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Biological Studies on Quinoa and Spirulina on Anemic Rats

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Abstract:
Iron deficiency anemia is a common type of anemia in which blood lacks adequate healthy red blood cells. This study aimed to determine the effect of quinoa and spirulina on iron deficiency-induced anemia in mice. Five experimental groups were fed a diet supplemented with quinoa and spirulina at 2.5, 5, and 7.5% as a mixture for four weeks, versus the control rat group fed a basal diet free of iron. At the end of the experiment, rat groups fed herbs-supplemented diets had significant dose-related increases in serum iron; additionally, there were variable dose-dependent increases in hemoglobin, hematocrit, and ferritin levels compared to the control group. The healthy status of anemic rats was assessed by estimating plasma concentration of enzyme activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), Urea, creatinine, and anemia parameters. Results showed an improvement in the above parameters at a 7.5% mixture followed by a 5% mixture of both quinoa and spirulina. So, the investigation suggested that incorporating quinoa and spirulina in daily diets would be an adequate food to prevent and treat anemia.

***Keywords**: Rats, Quinoa, Spirulina, Iron, Anemia*

Introduction

Iron-deficiency anemia is a global nutritional issue that occurs as a complication of nutritional and absorption disorders and is observed frequently over in all cases [1]. Insufficient dietary iron intake or absorption represents the main risk factor of the incidence of iron-deficiency anemia. Iron-deficiency has been strongly related with many human diseases including immune disorders [2], chronic inflammation, limitation of physical performance, neurological impairment and cognitive deficits [3].

Anemia is a decrease in number of red blood cells (RBCs) or lower than the normal quantity of hemoglobin in the blood. It can include a decrease oxygen-binding ability of each hemoglobin molecule due to deformity or deficiency in numerical development as in some other types of hemoglobin deficiency. Because hemoglobin (found inside RBCs) normally carries oxygen from the lungs to the capillaries, anemia leads to hypoxia (lack of oxygen) in organs [4].

Most anemia are caused by a lack of nutrients required for normal erythrocyte synthesis, principally iron, vitamin B12, and folic acid. Others results from a variety of conditions such as hemorrhage, genetic abnormalities chronic diseases states, or drug toxicity. The anemia that result from an inadequate intake of iron, protein, certain vitamins (B12,folic acid, pyridoxine, and ascorbic acid), copper, and other minerals are frequently called nutritional anemia. The most common nutritional anemia result from iron or folic acid deficiency [5].

Iron deficiency (IDA) is in the top 20 risk factors for the global distribution of disease burden [6]. WHO is working with the Egyptian government to address major challenges due to the prevalence of iron-deficiency anemia among 40% of children between 2–5 years, which may increase to 51% in rural children. Similar prevalence was reported among women of reproductive age and in pregnancy, in addition to being associated with other diseases [7]. Iron deficiency has adverse consequences for health as a major contributor to maternal and fetal morbidity and mortality, affects productivity, work capacity, cognitive development, resistance to infection, and pregnancy in women [8].

Quinoa could be used as a complementary crop in Egypt's marginal lands. It has a high potential for adaptation to semi-arid and arid environments and provides good nutrition. Quinoa has been cultivated in Egypt for ten years because of its adaptability to its marginal places and the country's arid climate. Egypt has a large area of arid, semi-arid, and marginal lands, which limit traditional crop productivity [9]. It is gluten-free and a good source of high-quality protein, fiber, carbohydrates, vitamins, minerals, phytochemicals, and bioactive peptides. The anti-nutritional factors present in quinoa, such as saponins, tannins, and phytic acids, can decrease the bioavailability due to forming insoluble complexes with minerals, such as zinc and iron, which can be relieved by germination [10].

Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose, or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin) which may be triterpenoid or steroid in nature [11]. El Hazzam et al. reported that quinoa seed bitterness is essentially due to the presence of quinoside A, whilst others related bitter tasting to surface activity, which can cause intensive foaming in aqueous solutions [12]

Nutritionally, Spirulina has a high protein and vitamin content, which makes it an excellent dietary supplement for people on vegetarian or vegan diets. Its protein is relatively lower in cysteine, methionine, and lysine when compared to animal products. Spirulina has antioxidant and inflammation-fighting properties, as well as the ability to help regulate the immune system. Animal studies indicated that spirulina may support gut health as people age. The studies on older mice proved that spirulina may preserve healthy gut bacteria during the aging process and improve the weight loss [13,14].

