rat) and feed efficiency ratio (FER) of rats fed negative control group, positive control group (hypercholesterolemia), hypercholesterolemia group fed on QSP at (30% and 40%) were summarized in Table (5). It could be observed that there were significant increase in BWG; FI and FER for control positive group (18.30± 2.07; 12.37± 0.21 & 0.828 ± 0.15) as compared to the healthy control group (negative), (13.28±1.35; 10.91± 0.11 & 0.682± 0.01), respectively. While, hypercholesterolemia rats received diet containing various ratios from 30% or 40% QSP recorded significant decrease values ($P< 0.05$) in BWG; FI and FER as compared to the positive control group, it seems that QSP gave protection effect against overweight. These results are in harmony, with those Vega-Galvez *et al*., (2010) and Barakat, (2011) they showed that hypercholesterolemia diet caused a significant increase in BWG as compared with healthy control group, also, consumption of 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.

Table 5: Effect of feeding on high cholesterol diet fortified with *QSP* on body weight gain, feed intake and feed efficiency ratio

Group \ Parameters		BWG *(g)*	FI (g/day)	(FER)
1	Control (-ve)	^{d} 13.28 ±1.35	^{c} 10.91 ± 0.11	^{d} 0.682 ± 0.01
2	Control (+ ve)	^{a} 18.30 ±2.07	^{a} 12.37 ±0.21	^{a} 0.828 ±0.15
3	(+ ve)+ QSP 30%	^{c} 14.35 ±1.14	^{bc} 11.20 ±0.03	^{b} 0.718 ± 0.16
4	(+ ve)+ QSP 40%	^{b} 15.06 ±0.13	^{b} 11.99 ±0.21	^{c} 0.703 ±0.75

**Results are expressed as means ±SD.*
**Values in each column which have different letters are significantly different ($p<0.05$).*

Effect of feeding on high cholesterol diet fortified with QSP on lipid profile of rats:

Hypercholesterolemia is a key factor in the predictive equations for cardiovascular disease. In combination, these plant food components may have a very significant impact on blood lipids and cardiovascular disease, which appeared to be complications of hypercholesterolemia (Fulgoni *et al*., 2013). To evaluate the effect of feeding on high cholesterol diet fortified with (30% and 40% QSP) on total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low and very low density lipoprotein cholesterol (LDL-C and VLDL-C) in hypercholesterolemia rats is presented in Table (6).

Table 6: Effect of feeding on high cholesterol diet fortified with *QSP* on lipid profile of rats

Parameters / Groups	mg/dl				
	Cholesterol	Triglyceride	HDL-C	LDL-C	VLDL-C
Control (-ve)	104.49± 2.94^{d}	57.28± 2.07^{d}	32.21± 1.30^{b}	62.68± 3.05^{d}	09.60± 1.3^{b}
Control (+ ve)	127.15± 1.50^{a}	70.85± 3.70^{a}	24.14± 1.26^{d}	89.82± 2.07^{a}	13.19± 1.6^{a}
(+ ve)+ QSP 30%	119.94± 2.01^{b}	64.14± 1.05^{b}	26.71± 1.52^{c}	82.11± 2.09^{b}	11.12± 0.6^{b}
(+ ve)+ QSP 40%	118.11± 2.42^{c}	61.43± 3.265^{c}	29.85± 1.85^{c}	77.28± 1.07^{c}	10.98± 1.8^{b}

All results are expressed as means ±SD. Values in each column which have different letters are significantly different ($p<0.05$).

It could be noticed that the positive control group fed on basal diet containing 2% cholesterol has shown a significant increase $P< 0.05$ in the mean values of TC; TG; LDL-C and VLDL-C (127.15±1.50; 70.85±3.70; 89.82±2.07 and 13.19±1.6), compared with the control negative group fed on the basal diet (104.49±2.94; 57.28±2.07; 62.68±3.05 and 9.60±1.3) respectively. On the contrary, raised level of HDL-C was associated with reduced risk of atherosclerosis, since high density lipoprotein in serum is thought to facilitate the translocation of excess cholesterol from the peripheral tissue to liver for further catabolism (Makni *et al*., 2008). Furthermore, our results are agreed with Barakat, (2011) and Wang *et al*., (2012) they stated that the increase in HDL-C ratio is one of the most important criteria of anti-hypercholesterolemia agent.

