Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

PROTECTIVE EFFECT OF QUINOA (*CHENOPODIUM QUINOA* WILLD.) SEEDS AGAINST HYPERCHOLESTEROLEMIA IN MALE RATS

Etab Saleh Alghamdi

Food and Nutrition Dept, Faculty of Home Economics, King Abdul Aziz Univ., Kingdom of Saudi Arabia, Jeddah, Saudi Arabia

ARTICLE INFO

Received:
03th Jul 2018
Received in revised form:
12th Oct 2018
Accepted:
17th Oct 2018
Available online:
26th Dec 2018

Keywords: *Quinoa Seeds, Cake, Organoleptic Evaluation, Physical Properties, Hypercholesterolemia, Liver Function, Kidney Function, Histopathology*

ABSTRACT

The primary goal of the current research is to determine the impact of dietary supplementation with quinoa seed powder (QSP) on hypercholesterolemia. More specifically, the study seeks to measure the effect of dietary supplementation with QSP on cholesterol levels, to determine if QSP use provides protection against hypercholesterolemia, in the selected population of male rats. Thirty-two male albino rats were fed a high cholesterol diet, and then fed fortified cake with different percentages (25, and 45%) of the QSP. These rats were put on an ideal diet for 14 days before being divided into four groups of eight rats each. The negative control group (Group 1) was fed a basal diet. The positive control group (Group 2), was fed a basal diet + 2% cholesterol to ensure the rats developed hypercholesterolemia. The first experimental group (Group 3) was fed the same high cholesterol diet as the second group, while adding 35% QSP to their diet, and the final experimental group (Group 4) was fed as the second group, with 45% QSP added to the diet. An experimental period of 60 days was established, after which the rats had their feed dishes removed, forcing an overnight fasting period, after which tissue samples were collected. In order to measure the total cholesterol and lipid profiles for each rat, aortic blood samples were collected. Also, the animals were tested for liver and kidney functions. Additionally, other nutritional parameters were recorded including food intake, weight gain or mass increase, and feed efficiency ratio. Finally, heart and liver were removed surgically for histopathological observation. From the obtained results, the researcher concluded that group of rats fed on diet with 2% cholesterol were at significantly increased risk for hypercholesterolemia. However, the results indicated that a diet fortified at 35% and 45% QSP improves weight gain and feed consumption, reduces lipid profiles, and reduces the risk to organ function, related to hypercholesterolemia, when compared to positive control group. More specifically, a diet with 45% QSP reduced the adverse effect of hypercholesterolemia.

To Cite This Article: Etab Saleh Alghamdi, (2018), "Protective Effect of Quinoa (*Chenopodium Quinoa* Willd.) Seeds against Hypercholesterolemia in Male Rats", ***Pharmacophore, 9(6),*** *11-21.*

Introduction

Quinoa is a nutritionally well-balanced food product with multiple functions associated with the reduction of chronic disease risk. According to [1] and [2], quinoa is one of the most versatile grain-like products available, and is available for purchase as flour, flakes and grains as well as in commercialized, or pre-packaged goods (e.g., baby food, bread, energy bars, noodles, muffins, pasta, snacks and drinks). Quinoa grains are also used as the primary ingredient in tortillas, pancakes, and in muesli or granola type products for breakfast, (replacing wheat flakes). Additionally, quinoa is also used as fiber in multiple industrial products, including cardboard, cellulose, starch, and oil [3].

In addition to this versatility, Quinoa also has the benefit of being extremely nutritionally valuable. Gordillo et al., (2016) and Tang et al., (2015b) discovered that quinoa seed has a higher biological value than other grains, or cereal grains [4, 5]. More specifically, it has a biological value of 73% while corn, wheat, and rice, have dietary values of only 36%, 49% and 56%, respectively. As a result, Quinoa's dietary value is really more similar to that of a meat product, being comparable to beef, which has a biological value of 74%. It also has a higher protein content than typical cereal grains, with protein content ranging from 12.9 to 16.5% [5]. As such, it may be useful in increasing the protein intake of those on a high cholesterol diet, and reduce the risk related to hypercholesterolemia.

Hypercholesterolemia can be a precursor to several human diseases, in part because it induces tissue damage. This tissue damage leads to oxidative modification of LDL, with excess production of lipid oxidation products and free radicals, which

Corresponding Author: Etab Saleh Alghamdi, Food and Nutrition Dept, Faculty of Home Economics, King Abdul Aziz Univ., Kingdom of Saudi Arabia, Jeddah. Email: asalghamdy2@kau.edu.sa

can increase the risk of heart diseases. Recently, the treatment of hypercholesterolemia with medicinal plants and dietary change has increased consumer demand for healthy, fresh foods. As a result, consumers are seeking natural, beneficial food items that require minimal time and effort for their preparation [6, 7].

Though quinoa is not widely consumed, further research is needed to determine the health benefits which it might provide. Current consumption is low, because of a lack of consumer knowledge regarding health and nutrition benefits, and because of the high cost associated with import of the grain. The primary goal of the current research is to evaluate the biochemical, and biological changes that occur in albino rats placed on high cholesterol diet to induce hypercholesterolemia, and to investigate the protective effect of quinoa seeds powder on hypercholesterolemia in rats, when used as a dietary supplement.

Materials and Methods

Materials

Dry quinoa seeds were purchased through the Agricultural Research Center in Cairo, Egypt. Cholesterol, cellulose, casein, vitamins and minerals were ordered via El-Gomhoria Pharmaceutical Company, in Cairo, Egypt. Corn oil and starch were obtained from the local market.

Thirty-two normal male rats, that all of them were reproductively and physically mature, (Sprague-Dawley) were obtained from the laboratory animal colony, via the Ministry of Health & Population, Helwan; Cairo - Egypt. The rats used as the sample population were received weighting approximately 150g, ± 10g per specimen. Kits were purchased from Bio-diagnostic Company in Egypt to determine serum triglycerides, cholesterol, LDL-C, uric acid, urea nitrogen, creatinine, and transaminases.

Methods

The quinoa seeds were prepared for use by washing and drying the whole raw seed products. These seeds, once cleaned, were crushed using an electric blender, to create a fine, flour-like powder. The seed powder was then mixed into the basal diet daily at the specified concentration for the control or experiment group that each rat is assigned to.