So, this study speculated that tested quinoa and spirulina might have a stimulatory effect on iron absorption. Therefore, the study examined whether tested herbs feeding rats prevents anemia by increasing iron absorption.

Materials and methods

The quinoa was obtained from National Research Center , Giza, Egypt. Spirulina was obtained from Elgomhoria Company formed-preparations chemicals and Medical Equipments, Dokki, Egypt. Tested quinoa was soaked for 12 h to remove the anti-nutritional compounds and spirulina were grinded into fine powder by using Electric grinder and kept in dusky Stoppard glass bottles in a cool and dry location till use according to Russo [15].

Thirty male albino rats weighing 140 ±10g were selected from the Institute of Medical Insect Research, Dokki, Egypt. All rats were fed on basal diet which prepared according to American Institute of Nutrition (AIN) [16] for 7consecutive for adaptation and then 4 groups with anemia was induced according to the bleed-out method reported by Delwatta et al. [17] (Anemic rats were considered when they presented hematocrit levels < 33% and hemoglobin < 11 g/dL).After this adaptation period, rats are divided into 5 groups, each group which consists of 6 rats as follows: Group (1): Rats implemented on the main meal over the duration of the experiment as a negative control group. Group (2): Anemic rats fed on basal diet as a positive control group. Group (3): Anemic rats fed on basal diet with 2.5% quinoa and 2.5% spirulina. Group (4): Anemic rats fed on basal diet with 5% quinoa and 5% spirulina. Group (5): Anemic rats fed on basal diet with 7.5% quinoa and 7.5% spirulina.

By the end of the experimental periods (28 days), rats were scarified using diethyl ether anesthesia at fasting state. Blood samples were collected and allowed to coagulate at room temperature; other portion of blood was added to EDTA (ethylene diamine tetracetic acid) and centrifuged at 3000 r.p.m for 10 minutes. Serum was carefully transferred into clean covet tubes and stored frozen at -20°C until the time of analysis.

Biochemical analysis

Serum Alkaline phosphatase (ALP) was determined according to the procedure of Moss [18]. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were carried out according to the method of Henry and Yound [19,20]. Serum uric acid was determined according to the method described by Fossati et al. [21]. Serum creatinine in plasma was determined according to the enzymatic method of Patton and Crouch [22]. Hemoglobin, Hematocrit and total iron binding capacity (TIBC) were determined according to Drabkin, Mc-Inory and Cavill's method, [23,24,25] respectively while, hemoglobin regeneration efficiency (HRE) was calculated according to the method and equations of Miller [26].

The obtained data were analyzed using one-way analysis of variance (ANOVA) followed by the student t-test for significant difference. Statistical significant difference was defined as $P<0.05$ according to Snedecor and Cochran [27].

Results and Discussion

Data presented in Table (1) showed the effect of different levels of quinoa and spirulina mixture on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of rats with anemia. The obtained results indicated that the highest BWG and FI levels recorded for negative control group, while positive control group recorded the lowest value with significant ($P\leq0.05$) differences, the mean values for BWG were 42.20 ± 1.22 g and 9.20 ± 0.12 g, respectively. The mean values for FI were 14.90 ± 0.37 g/28 day and 5.80 ± 1.33 g/28 day, respectively. For treated groups (induced anemia groups) the highest levels of BWG and FI recorded for the fifth group which contains 7.5% quinoa and 7.5% spirulina, while the lowest value recorded for the third group which contains 2.5% quinoa and 2.5% spirulina with significant ($P \leq 0.05$) differences. The mean values for BWG were 34.20 ± 1.22 g and 20.70 ± 0.13 g, respectively. The mean values for FI were 12.90 ± 0.37 g/28 day and 8.70 ± 1.22 g/28 day, respectively.

In case of FER, the obtained results indicated that the highest levels of FER recorded for negative control group, while positive control group recorded the lowest value with significant ($P \leq 0.05$) differences. The mean were 0.100 ± 0.004 and 0.057 ± 0.003, respectively. On the other hand, the highest FER levels of treated groups (induced anemia groups) recorded for the forth group which contains 7.5% quinoa and 7.5% spirulina, while the lowest value recorded for the third group which contains 2.5% quinoa and 2.5% spirulina with significant ($P \leq 0.05$) differences. The mean values were 0.095 ± 0.004 and 0.085 ± 0.002, respectively. There were no significant ($P > 0.05$) differences between forth (5% quinoa and 5% spirulina) and fifth (7.5% quinoa and 7.5% spirulina) groups. Anemia led to poor appetite which is mediated by the central nervous system by neuropeptides that adjust energy homeostasis. In addition, long-term anemia is mediated by insulin and by hormones synthesized by fatty tissue [28].

