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EFFECT OF QUINOA ON BIOLOGICAL, BIOCHEMICAL AND HISTOLOGICAL PARAMETERS OF ALBINO MALE RATS

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ABSTRACT:

This study was aimed to determine the effect of adding quinoa seeds powder (QSP) on biological, biochemical and histological parameters of albino male rats as well as preparing functional biscuit supplemented with quinoa powder. The results of QSP in the current study show that DPPH radical scavenging activity (%) was gradually increased with the increasing the concentration from 1.5 to 10. Whereas, IC_{50} was recorded 4.39. Wheat flour was substituted with quinoa powder at a rate of 10,15 and 20% in tested biscuit. The panel test revel that 20% quinoa biscuit was recorded the highest general acceptability values by the panelists. So, this quinoa percentage was used in the biological experimental design. Twelve male rats weighting 120±10g were divided into two main groups (6 each). The first group was fed on basal diet and the second group was fed on basal diet containing 20% QSP. Nutritional, biochemical and histological parameters revel that feed intake, body weight, feed efficiency ratio, AST, ALT, Creatinine(Cr), TG, TC, LDL and VLDL were insignificant decreased compared with control group. Whereas, urea, Blood Urea Nitrogen (BUN) and HDL were insignificant increased compared with control group. The results of histological examination for liver and kidney were similar to those of the previous studies. According to these findings quinoa may be used in several food products and might has a beneficial food property.

Key Words: Quinoa biscuit, Liver and Kidney functions, Lipid profile

INTRODUCTION

Quinoa is a grain crop grown primarily for its edible seeds. The plant has originated in the Andean region of South America where it was domesticated 3,000-4,000 years ago for human consumption **(Tang *et al.*, 2015).** Therefore, The Food and Agriculture Organization (FAO) of the United Nations announce 2013 to be the international year of quinoa. Because quinoa has a high nutritive value and desirable agricultural traits as tolerance to drought and salinity, it has gained increased demand in recent years .

The winter climate in Egypt provides good production of quinoa and it has already been cultivated in saline soils in Upper Egypt **(El-Assiuty *et al.*, 2014).** Quinoa is a species of the goosefoot genus

(Chenopodium quinoa). It is one of the seeds considered as pseudo cereals. This is also because of its remarkable composition and exceptional balance between carbohydrates, fat and protein (**James, 2009**). Also, protein composed of 16 amino acids being rich in lysine, threonine, and methionine **(Wright *et al.,* 2002).** The protein is of high quality containing much higher content of lysine than cereals and even milk and being devoid of gluten makes it suitable for celiac patients **(Stikic *et al.,* 2012).** Quinoa is moderately high in calories; starch present in the form of small granules; high viscosity make it useful for specialized industrial applications **(Tang *et al.,* 2002).** Moreover, quinoa rich in essential vitamins and minerals **(Konishi *et al.,* 2004).** Quinoa, according to sowing density, can grow from 1 to 3 m high. The seeds can germinate very fast, in a few hours after having been exposed to moisture. Quinoa crops is variety, it changes from white, yellow or light brown to red **(Valencia-Chamorro *et al.,* 2003).** Seed colurs go from white to grey and black, potentially having tones of yellow, rose, red and purple and violet, often with very colourful mixes in the same panicles **(Vega-Gálvez *et al.,* 2010).**

Quinoa is an excellent example of (functional food) that aims at lowering the risk of various diseases *(***Vega-Gálvez *et al.,* 2010).** On the other hand, **Pa´sko *et al.,*(2009)** concluded that functional properties given by strongly active compounds including, flavonoids, phenolic acids, fat soluble vitamins, fatty acids, high dietary fiber, trace elements and other compounds which can prevent oxidative stress, particularly for all cell processes requiring antioxidant protection of membranes, like neuronal activity, with minerals and amino acid contents with potential implications for aiding memory and lowering anxiety under stressful conditions **(Gorinstein *et al.,* 2007).** Quinoa contains isoflavones (daidzein & genistein), these hormones could be recognized by human estrogen receptors and act as antagonists of vessel contraction and reduce arterial resistance; by that, quinoa offer an advantage over other plant foods for human nutrition **(James, 2009).** Quinoa is a wonderful weight-loss food that may also be ingested after weight-loss exercises **(Pasko *et al.,* 2010).**