Chemical analysis of quinoa

Ash, crude fiber, moisture, and protein contents were measured by applying the method outlined by AOAC. (2000) [8]. Total carbohydrates were determined according to the methods designed by Abd El-Latif, (1990) [9]**.** Total antioxidant activity was determined according to the methods previously developed by Politeo et al., (2006) [10]**.** Vitamin contents (A, E, C, thiamine, riboflavin, niacin and folic acid) and minerals (Cu, Fe, Mn, Zn, Ca, P, Mg, Na and K) were measured based on the protocol described by J. Chrom. (1999 & 2006)**.** Finally, the amount of phenolic compounds was determined by HPLC according to the methods dictated in the J. Sci. Food Agric. (1999) [11].

Induction of hypercholesterolemia

Hypercholesterolemia was induced within the mouse population, by adding a 2% cholesterol powder into the rat's basal diet according to the methods previously developed by Hassarajani et al., (2007) [12].

Diet composition

All the rats were fed, using a basal diet, which was created following the formula developed by Reeves et al. (1993) [13].

Experimental design

The rats were adapted two weeks prior to the application of experimental conditions. Water was introduced ad-libitum. After adaptation was completed, the rats were sorted by random selection into four groups, which comprised the control and experiment groups. Each group was then fed based on the control and experiment diet for that classification for sixty days. The first group of rats, which represented the negative control group, was fed the basic basal diet only. The positive control group, Group 2, was fed the same basal diet and dietary amount as the first group plus 2% cholesterol. The third group, representing the first experimental group, was fed the same diet as Group 2, with not only the 2% added cholesterol, but also 35% added QSP. Finally, the fourth and final experimental group was fed the same diet as the previous group, but with 45% QSP. For each group of rats, the feed intake, body mass increase percentages, and feed efficiency or conversion ratios were measured based on the procedure and model established by Chapman et al., (1959) [14].

Blood Sampling

At the conclusion of the experiment period, food was removed from the rats' environment, and they were forced to fast overnight. After fasting, they were all humanly euthanized, and blood and tissue samples collected. Aortic blood samples were collected, and centrifuged at 3000 rpm for 15 minutes to separate the serum for further study. The serum was placed in dry and clean Wassermann tubes using a Pasteur pipette. The samples were then frozen at -20°C for preservation until analyses.

Biochemical analysis of serum

Total cholesterol [15], triglycerides [16], HDL-C [17], LDL-C and VLDL-C [18] were determined. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were both measured using Retiman & Frankel's (1957) approach [19]**.** Uric acid, urea nitrogen and creatinine were measured according to the procedure designed by Fossati et al., (1980), Patton & Crouch (1977) and Bartels et al., (1972), respectively [20-22].

Histopathological Examination

Tissues from the heart and liver were examined at the Histology laboratory, by the Faculty of Veterinary Medicine, Cairo University according to the methods and procedures established by Bancroft et al., (1996) [23].

Statistical analysis

Results are expressed based on the statistically mean ± SD. Data were statistically analysed using the "ANOVA," as calculated by the Computer software system SPSS (version 20).

Results and Discussion

Chemical composition of raw materials

Quinoa seed powder (QSP) was analyzed base on dry weight characteristics. The moisture, fat, protein, ash, total carbohydrates, crude fiber (g/100g DW), and total antioxidant activity by DPPH (µg/mL) were 9.8, 6.6, 14.7, 3.5, 62.5, 2.9 and 6.05% DW, respectively (Table 1).

The current study's statistical findings concur with those of Vega-Galvez et al., (2010), which showed that quinoa has a dietary intake value, for protein which is similar to the casein found in milk [24]. Quinoa is considered among the most valuable grain-based protein sources, because the protein levels in tested samples are higher than those measured in wheat, rice, maize, barley, corn and sorghum. This nutritional significance is evidenced by the fact that quinoa has been implemented in *National Aeronautics and Space Administration (NASA)*, and rations were designed for space travel due to its unique ability to meet the nutritional needs of astronauts during space missions [25, 26].

In addition, the quinoa had a higher antioxidant potential, when compared with other whole cereal grains, including barley buck, buckwheat millet, wheat, and rice according to Gordillo et al., (2016) [4]. Th exact antioxidant level of the quinoa, however, varies because the chemical constituents and antioxidant of the plant depend on several factors, including growing condition, season, maturity, genotype, post-harvest treatment and storage conditions, as described in greater detail by Navruz & Sanlier (2016) [27].

The nutritional value of QSP has led to its classification as a superfood, because it contains a wide variety of vitamins including: A, E, C, thiamine, riboflavin, niacin and folic acid. Results of nutritional analysis, given in Table (2), indicate that the quinoa used in this study contained these key vitamins with an average of 0.41 (µ/100g), 5.62 (ppm), 4.26, 0.39, 0.40, 1.13 and 75.63 (mg/100g), respectively. The current findings and dietary analysis align with previous measurements of nutritional values indicated in the research of both Alvarez et al., (2010a) and Hejazi (2016) [28, 29]**.** The data given in Table (2) also illustrated that the QSP contained the various minerals and elements including copper, iron, manganese, zinc, calcium, phosphorous, magnesium, sodium and potassium. Results indicated that QSP contained each mineral with an average values of 19.20, 73.10, 34.10, 42.00, 1064.01, 5602.30, 2534.00, 48.02 and 879.00 (mg/100g DW), respectively. These finding were as expected, given the nutritional values previously reported by Vega-Galvez et al., (2010) and Hejazi, (2016) [24, 29]. Previous researches, more specifically demonstrated that the levels of potassium, calcium and magnesium found in quinoa are in bio-available forms and sufficient quantities for human digestion and dietary use, which is necessary for maintaining a balanced human diet [24, 29].