Quinoa is rich in fiber and high-quality protein which is one of the few plant-based complete proteins, having the 9 essential amino acids. It contains more protein than any other grain while also packing in iron and potassium. Quinoa has 222 calories per serving, making it a suitable food for weight gain, with 39 grams of carbs. and 4 grams of fat too. Also, it contains a small amount of omega-3 fatty acids [10].

Spirulina has various benefits for improving weight loss, dyslipidemia and obesity. It is rich in a range of vitamins and minerals essential for maintaining body weight and healthy immune system, such as vitamins E, C, and B6 [29].

Table (1) Effect of tested plants on feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) of rats with anemia.

Parameters Groups	Body weight gain (g)	Feed intake (g/day)	Feed efficiency ratio
(G1): Negative control	42.20 a ± 1.22	14.90 a ± 0.37	0.100 a ± 0.004
(G2): Positive control	9.20 e ± 0.12	5.80 e ± 1.33	0.057 d ± 0.003
(G3): 2.5% quinoa and 2.5% spirulina	20.70 d ± 0.13	8.70 d ± 1.22	0.085 c ± 0.002
(G4): 5% quinoa and 5% spirulina	26.7 c ± 1.90	10.50 c ± 0.12	0.091b ± 0.001
(G5): 7.5% quinoa and 7.5% spirulina	34.2 b ± 1.22	12.90 b ± 0.37	0.095 b ± 0.004
LSD	2.54	1.86	0.004

Means under the same column bearing different superscript letters are different significantly ($P<0.05$)

Data presented in table (2) showed the effect of quinoa and spirulina mixture on liver enzymes (AST, ALT and ALP) of rats with anemia. The obtained results indicated that positive control group recorded the highest value of serum AST, ALT and ALP compared to negative control group. Treating anemic rats with the different concentrations of quinoa and spirulina reduced AST, ALT and ALP levels compared to positive control group. Group 7.5% quinoa and 7.5% spirulina recorded the lowest values compared to treated groups and positive control group. There were no significant (P>0.05) differences between group 2.5% quinoa & 2.5% spirulina and group 7.5% quinoa & 7.5% spirulina in case of AST and ALP. Iron precipitation in the liver leads to enlargement and elevation in liver enzymes. This may cause right upper quadrant pain and predispose to fibrosis, cirrhosis and cancer 8. The results of Cao et al. who shown that feeding quinoa for 8 weeks improved liver tissue and the level of transaminases such as ALT and AST in fatty liver and this due to the abundance of proteins, minerals, dietary fibers, essential amino acids, and bioactive compounds in quinoa seeds, which protect the liver from oxidative stress. Also, Betacyanins, rutin, quercetin, and other flavonoids also present in quinoa were proven to have an anti-inflammatory effects and perform as an anti-oxidative [30].

Table (2): Effect of tested plants on liver enzymes (AST, ALT and ALP) of rats with anemia.

Parameters / Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
(G1): Negative control	39.4 d ± 2.04	36.60 e ± 6.05	76.40 e ± 1.13
(G2): Positive control	71.30 a ± 2.31	69.50 a ± 1.43	95.20 a ± 2.36
(G3): 2.5% quinoa and 2.5% spirulina	64.90 b ± 0.12	64.70 b ± 2.13	90.10 b ± 1.13
(G4): 5% quinoa and 5% spirulina	60.80 b ± 5.13	59.90 c ± 6.34	85.10 c ± 0.22
(G5): 7.5% quinoa and 7.5% spirulina	55.10 c ± 3.46	54.40 d ± 4.88	79.40 d ± 0.16
LSD	2.65	3.44	4.98

Means under the same column bearing different superscript letters are different significantly (p<0.05)

Data presented in table (3) show the effect of quinoa and spirulina on kidney functions (mg/dl) of rats with anemia. The highest levels of creatinine and uric acid recorded for positive control group, while the negative control group recorded the lowest value with significant (P≤0.05) differences. For treated groups the highest values of creatinine and uric acid levels recorded for 2.5% quinoa and 2.5% spirulina group, while 7.5% quinoa and 7.5% spirulina group recorded the lowest value with significant (P≤0.05) differences. Minutolo et al. reported an interaction between anemia and high serum creatinine that increased the risk for CHD events [31]. Quinoa is a grain that is high in protein and fiber and is a good source of different vitamins and minerals, including magnesium, B vitamins, and iron. Quinoa is also a good source of antioxidants. Some research has shown that quinoa may help to improve kidney function in people with CKD. Quinoa may help to reduce inflammation and oxidative stress, both of which can contribute to kidney damage [30].