According to **(Ogungbenle, 2003)**, the quinoa seed flour has good gelation property, water-absorption capacity, emulsion capacity and stability, as well contains high percentages of D-xylose & maltose, and low contents of glucose & fructose, which allows its use in malted drink formulations. Quinoa flour is commonly used in baby foods, soup, cookies, muffins, breakfast cereal, flakes, pasta, snacks, drinks, beer, diet supplements, and extrudates **(Dogan and Karwe, 2003).** Puffed grains of quinoa are produced commercially in Peru and Bolivia, in addition of that, the plant is sometimes grown as a green vegetable and its leaves are eaten fresh in combination dishes or cooked as rice. Whole plant is also used a green fodder to feed cattle and poultry **(Repo-Carrasco and Serna, 2011).** The saponins obtained as a by-product in the processing of quinoa can be utilized by the cosmetics and pharmaceutical industries **(Bhathal *et al.,* 2015).** In general, quinoa seeds contain saponins in the seed coat, except sweet varieties, without saponin or containing less than 0.11% **(James, 2009).** In addition, quinoa based extruded products developed during the past years can be noted as new quinoa food products. Quinoa extrusion processes involve high pressure and temperatures, as well as shearing over short periods of time. This process restructures the starch and protein content of quinoa, thus producing different textures **(Viktória *et al.,* 2020).** In contrast to the puffed quinoa, there is no apparent nutritional degradation of quinoa in this process, probably due to shorter processing times **(Birbuet *et al.,* 2009).** This study was aimed to determine the effect of adding quinoa seeds powder (QSP) on biological, biochemical and histological parameters of albino male rats.

MATERIALS AND METHODS

Materials

Quinoa ***(Chenopodium quinoa* Willd.*)*** seeds were obtained from National Research Center, Giza, Egypt. Basal diet formula (AIN 93) was purchased from El-Gomhoriya Pharmaceutical Company, Cairo, Egypt. Starch, corn oil, and sucrose were obtained from the Egyptian local market. As well as chemical kits were obtained from Gamma Trade, Giza, Egypt.

Methods

Quinoa powder and tested biscuit formulas were prepared according to **Park and Morita, (2005) and Sukhcharn *et al.,* (2010),** respectively**.** DPPH and IC_{50} analyzed were carried out by the method described by **Brand-Williams *et al.,* (1995)**. Quinoa chemical composition was analyzed using **(AOAC 2012)**. Feed intake, body weight gain and feed efficiency ratio were calculated using the method of **Chapman *et al.,* (1959).** Liver transaminases (**AST** and **ALT**) were analyzed according to **Young (2001).** Urea, creatinine and blood urea nitrogen were assessed according to **Tabacco, (1979), Young, (2001) and Schrier, (2008),** respectively. Total cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein and very low-density lipoprotein were assessed using the methods described by **Allain *et al.,* (1974), Fossati and Principe,** (**1982**), **Friedewald *et al.,* (1972).** Histological examination was done according to **Bancroft and Stevens, (1996).** Statistical analysis was done using ANOVA test according to **Armitage and Berry, (1987)**.

Experimental Design:

Twelve adult male albino rats (Sprague-Dawley strain), weighing about (120±10g) were purchased from the Animal House, Agriculture Research Center, Giza, Egypt. Animals were housed in well conditions in biological Studies Lab of Faculty of Home Economics. Rats were kept in standard cages at room temperature (25 ± 3 °C) with a 12 h dark/light cycle. It was left for seven days as adaptation period and was allowed to feed standard laboratory basal diet (**Reeves *et al.*, 1993).** After the period of adaptation, animals were divided into two groups, as follows:

- **Group one:** Negative control group, rats (n=6) were fed on basal diet only during the experimental period (28 days)
- **Group two :** as the same as group 1, (n=6), but rats were fed on basal diet containing 20% quinoa powder which had the more general acceptability mean values by the forty trained panelists.