Types and concentrations of Phenolic compounds of QSP

The results, recorded in Table (3), indicated that QSP also contains considerable levels of phenolic compounds, with an average from 29481.012 to 67.373 *(µg/100g)*. It is evident from the data that salicylic, benzoic, e-vanillic and pyrogallol were the predominant phenolic compounds present in samples of QSP, which were at concentration of 29481.012, 11032.675, 7633.651 and 6327.491 (µg/100g), respectively. It also contains other phenolic compounds which were present in moderate concentrations, such as chlorogenic acid, p-coumaric, P-OH-benzoic, cinnamic and gallic acid. Finally, analysis revealed that vanillic, ellagic, coumarin, catechol; 3-oh-tyrosol, ferulic and caffeic acids were present, but were least abundant in QSP. Previous studies noticed that, these essential nutrients including polyphenols and flavonoids, are useful in decreasing nutritional deficit among consumers, and may combat many chronic diseases [30]. More specifically, according to Mbikay, (2012) [31] phenolic compounds have anti-dyslipidemic properties, which are shown to reduce plasma TC and TG (cardiovascular prevention), and provide anti-allergic, anti-inflammatory, antiviral and anti-carcinogenic benefits. Moreover, Tang et al., (2015b) revealed that "the total phenolic content" (mg/kg) in QSP "is 466.99, 634.66 and 682.05 for white, red and black Quinoa, respectively. [5]" Thus, quinoa presents at least 23 phenolic compounds, all of which have health related benefits. This was further confirmed by Asao & Watanabe, (2010) and Gordillo et al., (2016), whose studies confirmed that quinoa contains more phenols than whole cereals [4, 32].

Biological evaluation:

The mean values of body weight gain (BWG %) was calculated for all rats in the study population. The feed intake (g/day for each rat) and feed efficiency ratio (FER) were calculated for subjects in the negative control group, positive control group (hypercholesterolemia), hypercholesterolemia group fed additional QSP at 35% and hypercholesterolemia group fed

on QSP at 45%, as summarized in Table (4). It is observed that there were significant increases in BWG, FI and FER for the control positive group (18.22± 1.94, 12.36± 0.65 & 0.728 ± 0.18) as compared to the healthy control group (negative; 13.39±1.74, 10.80± 0.38 & 0.68± 0.16, respectively). In contrast, however, the rats with induced hypercholesterolemia received diet containing QSP ratios of 35% or 45%, had significantly lower values (P< 0.05) for their BWG, FI and FER when compared to the positive control group. This indicates that QSP provided some protection against weight gain, when incorporated into the diet. These findings are in harmony, with those of Vega-Galvez et al., (2010) and Barakat & Mahmoud (2011), whose studies showed that a hypercholesterolemic diet causes increase in BWG as compared with the BW and dietary content of a healthy control group [24, 33]. Also, consumption of QSP plays a role in regulating energy and maintains body weight balance. Moreover, they indicated that quinoa is an important instance of functional food, used to improve nutrient intakes and lower body weight, and possibly reducing the risk of various cholesterol related diseases.

Influence of *QSP* on lipid profile of hypercholesterolemic rats:

The influence of QSP on lipid profile in the rats from each group, is presented in Tables (5 & 6). It is noted that the positive control group, when fed using a basal diet, which contained 2% cholesterol, experienced a statistically significant increase (P< 0.05) in the mean values of TC, TG, LDL-C, and VLDL-C (127.13±3.01, 74.64±1.79, 93.82 ±2.07 and 14.19 ±1.6), compared with the control negative group, which was fed the basal diet only (101.09±1.23, 43.18±2.07, 64.68 ±3.05 and 08.60, ±1.3, respectively). On the contrary, HDL-C facilitates catabolism, by helping to transport excess cholesterol out of the peripheral tissue, and into the liver. [34]. Furthermore, our results closely correspond to those of Wang et al., (2012) which indicated that the increase in HDL-C ratio is one of the most significant identifiers for any anti-hypercholesterolemia agent [35].

Rats which were fed a high-cholesterol diet with two various levels from QSP fortified at 35% & 45%, had lower mean values of lipid profile compared with the positive control group. This might be as a direct result of reduced absorption of cholesterol, when accompanied by an increase in fecal bile acid and excretion of cholesterol, which is attributed to the dietary supplementation. In fact, the best results in lipid fractions for all treated groups was noticed in the group fed on a high cholesterol diet fortified with QSP at 45%, because this treatment improved levels of serum cholesterol and triglycerides. These findings closely correspond to the previous researches of Farinazzi et al., (2012) and Zevallos et al., (2014) [36, 37]**.**

Influence of *QSP* on liver enzymes of hypercholesterolemic rats:

The results of the analysis of the influence of QSP on the liver enzyme in rats is displayed in Table (7). Findings indicated that feeding rats on the basal diet containing 2% cholesterol resulted in a statistically significant increase (P< 0.05) in serum AST and ALT when compared to healthy rats group (33.26±3.41 and 21.05±2.43 vs. 16.59±5.51 and 09.23±3.04 U/L, respectively). The high levels of AST and ALT in serum are indicators of liver dysfunction. These findings align with those of Al-Dosari, (2011), which revealed that rats feeding on a high cholesterol diet for 70 day demonstrated a statistically significant effect, and increased the bilirubin levels and serum liver marker enzymes (GOT, GPT, GGT, ALP) [38].

Results also indicated that, feeding a high cholesterol diet, fortificated with QSP at 35% and 45% levels, resulted in a statistically significant reduction (p<0.05) in serum AST and ALT when compared with those of the positive control group. The best results of liver function recorded was among hypercholesterolemia rats fed on a diet fortified with 45% QSP, which is further confirmed by Zevallos et al., (2014) [37].

Effect of QSP on kidney functions of hypercholesterolemic rats:

The onset of renal diseases, or decreased kidney function, is marked by an increase in the concentrations of the metabolites in the blood. This may be due to increased activity rate of lipid peroxidation, as well as elevated triacylglycerol and cholesterol levels. Results presented in Table (8) summarize the analysis of the different ratios of QSP on serum urea nitrogen; creatinine, and uric acid in rats given a high cholesterol diet, when compared to the negative control group.

Serum urea nitrogen:

The ingestion of a persistently high cholesterol diet induced hypercholesterolemia in rats, resulting in a higher value of serum urea nitrogen in the blood. The serum urea nitrogen reached 146.08 ± 7.2 in the positive control group, when compared with negative control group which had serum urea nitrogen levels of 88.41±4.05 mg/dl. Thus, the increased levels may be related to the high cholesterol diet, and the kidneys' loss of function. It is therefore concluded that when the body is using large amounts of cholesterol in the diet, the serum urea nitrogen level will rise. The current results also indicated that the level of urea nitrogen at the conclusion of the experimental stage decreased gradually based on the concentration of QSP fed to the rats, and the ratios reached from 146.08 ± 7.21 to 132.4 ± 4.65 and 97.07 ± 4.80 mg/dl respectively for fortification diet with 35% and 45% QSP.