Rats which were fed on high-cholesterol diet with two various levels from QSP fortified at 30% & 40%, had lower mean values of lipid profile compared with positive control group might due to decrease of cholesterol absorption and biosynthesis and increase of faecal bile acid and cholesterol excretion. In fact, the best results in lipid fractions for all treated groups was noticed in group fed on high cholesterol diet basal diet fortified with QSP at 40%, because this treatment improved levels of serum cholesterol and triglycerides. The data were in the line with those of Farinazzi *et al*., (2012) and Zevallos *et al*., (2014)).

Effect of feeding on high cholesterol diet fortified with QSP on liver function of rats:

Results in Table (7) indicated that feeding on basal diet containing 2% cholesterol has shown a significant increase $P< 0.05$ in serum AST and ALT, as compared to healthy rats group (33.21±5.61 and 21.05±2.62 vs. 17.57±5.51 and 09.23±3.25 U/L), respectively. The high levels of AST and ALT in serum are indicators for liver dysfunction. These findings are in agreement with Al-Dosari, (2011) and Halaby *et al*., (2013) they revealed that the rats feeding on high cholesterol diet for 70 day showed significant increase in serum liver marker enzymes (GOT, GPT, GGT, ALP) and bilirubin levels.

Results indicated also that, feeding on high cholesterol diet fortifications with QSP at 30% and 40% resulted in significant decrease $p<0.05$ in serum AST and ALT as compared to positive control group. The best results of liver function recorded for hypercholesterolemia rats fed on diet fortified with QSP at 40%, confirmed by Zevallos *et al*., (2014).

Table 7: Effect of feeding on high cholesterol diet fortified with *QSP* on liver function of rats

Parameters / Groups	AST (u/l)	ALT (u/l)
Control (-ve)	17.57±5.51[d]	09.23±3.25[c]
Control (+ ve)	33.21±5.61[a]	21.05±2.62[a]
(+ ve)+ QSP 30%	26.20±2.74[b]	12.56±1.71[b]
(+ ve)+ QSP 40%	20.71±5.43[c]	10.40±1.81[d]

**Results are expressed as means ±SD.*
** Values in each column which have different letters are significantly different (p<0.05).*

Effect of feeding on high cholesterol diet fortified with QSP on kidney functions of rats:

Kidneys remove metabolic wastes such as urea nitrogen, uric acid and creatinine, so optimum chemical composition of body fluids is maintained. The concentrations of the metabolites increase in blood during renal diseases or renal damage may due to high activities of xanthine oxidase, lipid peroxidation, and increased triacylglycerol and cholesterol levels (Barakat & Mahmoud, 2011 and Halaby *et al*., (2013). Results presented in Table (8) summarize the effect of tested different ratios of QSP on serum urea nitrogen; creatinine and uric acid in rats fed high cholesterol diets and compared with healthy group.

Table 8: Effect of feeding on high cholesterol diet fortified with *QSP* on kidney functions of rats

Parameters / Group	Urea nitrogen	Creatinine	Uric acid
	mg/dl		
Control (-ve)	86.43±2.15[c]	0.85±0.05[c]	1.55±0.12[d]
Control (+ ve)	147.7±9.31[a]	1.26±0.33[a]	4.77±0.31[a]
(+ ve)+ QSP 30%	138.4±14.64[b]	0.99±0.05[b]	3.95±1.10[b]
(+ ve)+ QSP 40%	98.00±7.89[d]	0.91±0.03[c]	2.04±0.25[c]

**Results are expressed as means ±SD.*
**Values in each column which have different letters are significantly different (p<0.05).*

Serum urea nitrogen:

High cholesterol diet induced hypercholesterolemia rats had the higher values of serum urea nitrogen reached to 147.7 ± 9.31 compared with negative control group 86.43±2.15 mg/dl may be related to with high cholesterol diet the kidneys are not functioning properly or if the body is using large amounts of cholesterol in the diet, the serum urea nitrogen level will rise. Our results indicated also, that the level of urea nitrogen at the end of experimental period decreased gradually according to the concentration, the ratios reached from 147.7 ± 9.31 to 138.4 ± 14.64 and 98.00± 7.89 mg/dl respectively, for fortification diet with QSP at 30% and 40%.