Table (3): Effect of quinoa and spirulina on kidney functions (mg/dl) of rats with anemia.

Groups / Parameters	Creatinine mg/dl	Uric Acid mg/dl
(G1): Negative control	0.52 d ± 0.98	1.01 e ± 0.17
(G2): Positive control	1.61 a ± 0.01	3.30 a ± 0.23
(G3): 2.5% quinoa and 2.5% spirulina	1.30 b ± 0.03	2.99 b ± 0.07
(G4): 5% quinoa and 5% spirulina	1.02 c ± 0.11	2.06 c ± 0.27
(G5): 7.5% quinoa and 7.5% spirulina	0.73 d ± 0.36	1.76 d ± 0.85
LSD	0.10	0.22

Each value represents mean ± standard deviation. Mean under the same column bearing different superscript letters are different significantly (p ≤ 0.05).

Data presented in table (4) show the effect of quinoa and spirulina on serum minerals concentration and hemoglobin indices of rats with anemia. In case of serum Fe levels, the highest value recorded for negative control group, while the lowest value recorded for positive control group with significant differences. The mean values were 119.11 and 66.30 µg/dl, respectively. For treated groups the highest value of serum Fe level recorded for 7.5% quinoa and 7.5% spirulina group, while 2.5% quinoa and 2.5% spirulina group recorded the lowest value with significant (P≤0.05) differences. The mean values were 95.80 and 78.25 µg/dl, respectively. As for HRE, the highest value recorded for negative control group, while the lowest value recorded for positive control group with significant (P≤0.05) differences. The mean values were 59.95 and 46.14, respectively. For treated groups the highest value of HRE level recorded for 7.5% quinoa and 7.5% spirulina group, while 2.5% quinoa and 2.5% spirulina group recorded the lowest value with significant (P≤0.05) differences. The mean values were 55.18 and 50.15, respectively. Additionally to there were no significant differences between 7.5% quinoa and 7.5% spirulina and 5% quinoa and 5% spirulina groups. In case of serum ferritin, the highest value recorded for negative control group, while the lowest value recorded for positive control group with significant differences. The mean values were 175.97 and 80.08 nanog/L, respectively. For treated groups the highest value of serum ferritin levels recorded for 7.5% quinoa and 7.5% spirulina group, while 2.5% quinoa and 2.5% spirulina group recorded the lowest value with significant (P≤0.05) differences. The mean values were 108.65 and 89.03 nanog/L, respectively. For TIBC, the highest value recorded for positive control group, while negative control group recorded the lowest value with significant (P≤0.05) differences. The mean values were 266.98 and 206.76, respectively. For treated groups the highest value of TIBC levels recorded for 2.5% quinoa and 2.5% spirulina group, while 7.5% quinoa and 7.5% spirulina group recorded the lowest value with significant (P≤0.05) differences. The mean values were 250.77 and 230.94, respectively. In case of serum hemoglobin and hematocrite levels, the highest value recorded for negative control group, while the lowest value recorded for positive control group with significant (P≤0.05) differences. For treated groups the highest values of serum hemoglobin and hematocrite levels recorded for 7.5% quinoa and 7.5% spirulina group, while 2.5% quinoa and 2.5% spirulina group recorded the lowest value with significant (P≤0.05) differences.

In this study, the primary cause of anemia was considered to be the feeding on iron-deficient diet (malnutrition) for a long period (4 weeks) through the adaptation feeding course before incorporation of herbs together with normal load of iron into the experiment diets. The hemoglobin concentration decreased constantly during the feeding period of iron-free diets in all the rat groups. It was evident that iron deficiency contributed to this anemia, because typical symptoms of iron-deficiency anemia such as decreases in hemoglobin and serum iron concentrations and increases in total iron binding capacity were observed [32]. A single cup of quinoa contains 2.8 mg of iron or 15% of RDI. Quinoa flour (Chenopodium quinoa Willd) of the Negra Collana variety and kanihua flour (Chenopodium pallidicaule Aellen) of the Ramis variety are anti-anemic nutraceutical alternatives due to their high protein content, the balance of essential amino acids, good content of vitamins and minerals such as iron which makes it possible to have endogenous nitrogen sources for subsequent uses in the synthesis of proteins such as hemoglobin, ferritin, and other proteins; as well as the balanced composition of essential and non-essential amino acids, vitamins, fatty acids, and fiber that improve the nutritional status of the anemic patient [32,33]. Spirulina contains high iron, about 28.5 mg /100 g. of which 58 times more than in spinach, and 18 times higher than that found in meat. Consuming 100 gr of spirulina can fulfill 158% of iron needed in a day. Spirulina could prove beneficial to increased Hb concentration and reducing the risk of anemia. Consuming spirulina for six weeks had a significant effect of lowering anemia by rising 60% of Hb concentration [34,35].