Results from the current study closely correspond with those of Zevallos et al., (2014) which demonstrated that renal damage in hypercholesterolemia may be related to increase in serum urea nitrogen level which indicates glomerular and dysfunction of the kidney at the tubular levels [37].

Serum creatinine:

High creatinine levels indicate that a person is experiencing kidney failure, and may occur as a result of increased cholesterol levels as revealed by Barakat & Mahmoud (2011) [33]**.** The current study's findings, as shown in Table (8) indicate that the level of serum creatinine raised to a statistically significant level (p< 0.05) in groups of rats given a high cholesterol diet,

when compared to the negative control groups (1.24 ± 0.33 *vs* 0.84 ± 0.05). The mean values and standard deviation of serum creatinine for those rats whose diet was fortified with 35% and 45% QSP were 0.96±0.05 and 0.89±0.03 mg/dl, respectively. These results indicated that there was improvement in the serum creatinine level in groups fed on 45% QSP compared with the other groups of rats.

Serum uric acid

It is observed that the control positive group, that were fed a high cholesterol diet, have a statistically significant growth ($p< 0.05$) of serum uric acid levels when compared to those individuals in the control negative group fed the basal diet (4.49±0.21 *vs* 1.56±0.16 mg/dl). It was also observed that administration of 35% and 45% QSP significantly reduced the uric acid level. The ratios for those rats supplemented with 35% and 45% QSP were 3.64±1.11 and 2.21±0.23 mg/dl, respectively.

Data revealed that a highly significant reduction of all parameters including urea nitrogen, creatinine and uric acid was observed in the group fed a high cholesterol diet fortified with QSP at 45%. This indicates that dietary management is an essential component of care for patients with hypercholesterolemia. This was previously determined by the research of Zevallos et al., (2014) [37].

Physical measurements of cake fortified with quinoa seeds powder

Objective evaluation results of the parameters for control cake with wheat flour and fortified cake with various levels of quinoa seeds powder at 25%, 35% and 45% are illustrated in Table (9). It was found that the percentage of weight, relative to control value, decreased upon increasing the level of quinoa seeds powder by 97, 95 & 91%, respectively. At the same time, percent of volume relative of control cake compared to the cake that fortified with quinoa seeds at different ratios decreased by 97.77; 96.49 & 87.25, respectively. This reduction in volume could be related to lower gluten content of the cake, fortified with quinoa seeds which had high water absorption, therefore less water is available for gluten formation.

Density as a percentage of control value increased with increasing level of quinoa seed powder, including 0.41, 0.44, 0.46 & 0.48 g/cm^3, respectively. This may be the result of an inverse relationship between volume and density, which determined that as the volume decreases, the density increases. The present results are aligned with those obtained by Titcomb & Juers (1996) who found that addition of cereal bran to the recipe for baked goods may results in a decrease in volume for the final baked product [39]. Jinshui & Benedito (2002) mentioned that fiber increased water absorption and adversely affect volume and specific volume in bakery products [40]. It was confirmed by Bouaziz et al., (2010) that specific volume of bread decreases as dietary fiber increase [41].

Organoleptic evaluation of fortified Cakes with Quinoa seeds powder

Organoleptic evaluations of fortified cake with three different levels of quinoa seed powder (25%; 35% and 45%) are presented in Table (10). The results showed no significant differences ($P < 0.05$) between the tested fortified cakes at 25%; 35% and 45% quinoa seed powder, when compared to the control sample with regard to odor; volume and external color. Fortified cakes with 25% and 35% quinoa seed powder had lower scores for taste (15.5±0.9 and 16.6±0.6, respectively), interior color (8.9±0.8 and 8.6±1.4, respectively) and general appearance (13.3±1.5 and 13.2±1.3, respectively) which differ significantly at ($P < 0.05$) compared to the control cake scores (19.0±1.1; 9.6 ±0.7 and 14.3 ±0.2, respectively). At the same time, fortified cakes at 25%; 35% and 45% quinoa seed powder had lower scores for texture (13.2±0.9; 13.5±1.2 and 13.7±1.2, respectively), which differ significantly at ($P < 0.05$) when compared to the control cake score (14.1±0.3).

These findings align with previous research claims made by Sindhuja et al., (2005), which revealed that quinoa seeds can be used as carriers of nutrition, resulting in an improved diet, and can be utilized as a functional food ingredient [42]. QSP can be ground or riced and then implemented in the baking of various recipes, including breads, cookies, muffins, pancakes, pasta, muffins, and puddings [43, 44].