RESULTS AND DISCUSSION

Regarding to the panel test, **Table (1)** illustrate the mean values of sensory evaluation which was contain colour, texture, taste, general acceptability (GA) among control sample contains 100% wheat flour vs. the second sample contains 10% quinoa powder & 90% wheat flour (T1), the third sample contains 15% quinoa powder & 85% wheat flour (T2) and the fourth sample contains 20% quinoa powder & 80% wheat flour (T3).

Based on colour test among control vs. treatments, mean values reveal that third treatment which contained 15% quinoa recorded the highest value followed by 20% replacement, 10% replacement and control with mean values 3.95, 3.85, 3,43 and 3.35, respectively. Pertaining to texture, mean values of the fourth sample recorded the highest mean value followed by third sample, second sample and control group with mean values 4.33, 3.95, 3.93 and 3.90, respectively. Taste mean values showed that the highest mean value was in fourth group, which contain 20% quinoa powder followed by third group, control group and second group, which were recorded 4.28, 3.90, 3.75 and 3.60, respectively. General acceptability mean values among control and treated quinoa samples reveled that 20% quinoa replacement was the highest value followed by control group, third and second treatment. According to the sensory evaluation test which was done by well-trained panelists, the best general acceptability result was in group four which contained 20% quinoa replacement. Therefore, the biological experimental design was used this formula. These findings are in the same line with the research result, which reported that 20% of quinoa flour biscuit substituted from wheat flour, was the best product among tested formulas **(Makpoul and Ibrahem, 2015)**.

Table (1): Sensory evaluation of control and quinoa biscuit treatments

Treatment	Colour	Texture	Taste	General acceptability
control	3.35±0.22[b]	3.90±0.17[b]	3.75±0.16[c]	3.90±0.16[a]
T1	3.43±0.17[b]	3.93±0.18[b]	3.60±0.14[c]	3.46±0.22[b]
T2	3.95±0.14[a]	3.95±0.16[b]	3.90±0.16[b]	3.55±0.17[b]
T3	3.85±0.20[a]	4.33±0.14[a]	4.28±0.12[a]	3.94±0.18[a]

(T1), sample contains 10% quinoa powder & 90% wheat flour.

(T2), sample contains 15% quinoa powder & 85% wheat flour.

(T3), sample contains 20% quinoa powder & 80% wheat flour.

Results in **Table (2)** showed that quinoa flour contain. carbohydrate, protein, fat, moisture, fibers and ash contents at 68.90, 10.11, 7.00, 9.05, 3.34 and 1.60 %, respectively. These results of the present study are confirmed by **Niro *et al.*, (2019)** who demonstrated the chemical composition of quinoa flour contains carbohydrate, protein, fat, moisture, fibers and ash contents at a ratio of 64.20, 14.10, 6.10, 13.30, 6.10 and 2.40 %, respectively.

Table (2): Chemical composition of quinoa flour

Item	%
Carbohydrate	68.90
Protein	10.11
Total Fat	7.00
Moisture	9.05
Total Fibers	3.34
ASH	1.60

Results in **Table (3)** indicated that quinoa had more powerful antioxidant activity, which was presented as DPPH. The DPPH values were 18.70, 38.30, 75.94 and 92.72 % at tested levels 1.5, 3, 6 and 10 % of samples, respectively. The value of $\mathbf{IC_{50}}$ was 4.39.