Table (4): Serum minerals concentration and hemoglobin indices in rats fed control and herbs containing diets for 4 weeks

Parameters Groups	Serum Fe (µg/dl)	HRE1	Serum ferritin (nanog/L)	TIBC (µg/dl)	Haemoglobin (g/L)	Hematocrit (%)
(G1): Negative control	119.1a±4.55	59.95a±6.01	175.9a±6.97	206.7e±7.9	13.97a±3.55	46.76a±4.27
(G2): Positive control	66.30e±2.10	46.14d±4.81	80.08e±5.85	266.9a±9.0	8.06e±1.06	31.08e±5.86
(G3): 2.5% quinoa and 2.5% spirulina	78.25d±4.10	50.15c±4.05	89.03d±6.92	250.7b±7.0	9.45 d ± 0.89	34.03d±0.65
(G4): 5% quinoa and 5% spirulina	84.30c±2.12	53.16b±3.02	97.96c±7.99	242.0c±3.8	10.87c±0.76	37.06c±1.06
(G5): 7.5% quinoa and 7.5% spirulina	95.80b±6.62	55.18b±7.01	108.65b±8.9	230.9d±7.0	11.35b±1.05	40.23b±1.78
LSD	4.99	1.34	3.54	5.98	0.33	2.06

Means under the same column bearing different superscript letters are different. HRE1: Hemoglobin regeneration efficiency, TIBC2: total iron-binding capacity

Conclusion

From the obtained results, the mixture of quinoa and spirulina at 7.5% from each material recorded the best result. The 2.5% quinoa and 2.5% spirulina group recorded the lowest value. This mixture improved the body weight, FER liver enzymes, and kidney functions of anemic rats. The highest serum hemoglobin and hematocrit levels values were recorded for the 7.5% quinoa and 7.5% spirulina group. So, 7.5% quinoa and 7.5% spirulina supplementation can prevent anemia and improve iron absorption through anemic rats with caution and not long-term use.

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دراسات بيولوجية علي الكينوا والسبيرولينا في الفئران المصابة بالأنيميا

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قسم التغذية وعلوم الأطعمة .كلية الاقتصاد المنزلي .جامعة المنوفية، شبين الكوم، مصر

الملخص العربي:
في هذا البحث تم تقييم الفوائد الصحية المحتملة لمسحوق الكينوا والسبرولينا كمكمل غذائي وظيفي لتحسين امتصاص المعادن وكذلك لمنع وعلاج فقر الدم المرتبط بنقص تناول الحديد في الفئران. وعليه فقد تم تغذية خمس مجموعات تجريبية بنظام غذائي مكمل بمستويات مختلفة من الكينوا والسبيرولينا. تم إضافة مسحوق الكينوا والسبرولينا بمستويات 2.5 و 5 و7.5% كمخلوط لمدة 4 أسابيع مقابل مجموعة الفئران الضابطة التي تغذت على وجبة عالية في محتواها من الألياف و خالية من الحديد لمدة 4 أسابيع. في نهاية التجربة، تميزت مجموعات الفئران التي تغذت على أعشاب مكملة بزيادات معنوية مرتبطة بالجرعة في مستوى الحديد في الدم، بالإضافة إلى وجود زيادات متغيرة في المستويات المقاسة للهيموجلوبين والهيماتوكريت والفيريتين في مجموعات الأعشاب التي تتغذى في بطريقة تعتمد على الجرعة مقارنة بمجموعة التحكم. تم تقييم أنشطة إنزيمات الأسبارتات أمينوترانسفيراز وألانين أمينوترانسفيراز، اليوريا والكرايتين ومقاييس الدم. وفي النهاية أظهرت النتائج تحسنا في حالة الأعشاب المختبرة عند مستوى مخلوط 7.5٪. وبالتالي، يمكن اعتبارها مكمل غذائي فعال للغاية للوقاية من فقر الدم وعلاجه.

الكلمات الأفتتاحية: فئران، كينوا، سبيرولينا، الحديد، الأنيميا