Histopathological results

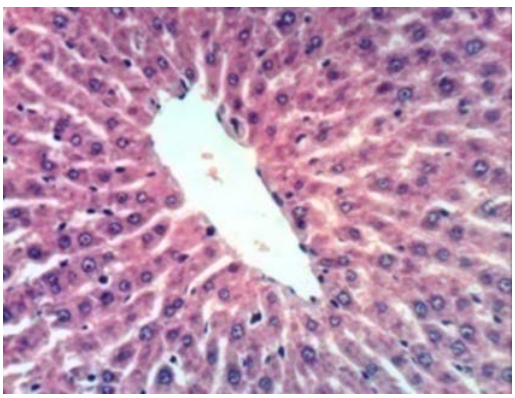
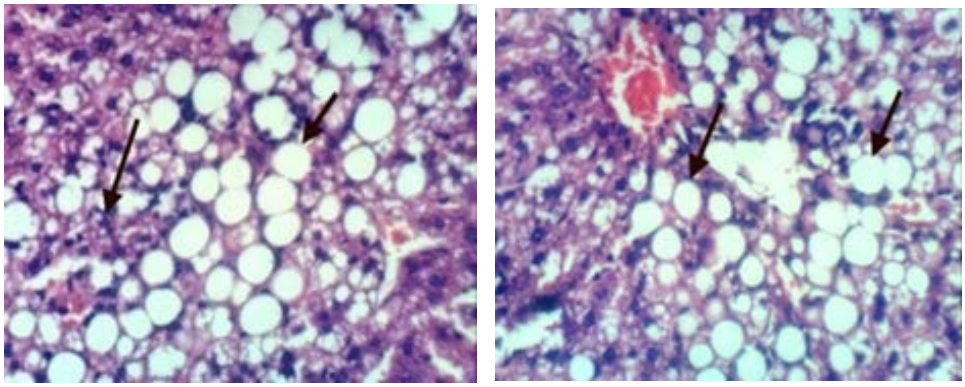
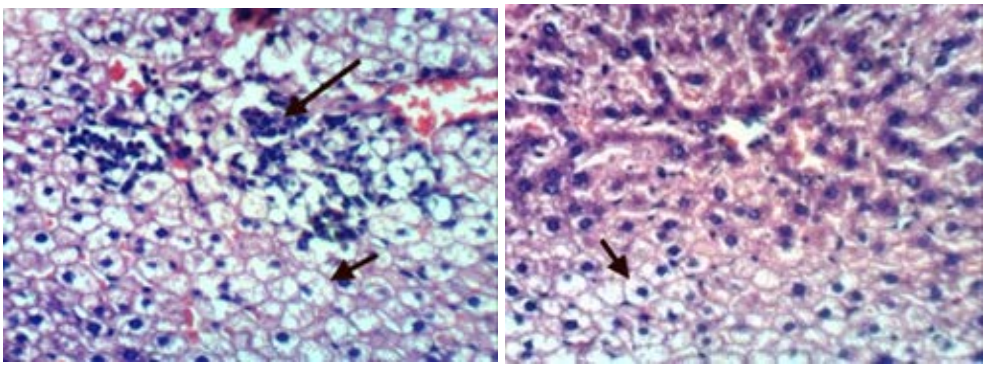


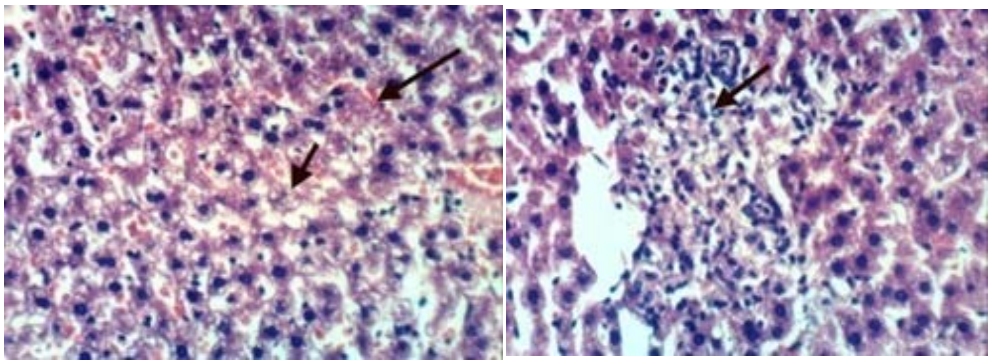
Figure 1: Microscopic Image of liver tissue sample from the control negative group of rat population. Image demonstrates a normal histological structure of the hepatic lobule (H & E X 400).



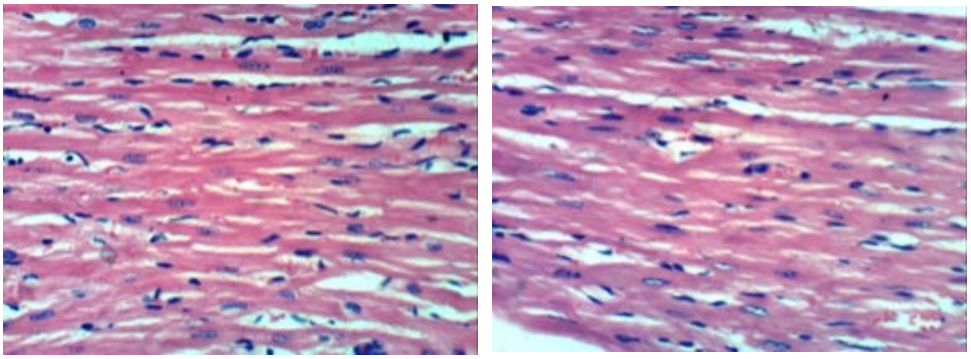
Figures 2 & 3: Microscopic Image of liver tissue sample from the control positive group, demonstrates evidence of inflammatory cell infiltration and macrovascular steatosis of hepatocytes infiltration (H & E X 400).



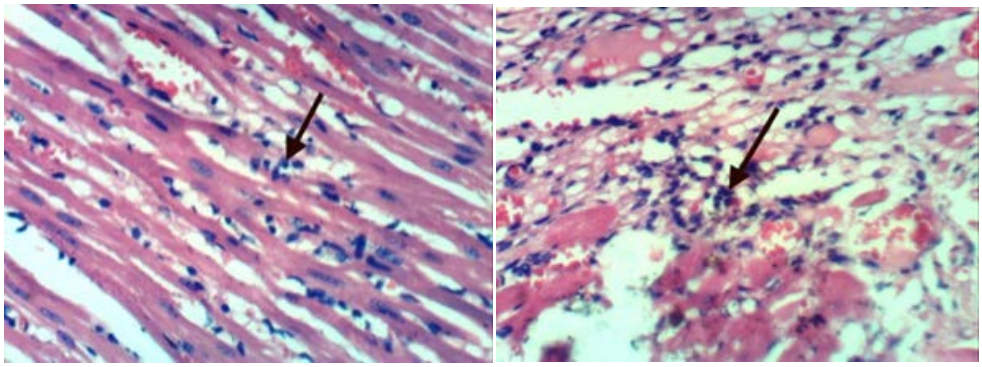
Figures 4 & 5: Liver tissue sample from the first experiment group, fed positive diet with 35% QSP showed steatosis of hepatocytes and infiltration of inflammatory cell (H & E X 400). Also, Figure (5): showed vacuolization of hepatocytes (H & E X 400).