The oxidative stress (OS) emerges from the imbalance between the productions of reactive oxygen species (ROS). Persistent OS may cause severe damages to proteins, lipids, DNA, and even result in cell death. Excessive free radicals and ROS are harmful to human health and trigger many diseases (**Wang and Zhao, 2019).**

Scavenging of DPPH free radical is one of the most important characteristics used to determine antioxidant activity. The presence of hydroxyl may contribute to the radical scavenging activity. Plant phenolics and flavonoids in the extract reduced DPPH radicals by their ability to donate hydrogen. Plant flavonoid is believed to reside in their free radical-scavenging capacity and their antioxidant activity increases with an increase in the number of hydroxyl groups that they bear and a decrease in their glycosylation **(Nurhanan *et al.*, 2012).**

Determination of the half-maximal (50%) inhibitory concentration (IC_{50}) is essential for understanding the pharmacological and biological characteristics of a chemotherapeutic agent **(Yifeng He *et al.*, 2016).** The IC_{50} values were calculated from the relationship curve of Radical scavenging activity (RSA) versus concentrations of the respective sample curve. The IC_{50} was calculated from the scavenging activities (%) versus concentrations of the respective sample curve **(Rivero-Cruz *et al.*, 2020).**

Table (3): The antioxidant activity of quinoa powder and its extract

Sample	DPPH Radical-Scavenging Activity				IC_{50} %
	1.5	3	6	10	
	%				
Quinoa	18.70	38.30	75.94	92.72	4.39

_DPPH : Diphenyl-1-picrylhydrazyl

Table (4) showed that feed intake (FI) (g/day), and feed efficiency ratio (FER) recorded non-significant different between quinoa group and (-ve) control group. Whereas body weight gain of quinoa group significantly decreased compared with control group with mean values -30.00 vs 35.50%, respectively.

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. **Alghamdi, (2018)** reported that a significant decrease in body weight gain (BWG) associated with quinoa consumption in high cholesterol diet-induced rats.

Ali , (2019) reported that rats fed on quinoa powder at a ratio 5 and 10% showed significant reduction in BWG and FIR compared with other research groups. **Simnadis *et al.*, (2015)** investigated physiological effect of consuming quinoa seeds to decrease weight gain and improve lipids profile. These physiological effects were attributed to the presence of protein, saponin, and 20- hydroxyecdysone in quinoa seeds, in addition to increasing the permeability of intestinal epithelial cell, decrease the capacity to nutrients active absorption for development and growth of animals.

Table (4): Effect of quinoa powder on feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) of albino male rats

Group \ Parameter	FI(g/d)	BWG%	FER
G1:-ve control	17.77	35.50 ± 04.68^{a}	00.07 ± 00.01^{a}
G2: 20% Quinoa	17.64	-30.00 ± 04.06^{b}	00.06 ± 00.01^{a}

-Mean values are expressed as means ± SE.

-Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.

Regarding to Table (5) rats fed on basal diet supplemented with 20% quinoa powder had non-significantly decreased in AST compared with (–ve) control group with mean values 08.40 vs 08.66 U/L, respectively. Whereas, ALT in group 2 which fed on 20% quinoa powder was not significant decreased compared with –ve control group with mean values 07.80 vs 08.20 U/L, respectively.

The results of the present study agree with **Alghamdi, (2018)** who reported that, mean values of serum AST & ALT were non-significantly differ between groups fed on diet supplemented with quinoa powder compared with –ve control group. **Farinazzi-Machado *et al.*, (2012)** reported that after 30 days, the use of quinoa did not lead to hepatic damages, as observed by the AST, ALT. No significant difference was found in ALT values, but showed a decrease in AST values. **Paśko *et al.*, (2010)** showed that the hypocholesterolemic effect of quinoa could be produced by the fiber, saponins or squalene, which are also present in these seeds. Dietary fiber from these grains can inhibit absorption of dietary cholesterol and can bind to biliary acid, which may increase cholesterol catabolism or fermentation of the fiber in the colon and produce short-chain fatty acids contributing to decrease of cholesterol synthesis in liver.

Table (5): Effect of quinoa powder on serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

Group \ Parameter	AST	ALT
	(U/L)	
G1: Control	08.66±00.99 [a]	08.20±00.73 [a]
G2: 20 % Quinoa	08.40±01.26 [a]	07.80±00.37 [a]

All result expressed as mean ± SE.
Values in each column, which have similar letters, are non-significantly different (p<0.05).