Results of the present study agree with those of (Zevallos *et al*., 2014) they published that administration of hypercholesterolemia rats caused significant increase in blood total lipids, uric acid and urea nitrogen contents, the same authors showed that the increase in serum urea nitrogen level in hypercholesterolemic control group indicates impairment in the normal kidney function of the animal, as the mechanism of removing it from the blood might have been affected. It may also be an indication of dysfunction at the glomerular and tubular levels of the kidney, it is well known that, many biochemical and histopathological findings confirmed renal damage in hypercholesterolemia conditions.

Serum creatinine:

Increased significantly ($p< 0.05$) in groups of rats fed on high cholesterol diet as compared to the negative control groups (1.26 ± 0.33 *vs* 0.85 ± 0.05), confirmed by Tricia, (2007) who showed that high creatinine levels indicate a person is experiencing kidney failure, also may due to increased cholesterol levels confirmed by Barakat & Mahmoud, (2011). From Table (8) the mean values and standard deviation of serum creatinine for different fortification with QSP at 30% and 40% were

0.99±0.05 and 0.91±0.03 mg/dl respectively. These results indicated that there was improved in the serum creatinine levels in groups fed QSP at 40% compared with the other groups of rats.

Serum uric acid:

It could be observed that the control positive group fed on high cholesterol diet has shown a significant increase ($p< 0.05$) in serum uric acid compared with those of the control negative group fed on basal diet (4.77±0.63 vs 1.55±0.31 mg/dl). It could observe also that oral administration of QSP at 30% and 40% reduced the uric acid level significantly. The ratios were (3.95±1.10 and 2.04±0.25mg/dl) respectively and the concentration of uric acid was reduced by 20.76% and 41.77% respectively.

Data reveled that the highly significant reduction of all parameters including urea nitrogen, creatinine and uric acid observed in the group fed on high cholesterol diet fortified with QSP at 40%. In fact, dietary management is an essential component for patients with hypercholesterolemia disease. Confirmed by Zevallos *et al*., (2014) they showed that the current trends demonstrate a significant increase in this segment of our healthcare population and gastroenterologists can anticipate increased interaction with patients who have hypercholesterolemia disease.

Histopathological results:

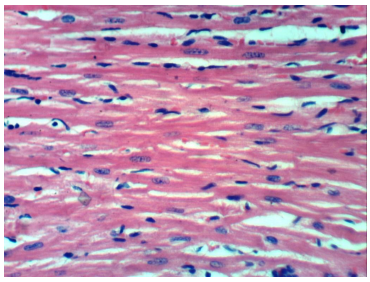

Fig. 1: Microscopically, heart of rat from control negative group fed on basal diet, showed no histopathological changes and normal cardiac myocytes (H & E X 400).

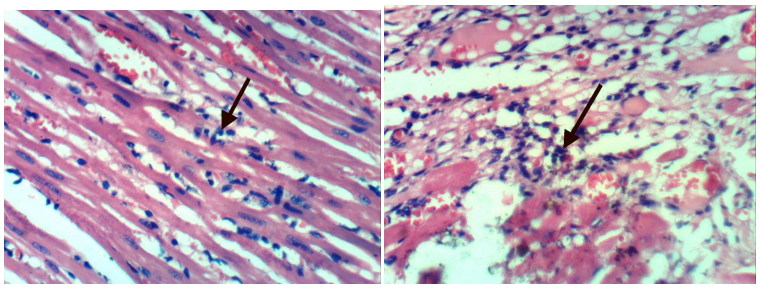

Fig. 2 & 3: Heart of rat from group fed on basal diet and 2% cholesterol showed myocarditis. Notice necrosis of focal myocytes associated with inflammatory cells infiltration between cardiac muscles (H & E X 400).