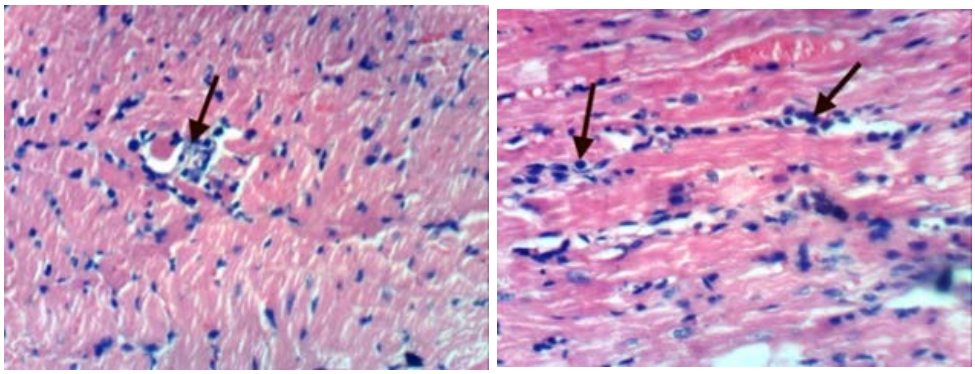
Figures6 & 7: Liver tissue sample from the second experiment group, fed on a positive diet with 45% QSP showed vacuolization of hepatocytes and congestion of the hepatic sinusoids (H & E X 400). Also, it showed focal hepatic necrosis, which may be related to the increase of inflammatory cell infiltration (H & E X 400).



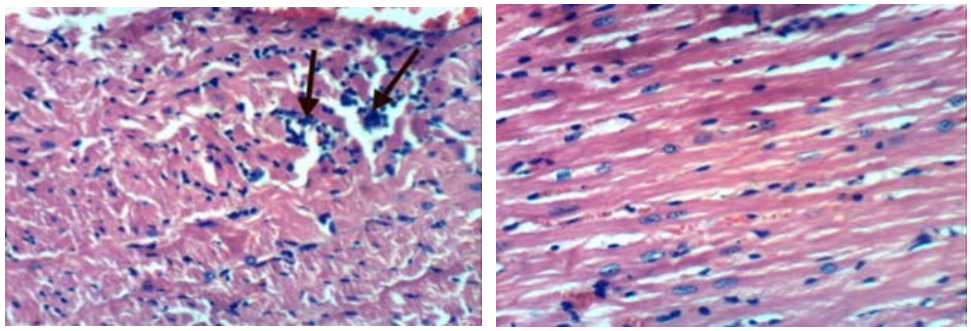
Figures 8 & 9: Heart of rat from negative group showed normal cardiac myocytes (H & E X 400).



Figures 10 & 11: Heart of rat from positive group fed the basal diet and 2% cholesterol showed myocarditis. Notice inflammatory cell infiltration between cardiac muscles (H & E X 400).



Figures 12 & 13: Heart of rat from group fed on basal diet + 2% cholesterol + 35% QSP showed myocarditis. Notice inflammatory cells infiltration between cardiac muscles (H & E X 400).



Figures 14 & 15: Heart of rat from group fed on basal diet + 2% cholesterol + 45% QSP showed myocarditis. Notice necrosis of focal myocytes associated with inflammatory cell infiltration (H & E X 400). At the same time, Figure (15) showed no histopathological changes (H & E X 400).

Recommendation

- Food industries should be encouraged to use quinoa seeds for fortification, and it should be included in wide scale in factories, medicines and processed food products.
- Nutrition education programs need to be designed to educate the public about the positive impact of quinoa seeds on decreasing the risk of hypercholesterolemia.

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

Component (g / 100g DW)	Quinoa seed powder
Moisture	9.8
Fat	6.6
Protein	14.7
Ash	3.5
Total carbohydrates	62.5
Crude fiber	2.9
Total antioxidant activity DPPH (µg/mL)	6.05

Table 2: Chemical composition of quinoa seeds powder (mg/100g dry weight basis)

Vitamins (%)	Quinoa seed powder	Elements (mg/100g DW)	Quinoa seed powder
Vitamin A (µ/100g)	0.41	Copper	19.20
Vitamin E (ppm)	5.62	Iron	73.10
Vitamin C (mg/100g)	4.26	Manganese	34.10
Thiamine (mg/100g)	0.39	Zinc	42.00
Riboflavin (mg/100g)	0.40	Calcium	1064.01
Niacin (mg/100g)	1.13	Phosphorous	5602.30
Folic acid (mg/100g)	75.63	Magnesium	2534.00
----	----	Sodium	48.02
		Potassium	879.00

Table 3: Types and concentrations of phenolic compounds of quinoa seed powder.

Phenolics	Phenolic compounds (µ g/100g) of Quinoa	Phenolics	Phenolic compounds (µ g/100g) of Quinoa
Gallic	1782.872	Ferulic	572.180
Pyrogallol	6327.491	Iso-ferulic	104.466
4-Amino-benzoic	101.779	Reversetrol	67.373
3-oh-Tyrosol	603.679	Ellagic	881.392
Protocatchuic	381.711	e-vanillic	7633.651
Chlorogenic	3765.182	Alpha-coumaric	180.172
Catechol	761.490	Benzoic	11032.675
Epicatechein	372.170	3,4,5-methoxy-cinnamic	271.902
Caffeine	116.461	Coumarin	784.638
P-OH-benzoic	2973.782	Salycilic	29481.012
Caffeic	528.512	P-coumaric	2873.122
Vanillic	982387	Cinnamic	2732.735

Table 4: Effect of feeding on high cholesterol diet fortified with QSP on body weight gain, feed intake, and feed efficiency ratio**.**

Parameters / Groups	Body Weight Gain (BWG %)	Feed Intake (g/day)	Feed efficiency ratio (FER)
Control (-ve)	13.39^{c} ±1.74	10.80^{b} ±0.38	0.680^{b} ±0.16
Control (+ ve)	$18.22.^{a}$ ±1.94	$12..36^{a}$ ±0.65	0.728^{a} ±0.18
Control (+ ve) with QSP 35%	15.26^{b} ± 2.06	10.95^{b} ±0.41	0.089^{c} ± 0.75
Control (+ ve) with QSP 45%	13.29^{c} ± 1.09	10.07^{bc} ±0.23	0.098^{c} ± 0.16

*Results are expressed as means ±SD.

*Values represented per column, have shown a statistically significant difference ($p<0.05$).

Table 5: Effect of feeding on high cholesterol diet fortified with quinoa seed powder on cholesterol and triglyceride of rats.