Regarding to Table (6), rats fed on basal diet supplemented with 20% quinoa powder had non-significant changed in urea, creatinine and BUN compared with –ve control group with mean values. 31.02 vs 30.93, 0.76 vs. 0.93 and 14.49 vs 14.45 mg/dl, respectively.

The results of the present study are in agreement with those obtained by **Arafa *et al.*, (2016)** who reported that non-significant changes in kidney functions among groups fed quinoa vs –ve control group. **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.

Table (6): Effect of quinoa powder on serum urea, creatinine and blood urea nitrogen (BUN)

Parameter / Group	Urea	Creatinine	BUN
	(mg/dl)		
G1: Control	30.93±06.03 [a]	00.93±00.02 [a]	14.45±02.84 [a]
G2:20% Quinoa	31.02±03.83 [a]	00.76±00.03 [a]	14.49±01.22 [a]

All result expressed as mean ± SD.
Values in each column, which have similar letters, are non-significantly different (p<0.05).

Pertaining to Table (7) rats fed on basal diet supplemented with 20% quinoa powder had non-significantly changed in triglycerides, cholesterol, HDL, LDL and VLDL compared with –ve control group with mean values 13.46 vs 14.16, 18.79 vs 19.95, 7.60 vs 7.18, 8.50 vs 9.94 and 2.69 vs 2.83 mg/dl, respectively.

Table (7): Effect of quinoa powder on lipid profile (mg/dl)

Parameter / Group	Triglycerides	T- Cholesterol	HDL	LDL	VLDL
G1: Control	14.16±02.68a	19.95±04.61a	07.18±.96a	09.94±03.21a	02.83±0.68a
G2: 20%Quinoa	13.46±03.05a	18.79±02.56a	07.60±1.48a	08.50±01.42a	02.69±0.61a

All result expressed as mean ± SD.
Values in each column which have similar letters are non-significantly different (p<0.05).

These results of the present study agree with those obtained by **Paśko, (2010)** who reported that the plasma cholesterol, HDL and LDL concentrations were reduced by consumption of quinoa seeds. The hypocholesterolemic effect of quinoa could be produced by the fiber, saponins which are present in these seeds. Dietary fiber from quinoa can inhibit absorption of dietary cholesterol.

The histopathological examinations of the liver are shown in **Photos 1 and 2**. **Photo 1** showed that the liver of negative control group (fed on a basal diet) revealed the normal histological structure of hepatic lobule, whereas, other sections showed hepatocellular vacuolar degeneration of some hepatocytes. Moreover, some sections from rat demonstrated no histopathological changes. However, **Photo2** showed that liver of group2 (20% quinoa) described slight Kupffer cells activation, vacuolar degeneration of some hepatocytes and congestion of central vein. Some sections from rat revealed no histopathological changes. **Saleem, *et al.*, (2014),** found that the Chenopodiaceae family, such as Chinopodium album and Chinopodium murale, have significant hepatoprotective and antioxidant activity due to high concentrations of phytochemical compounds such as flavonoids and phenolic acids.

The histopathological examinations of the kidney are shown in **Photos 3 and 4**. **Photo 3** showed that the kidney of negative control group (fed on a basal diet) revealed the normal histological structure of renal parenchyma (normal renal cortex and renal medulla). Some examined sections showed slight vacuolization of epithelial lining some renal tubules. whereas, other sections from this rat revealed no histopathological alterations. Furthermore, kidneys of rats. **Photo4** showed that kidney of group 2 (20% quinoa) revealed no histopathological alterations except slight congestion of intertubular blood capillaries. **Repo-Carrasco, (2003),** reported that quinoa contains some vitamins bioactive ingredients are important because they act as antioxidants in kidney cell membranes, such as selenium, magnesium, phyto-sterols, folic acid, and tocopherols, which are thought to have antioxidant anti-carcinogenic properties or work as anti-inflammatory agents.