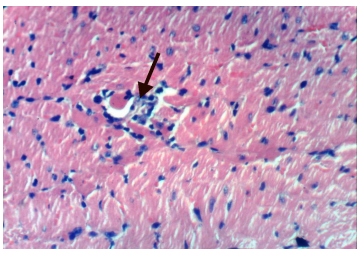

Fig. 4: Heart of rat from group fed on positive diet with 30% QSP showed myocarditis. Notice necrosis of focal myocytes associated with inflammatory cells infiltration (H & E X 400).

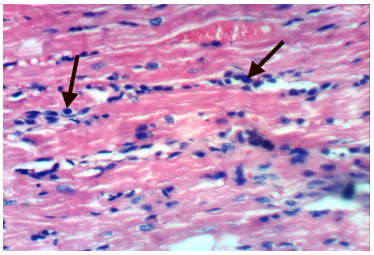

Fig. 5: Heart of rat from group fed on positive diet with 30% QSP showed myocarditis. Notice inflammatory cells infiltration between cardiac muscles (H & E X 400).

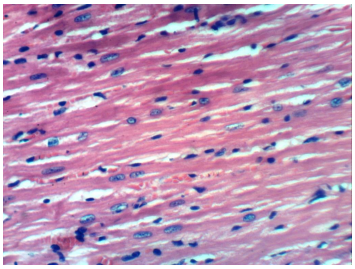

Fig. 6: Heart of rat from group fed on positive diet with 40% QSP showed no histopathological changes (H & E X 400)

Recommendation

Quinoa seeds should be recommended for production on a commercial scale in the Egyptian meal, factories and medicines; such seeds have the capability to give more protection against hypercholesterolemia disease and to improve blood lipid levels as well as reducing hazards on liver and kidney functions.

References

Abd El-Latif, B.M., 1990. Improvement of some bakery products thesis. Ph.D. F. Tech. Agric. Moshtohor, Zagazig Univ.

Al-Dosari, M., 2011. Hypolipidemic and antioxidant activities of avocado fruit pulp on high cholesterol fed diet in rats. Afr. J. Pharm. Pharmacol., 5: 1475-1483.

Allain, C., L. Poon and C. Chan, 1974. Enzymatic determination on total serum cholesterol.Clin.chem., 20: 470-475.

Alvarez, J.L., H. Wijngaard, E.K. Arendt and E. Gallagher, 2010a. Polyphenol composition and in vitro antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. Food Chemistry, 119: 770-778.

AOAC., 2000. Association of Official Analytical Chemist Official Methods of Analysis 17th ed., Washington, USA.

Armitage, P. and G. Berry, 1987. Statistical method in medical research. Blackwell, Oxford, UK, 93-213.

Asao, M. and K. Watanabe, 2010. Functional and Bioactive Properties of Quinoa and Amaranth. Food Sci Technol Res., 16: 163-8.

Bancroft, D., A. Stevens and R. Turner, 1996. Theory and practice of histological techniques. 4th ed, Churchill Living Stone, Edinburgh, Landon, Melbourne

Barakat, L., 2011. Hypolipidemic and Antiatherogenic Effects of Dietary Chitosan and Wheatbran in High Fat- High Cholesterol Fed Rats. Australian Journal of Basic and Applied Sciences, 5(10): 30-37.

Barakat, L. and R. Mahmoud, 2011. The antiatherogenic, renal protective and immunomodulatory effects of purslane, pumpkin and flax seeds on hypercholesterolemic rats. North American Journal of Medical Sciences, 3(9): 351-357.

Bartels, H., M. Bohemer and C. Heirli, 1972. Colorimetric kinetic method of creatinine. Clin. Chem. Acta. 37: 193.