Parameters / Groups	mg/dl	
	Cholesterol	**Triglyceride**
Control (-ve)	101.09 ± 1.23^{d}	43.18 ± 2.67^{d}
Control (+ ve)	127.13 ± 3.01^{a}	74.64 ± 1.79^{a}
Control (+ ve) with QSP 35%	121.06 ± 2.12^{b}	45.04 ± 2.46^{b}
Control (+ ve) with QSP 45%	112.13 ± 1.72^{c}	44.46 ± 1.29^{c}

All results are expressed as means ±SD.

*Values represented per column, have shown a statistically significant difference ($p<0.05$).

Table 6: Effect of feeding on high cholesterol diet fortified with quinoa seed powder on HDL-C, LDL-C, and VLDL-C.

Parameters / Groups	mg/dl		
	HDL-C	**LDL-C**	**VLDL-C**
Control (-ve)	27.21 ± 1.30^{a}	64.68 ± 3.05^{d}	08.60 ± 1.3^{b}
Control (+ ve)	20.14 ± 1.26^{d}	93.82 ± 2.07^{a}	14.19 ± 1.6^{a}
Control (+ ve) with QSP 35%	25.71 ± 1.52^{c}	86.11 ± 2.09^{b}	9.12 ± 0.6^{b}
Control (+ ve) with QSP 45%	24.85 ± 1.85^{b}	78.28 ± 1.07^{c}	8.98 ± 1.8^{b}

Table 7: Effect of feeding on high cholesterol diet fortified with quinoa seed powder on liver function of rats.

Parameters / Groups	AST (u/l)	ALT (u/l)
Control (-ve)	16.59 ± 5.51^{d}	09.23 ± 3.04^{c}
Control (+ ve)	33.26 ± 3.41^{a}	21.05 ± 2.43^{a}

Control (+ ve) with QSP 35%	25.20±2.67[b]	11.56±1.71[b]
Control (+ ve) with QSP 45%	19.83±4.42[c]	9.89±1.36[d]

*Results are expressed as means ±SD.
*Values represented per column have shown a statistically significant difference (p<0.05).

Table 8: Effect of feeding on high cholesterol diet fortified with quinoa seed powder on kidney functions of rats.

Parameters / Group	Urea nitrogen	Creatinine	Uric acid
	mg/dl		
Control (-ve)	88.41±4.05[c]	0.84±0.05[c]	1.56±0.16[d]
Control (+ ve)	146.08±7.21[a]	1.24±0.33[a]	4.49±0.21[a]
Control (+ ve) with QSP 35%	132.34±4.65[b]	0.96±0.05[b]	3.64±1.11[b]
Control (+ ve) with QSP 45%	97.07±4.80[d]	0.89±0.03[c]	2.21±0.23[c]

*Results are expressed as means ±SD.
*Values represented per column have shown a statistically significant difference (p<0.05).

Table 9: Physical properties of cake fortified with different levels of quinoa seeds powder compared to control.

Parameters	Control	Quinoa seeds powder		
		25%	35%	45%
Weight before baking (g)	450	450	450	450
Weight after baking (g)	359± 0.5[d]	376± 0.2[c]	382± 0.3[b]	395± 0.2[a]
Change in weight after baking (%)	12.22	15.11	16.44	20.22
Weight relative to control (%)	100	97	95	91
Loaf volume (cm^3)	855	846	836	825
Volume relative to control (%)	100	97.77	96.49	87.25
Loaf height (cm)	8.8± 0.1[a]	8.7± 0.2[b]	8.7± 0.3[b]	7.8± 0.2[c]
(cm^3/g) Specific volume	2.38	2.25	2.18	2.08
Blank density (g/cm^3)	0.41	0.44	0.46	0.48

Each value represents the mean of 3 replications and is expressed as mean ± SD. Means in the same line with different superscript letters demonstrate a statistically significant difference at $p < 0.05$.

Table 10: Organoleptic evaluation of cake prepared with different levels of quinoa seeds powder.

Properties	Treatment				
	Control	QSP 25%	QSP 35%	QSP 45%	LSD
Taste 20	19.0±1.1[a]	15.5±0.9[d]	16.6±0.6[c]	18.7±0.6[b]	0.9
Odor 20	18.9 ±1.0	17.8±1.1	18.0±0.9	18.7±0.9	n.s
Texture 15	14.1±0.3[a]	13.2±0.9 [bc]	13.5±1.2[b]	13.7±1.2[bc]	0.8
Volume 10	9.2 ±1.1	8.6±1.7	8.9±1.4	9.2±1.0	n.s
Color interior 10	9.6 ±0.7[a]	8.9±0.8[b]	8.6±1.4[b]	9.4±1.4[a]	0.8
Color external 10	9.6 ±0.3	9.1±1.2	9.5±1.3	9.3±1.3	n.s
General appearance 15	14.3 ±0.2[a]	13.3±1.5[b]	13.2±1.3[b]	14.0±1.3[a]	0.6

LSD: Level Significant Difference n.s.: non-significant.
Means with the different letter superscripts in the column denote significant at ($P < 0.05$).

References

1. Fuentes,F. & Bhargava,A. (2011). Morphological analysis of quinoa germplasm grown under lowland desert conditions. Journal of Agronomy and Crop Science, 197: 124-134.
2. Machado,F.; Barbalho,M.; Oshiiw,A.; Goulart,R. & Junior,O. (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, abr.-jun. 2012
3. Ruini,L.; Ciati,R.; Pratesi,C.; Marino,M. & Principato,L. (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.
4. Gordillo,S.; Díaz-Rizzolo,D.; Roura,E.; Massanés,T. & Gomis,R. (2016). Quinoa (Chenopodium quinoa Willd), from Nutritional Value to Potential Health Benefits: An Integrative Review. J Nutr. Food Sci. 6 (3): PP: 2-10.
5. Tang,Y.; Li,X.; Chen,P.; Zhang,B. & Hernandez,M. (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.