The histopathological examinations of liver

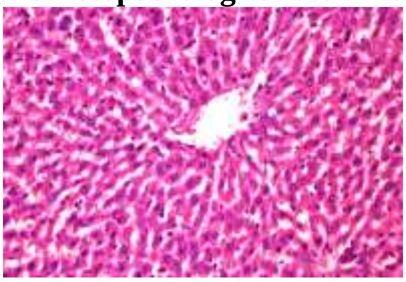
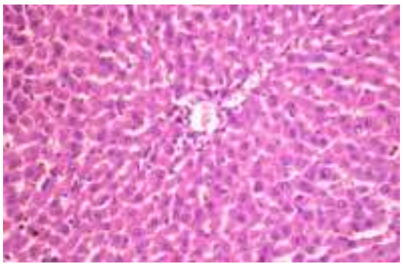


Photo (1) Liver of rat from negative control group **(H & E X 400)**



Photo(2) Liver of rat from quinoa group **(H & E X 400)**

The histopathological examinations of kidney

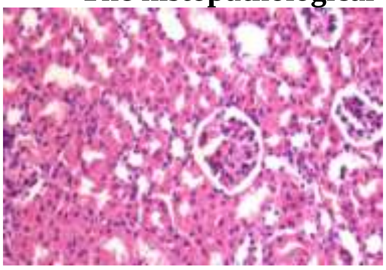


Photo (3) kidney of rat from negative control group **(H & E X 400)**

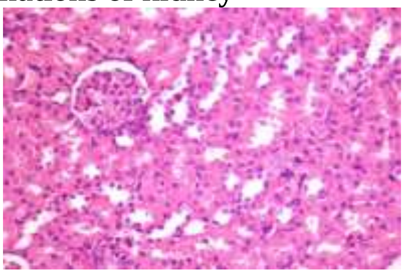


Photo (4) kidney of rat from quinoa group **(H & E X 400)**

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تأثير الكينوا على القياسات البيولوجية والبيو كيميائية والنسيجية في ذكور الفئران

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تعتبر بذور الكينوا من المكملات الغذائية الهامة للغاية وتعتبر كمصادر غذائية بديلة في بعض الدول. وتهدف هذه الدراسة إلى تحديد تأثير إضافة مسحوق بذور الكينوا على الخصائص البيولوجية والكيميائية والهستولوجية لذكور الفئران وكذلك تحضير وصفه البسكويت مع مسحوق الكينوا. اما فيما يخص DPPH فقد زاد تدريجياً مع زيادة التركيز من 1.5 الي 10. في حين أن IC_{50} سجل نتيجة 4.93 لدقيق الكينوا. تم استبدال مسحوق الكينوا بنسبة 10، 15، 20 ٪ من دقيق القمح في وصفات البسكويت المختبرة. التقييم الحسي أشار الى ان بسكويت الكينوا الذي تم استخدام أعلي نسبة كينوا به هو الأفضل من حيث التقبل العام ولذا تم استخدام هذه النسبة في تصميم التجربة البيولوجية. تم استخدام اثني عشر فأراً بأوزان 120±10 جرام وتم تقسيمها الى مجموعتين (كل مجموعه 6 فئران) وتم تغذية المجموعة الأولي على النظام الغذائي

القياسي في حين تم تغذية المجموعة الثانية على نفس النظام الغذائي مع استبدال نسبة 20% من دقيق القمح بدقيق الكينوا. وقد أشارت الخصائص البيولوجية والكيميائية والهستولوجية الي أن الغذاء المتناول أدى الي زيادة وزن الجسم، كفاءة التغذية، AST، ALT، CR، TG، TC، LDL، VLDL انخفضت غير معنوياً بالمقارنة مع المجموعة الضابطة. في حين أن اليوريا، نيتروجين اليوريا في الدم، بروتين الدهني عالي الكثافة حدث به زيادة غير معنوية مقارنة بالمجموعة الضابطة.. كان الفحص الهستولوجي للكبد والكلى كان على نفس النسق مع جميع الدراسات المذكورة أعلاه. وفقًا لهذا الدراسة، يمكن استخدام الكينوا في العديد من المنتجات الغذائية وقد يكون لها خصائص غذائية مفيدة.