Chapman, d., R. Castilla and J. Cambell, 1959. Evaluation of protien in foods:A method for the determination of protien efficiency ratio .cam.J.biochem.physical., 37: 697-686.

Cooper, R., 2015. Re-discovering ancient wheat varieties as functional foods. J Tradit Complement Med., 5: 138-143.

Fassati, p. and l. Prencipe, 1982. Triglycerides determination after enzymatic hydrolysis. clin. chem., 28: 2077.

Fossati, P., L. Prencipe and G. Berti, 1980. Enzymatic colorimetric method of determination of uric acid in serum. Clin. Chem., (18): 499-502.

Friedewald, W., R. Leve and D. Fredrichson, 1972. Estimation of concentration of low-density lipoproteins separated by three different. Clin.Clem., 18: 499-502.

Fulgoni, V., M. Dreher and A. Davenport, 2013. Avocado consumption is associated with better diet quality and nutrient intake, and lower metabolic syndrome risk in US adults: results from the National Health and Nutrition Examination Survey (NHANES) 2001–2008 Nutr J. 12: 1.

González, M., G. Wells Moncada, S. Fischer and O. Escuredo, 2014. Chemical characteristics and mineral composition of quinoa by nearinfrared spectroscopy. J Sci Food Agric, 94: 876-881.

Gordillo, S.B., D. Díaz-Rizzolo, E. Roura, T. Massanés and R. Gomis, 2016. Quinoa (Chenopodium quinoa Willd), from Nutritional Value to Potential Health Benefits: An Integrative Review. J Nutr. Food Sci., 6(3): 2-10.

Halaby, M.S., E.M. Elmetwaly and A.A. Omar, 2013. Effect of Moringa Oleifera on serum lipids and kidney function of hyperlipidemia rats. Journal of Applied Sciences Research, 9(8): 5189-5198.

Halaby, M.S., M.H. Farag and A.Z. Mohammed, 2013. Influence of Kiwifruits on hypercholesterolemia in male albino rats. Egyptian J. of Nutrition and Health. Published by Society of Feeding mind, Combating malnutrition., 8(1): 21-36.

Hassarajani, S., T. Souza, S. Mengi and Chattopadhayay, 2007. Efficacy study of the bioactive fraction (F-3) of Acoruscalamus in hyperlipidemia. Indian J Pharmacol., 39: 196-200.

Hejazi, M., 2016. Preparation of different formulae from quinoa and different sources dietary fiber to treat obesity in rats. Nature and Science., 14(2): 55-65.

ISO 5508, 1990.

ISO 5509, 2000.

J.og.Agric. and Food Chem, 2000. Flavonoids Official methods (ISO) 48: 5834-5841.

J.Sci.Food Agric, 1999. Phenolic compounds. Official methods (ISO) 79: 1625-1634.

Journal of chromatography A, 1999. 721: 247-259.

Journal of chromatography B, 2006. 830: 41-46.

Lako, J.V., M. Trenerry, N. Wahlqvist, S. Wattanapenpaiboon and R. Southeeswaran, 2007. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. Food Chemistry, 101(4): 1727-1741.

Lamothe, L., S. Srichuwong, B. Reuhs and B. Hamaker, 2015. Quinoa (Chenopodium quinoa W.) and amaranth (Amaranthus caudatus L.) provide dietary fibres high in pectic substances and xyloglucans. Food Chem, 167: 490-496.

Lopes, V., 1977. Clin. Chem., 23: 882.

Machado, F.M., M.B. Barbalho, A.M. Oshiiw, R. Goulart and O.P.J unior, 2012. Use of cereal bars with quinoa (Chenopodium quinoa W.) to reduce risk factors related to cardiovascular diseases. Tecnol. Aliment., Campinas, 32(2): 239-244.

Makni, M., N. Fetoui, H. Gargouri, T. Jaber, A. Boudawar and N. Zeghal, 2008. Hypolipidemic and hepatoprotective effects of flaxseed and pumpkin seed mixture in ω-3 and ω-6 fatty acids in hypercholesterolemic rats. Food Chem. Toxicol., 46: 3714-3720.