6. Olorunnisola,O.; Bradley,G. & Afolayan,A. (2012).Protective Effect of T. violacea Rhizome Extract Against Hypercholesterolemia-Induced Oxidative Stress in Wistar Rats. Molecules 17, 6033-6045.
7. Yang,H. & Ludewig,U. (2014) Lysine catabolism, amino acid transport, and systemic acquired resistance: what is the link? Plant Signal Behav 9: e28933.
8. AOAC. (2000). Association of Official Analytical Chemist Official Methods of Analysis 17th ed., Washington, USA.
9. Abd El-Latif,B.M., (1990). Improvement of some bakery products thesis. Ph.D. F. Tech. Agric. Moshtohor, Zagazig Univ.
10. Politeo,O.; Jukic,M. & Milos,M. (2006). Chemical composition and antioxidant activity of essential oils of twelve spice plants. Croat. Chem. Acta., 79 (4): 545-552.
11. J.Sci.Food Agric. (1999). Phenolic compounds. Official methods (ISO) 79: 1625-1634.
12. Hassarajani,S.; Souza,T.; Mengi,S. & Chattopadhayay,A. (2007). Efficacy study of the bioactive fraction (F-3) of Acoruscalamus in hyperlipidemia. Indian J Pharmacol 39: 196-200.
13. Reeves,P.; Nielsen,F. & Fahey,G. (1993). J Nutr. Nov; 123 (11):1939.
14. Chapman,d.; Castilla,R. & Cambell,J.(1959). Evaluation of protien in foods: A method for the determination of protein efficiency ratio .cam.J.biochem.physical. 37: 697-686.
15. Allain,C., Poon,L. & Chan,C. (1974). Enzymatic determination on total serum cholesterol.Clin.chem. 20: 470-475.
16. Fassati,p. & Prencipe,l.. (1982). Triglycerides determination after enzymatic hydrolysis. clin. chem., 28:2077.
17. Lopes,V. (1977). Clin. Chem. 23:882.
18. Friedewald,W.; Leve,R. & Fredrichson,D. (1972). Estimation of concentration of low-density lipoproteins separated by three different. Clin.Clem.18: 499-502.
19. Retiman,S. & Frankel,S. (1957). A colorimetric method for the determination of serum glutamic oxalo-acetic and glutamic pyruvic transamin-ases. Am. J. Clin. Path. 28–56.
20. Fossati,P.; Prencipe,L. & Berti,G. (1980). Enzymatic colorimetric method of determination of uric acid in serum. Clin. Chem. (18) 499-502.
21. Patton,C. & Crouch,S. (1977). Enzymatic colorimetric method for determination of urea in serum. Anal. Chem. 49: 464 – 269.
22. Bartels,H.; Bohemer,M. & Heirli,C. (1972). Colorimetric kinetic method of creatinine. Clin. Chem. Acta. 37: 193.
23. Bancroft,D.; Stevens,A. & Turner,R. (1996). Theory and practice of histological techniques. 4th ed, Churchill Living Stone, Edinburgh, Landon, Melbourne
24. Vega-Galvez.A; Miranda, M.; Vergara, J.; Uribe, E. & Puente, L. (2010). Nutrition facts and functional potential of quinoa (Chenopodium quinoa Willd.), an ancient Andean grain: a review. J Sci Food Agric 90: 2541-2547.
25. Cooper,R. (2015). Re-discovering ancient wheat varieties as functional foods. J Tradit Complement Med 5: 138-143.
26. USDA (2015) National Nutrient database for Standard Reference Release.
27. Navruz,V. & Sanlier,N. (2016). Nutritional and health benefits of quinoa (Chenopodium quinoa Willd.). Journal of Cereal Science 69; 371-376.
28. Alvarez,J.; Wijngaard,H.; Arendt,E. & Gallagher,E. (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.
29. Hejazi,M. (2016). Preparation of different formulae from quinoa and different sources dietary fiber to treat obesity in rats. Nature and Science; 14 (2) pp: 55-65.
30. Lako,J.; Trenerry,M.; Wahlqvist,N. ; Wattanapenpaiboon,S. & Southeeswaran, R. (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.
31. Mbikay,M., (2012). Therapeutic Potential of Moringa oleifera Leaves in Chronic Hyperglycemia and Dyslipidemia: A Review. Front Pharmacol., 3: 24.
32. Asao,M. & Watanabe, K. (2010). Functional and Bioactive Properties of Quinoa and Amaranth. Food Sci Technol Res 16: 163-8.
33. Barakat,L. & Mahmoud,R. (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.
34. Makni,M.; Fetoui,N.; Gargouri,H.; Jaber,T.; Boudawar,A. & Zeghal,N. (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.
35. Wang,L.; Sun,J.; Yi,Q.; Wang,X. & Ju,X. (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.

36. Farinazzi,F.; Barbalho,S.; Oshiiwa,M., Goulart,R. & Pessan,O. (2012). Use of cereal bars with quinoa (Chenopodium quinoa W.) to reduce risk factors related to cardiovascular diseases. Cienc. Technol. Aliment. Campinas 32 (3), 239-244.
37. Zevallos,V.; Herencia,;L.; Chang,F.; Donnelly,S.; Ellis,H. & Ciclitira,P. (2014). Gastrointestinal effects of eating quinoa (Chenopodium quinoa Willd.) in celiac patients. Am J Gastroenterol 109: 270–8.
38. 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.
39. Titcomb,S. & Juers,A. (1996). Reduced calorie, high fiber content breads and methods of making some. Us Patent (4): 590, 76
40. Jinshui,W. & Benedito,C. (2002). Effect of the addition of different fibers on wheat dough performance and bread quality. Food Chemistry, 79 (2): 221-226, 27.
41. Bouaziz,M.; Amara,W.; Attia,H., Blecker,C. & Besbes,S. (2010). Effect of the addition of defatted date seeds on wheat dough performance and bread quality. Journal of Texture Studies, 41:511–531.
42. Sindhuja,A.; Sudha,M. & Rahim,A. (2005). Effect of incorporation of amaranth flour on the quality of cookies. European Food Research and Technology, vol.221, pp.597-601.
43. Grobelnik,M.; Turinek,M.; Jakop,M.; Bavec,M. & Bavec,F. (2009). Nutrition value and use of grain amaranth: potential future application in bread making. Agricultura, no.6, pp.43-53.
44. Schoenlechner,R; Drausinger,J.; Ottenschlaeger,V.; Jurackova,K. & Berghofer,E. (2010) Functional properties of gluten-free pasta produced from amaranth, quinoa and buckwheat. Plant Foods Hum Nutr 65: 339-349.