Mbikay, M., 2012. Therapeutic Potential of Moringa oleifera Leaves in Chronic Hyperglycemia and Dyslipidemia: A Review. Front Pharmacol., 3: 24.

Navruz, V. and N. Sanlier, 2016. Nutritional and health benefits of quinoa (Chenopodium quinoa Willd.). Journal of Cereal Science., 69: 371-376.

Olorunnisola, O., G. Bradley and A. Afolayan, 2012. Protective Effect of T. violacea Rhizome Extract Against Hypercholesterolemia-Induced Oxidative Stress in Wistar Rats. Molecules, 17: 6033-6045.

Patton, C. and S. Crouch, 1977. Enzymatic colorimetric method for determination of urea in serum. Anal. Chem., 49: 464-269.

Politeo, O., M. Jukic and M. Milos, 2006. Chemical composition and antioxidant activity of essential oils of twelve spice plants. Croat. Chem. Acta., 79(4): 545-552.

Ramos, M., A. Moreno, G. Cevallos, M. Navarro, L. Siciliano, H. Mondragón and M. Ortega, 2012. Hypolipidemic Effect of Avocado (Persea americana Mill) Seed in a Hypercholesterolemic Mouse Model. Plant Foods Hum Nutr., 67: 10-16.

Reeves, P., F. Nielsen and G. Fahey, 1993. J Nutr. Nov; 123(11): 1939.

Retiman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalo-acetic and glutamic pyruvic transamin-ases. Am. J. Clin. Path., pp: 28-56.

Ruini, L., R. Ciati, C. Pratesi, M. Marino and L. Principato, 2015. Working toward healthy and sustainable diets: the "Double Pyramid Model" developed by the Barilla center for Food and Nutrition to raise awareness about the environmental and nutritional impact of foods. Frontiers in Nutrition, 2: 1-6.

Tang, Y., X. Li, P. Chen, B. Zhang and M. Hernandez, 2015b. Characterisation of fatty acid, carotenoid, tocopherol/tocotrienol compositions and antioxidant activities in seeds of three Chenopodium quinoa Willd. genotypes. Food chemistry, 174: 502-508.

Tang, Y., X. Li, B. Zhang, P. Chen and R. Liu, 2015. Characterisation of phenolics, betanins and antioxidant activities in seeds of three Chenopodium quinoa Willd. genotypes. Food Chem, 166: 380-388.

Tricia, E., 2007. What is Creatine. Copyright © by Conjecture Coporation. http://www.wisegeek.com/what-is-creatine.htm

USDA, 2015. National Nutrient database for Standard Reference Release.

Vega-Galvez, A., M. Miranda, J. Vergara, E. Uribe and L. Puente, 2010. Nutrition facts and functional potential of quinoa (Chenopodium quinoa Willd.), an ancient Andean grain: a review. J Sci Food Agric, 90: 2541-2547.

Wang, L., J. Sun, Q. Yi, X. Wang and X. Ju, 2012. Protective Effect of Polyphenols Extract of Adlay (Coix lachryma-jobi L. var. ma-yuen Stapf) on Hypercholesterolemia-Induced Oxidative Stress in Rats. Molecules 2012, 17, 8886-8897.

Yang, H. and U. Ludewig, 2014. Lysine catabolism, amino acid transport, and systemic acquired resistance: what is the link? Plant Signal Behav, 9: e28933.

Zevallos, V., L. Herencia, F. Chang, S. Donnelly, H. Ellis and P. Ciclitira, 2014. Gastrointestinal effects of eating quinoa (Chenopodium quinoa Willd.) in celiac patients. Am J Gastroenterol., 109: 270-8.

Zhang, M., S. Swarts, L. Yin, C. Liu, Y. Tian, Y. Cao, M. Swarts, S. Yang, S. Zhang, K. Zhang, S. Ju, D. Olek, L. Schwartz, P. Keng, R. Howell, L. Zhang, P. Okunieff, 2011. Antioxidant properties of quercetin. Adv. Exp. Med. Biol., 915: 283-289.