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Effect of Different Levels of *Molina* (*Lagenariasiceraria*) on the Healthy Status of Rats with Anemia

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Abstract:

The potential health benefits of *molina* as functional food supplement to improve iron absorption as well as to prevent and treat anemia associated with deficiency in iron intake were evaluated in rats. Three experimental groups were fed diet supplemented with different levels of *Molina* for 4 weeks versus the control rat group fed basal diet free in iron. At the end of the experiment, rat groups fed levels of *molina* supplemented diets were characterized by significant dose-related increases in the level of serum Fe (69.11 ± 4.55 to 84.3 ± 2.12 $\mu\text{g/dl}$). In addition, there were variable increases in the measured levels of hemoglobin (11.11 ± 1.1 to 14.25 ± 1.1 g/L), hematocrit (39.15 ± 0.15 to 42.15 ± 1.27 %) and ferritin (49.55 ± 2.25 to 65.12 ± 0.15 $\mu\text{g/dl}$) in levels of tested plantfed groups in a dose-dependent fashion compared with the control group. These data suggested that 10% of *molina* could provide with iron absorption and bioavailability of iron when incorporated in daily diets and therefore, could be considered as a very effective food supplement to prevent and treat anemia.

Key Words: Ascorbic acid, *Lagenariasiceraria* and iron absorption.

Introduction

Iron-deficiency anemia is a global nutritional problem occurring as a complication of nutritional and absorption disorders and is observed frequently over ages

(Makrides *et al.*, 2003). Shortage in dietary iron intake or absorption represents the major risk factor of the incidence of iron-

deficiency anemia . Iron-deficiency has been strongly related with many human diseases including immune disorders

(**Kim et al., 2002**), chronic inflammation, restriction of physical performance , neurological impairment and cognitive deficits (**Kruger and Schroeder, 2001**).

Molina (L. siceraria) (Family *Cucurbitaceae*) is a climber or trailer of Asian and African origin with subglobose ellipsoid or lageniform fruit . The plant is cultivated for its fruit, which is used as vegetable . It has highly rich ethnomedicine and is recognized to have cardiogenic, hepatogenic, anti-hyperglycemic, and antihyperlipidemic properties . The fruit has also been exhibited to possess fibrinolytic , antithrombotic , and anti-atherosclerotic activities (**Ahmed and Fatima, 2014**) . Antioxidant properties of the fruit have been studied in detail demonstrating it having remarkable antioxidative and free radical scavenging potential . It possesses considerable antimicrobial properties against a number of microorganisms . It has also been shown to possess antihyperlipidemic properties in animal models (**Ghuleet al., 2016**)

Molina has been found to contain ascorbic acid, caffeoylquinic acid, cucurbitacins, pectin, β -carotene, iso-fucoesterol, campesterol, spinasterol, kaempferol, palmitic acid, oleanolic acid, linoleic acid, quercetin and iso-quercetin (**Malik et al., 2017**). Therefore the present work was designed to study the effects of different levels of *Molina* on some biological and biochemical parameters of anemic rats.

Materials and Methods

Molina fruits were obtained from the local market, Cairo, Egypt. All chemicals and diagnostic kits were purchased from El-Gomhoria for trading Drugs, chemicals and medicals this company Co., Cairo, Egypt.

Preparation of the tested material: fruits were dried at 40 °C for three days and ground into fine powder by using electric grinders, in 80mg and kept in dark , stoppered glass bottles in a cool and dry location till use according to **Russo (2001)**

Experimental animals: This study was carried out on twenty four adult male Sprague Dawley albino rats weighing 155 ± 5 g body weight (6 rats in each group). The rats were obtained from Laboratory Animal Colony, Helwan, Egypt. Rats were kept for one week for acclimatization to the laboratory conditions, and fed on basal diet and provided with water and food ad libitum.

Basal diet (AIN-93M) was prepared according to **Reeves et al. (1993)**, Anemic diet which was used free in iron and vitamin C as reported by **Schermer (1967)**. Different levels of Molina fruit (5, 10 and 15%) were added which substituted from the amount of corn starch.

Experimental design: Rats were divided into four groups consisting of six rats per each. The groups were fed on anemic diet during the experimental period. After 28 days that was required to induce anemia as stated by **Schermer (1967)**, the first group was left as a control group, while the rest were given daily 5, 10 and 15% Molina. During the experiment period, the feed intake were determined daily and body of rats weight were weighed once a week. Body Weight Gain (BWG) and Feed Efficiency Ratio (FER) were calculated at the end of the experimental period such as mentioned in **Chapman et al., (1959)** according to the following equations:

$BWG (g) = \text{final weight (g)} - \text{initial weight (g)}$

$FER = \text{weight gain (g)} / \text{feed intake (g)}$

Collection of blood samples: At the end of the experimental period, rats were sacrificed fasted following a 12 h fast sacrificed. The rats were lightly anaesthetized by diethyl ether and about 7 ml of blood was withdrawn from the hepatic portal vein into dry centrifuge plastic tubes. Blood samples were centrifuged for 20 min at 3000 rpm to separate the serum samples which were kept in tubes at -20 C till biochemical analysis (**Drury and Wallington 1980**).

Analysis methods: Serum total cholesterol was calorimetrically determined according to **Allain *et al.* (1974)** and triglyceride was determined calorimetrically according to **Wahlefeld (1974)**. High Density Lipoprotein cholesterol (HDL-c) was determined calorimetrically according to **Richmond (1973)**. Low Density Lipoprotein cholesterol (LDL-c) and Very Low Density Lipoprotein cholesterol (VLDL-c) were calculated mathematically according to **Friedewald *et al.* (1972)** as follow:

$$\text{LDL-c} = \text{TC} - [\text{HDL-c} + (\text{TG}/5)]$$
$$\text{VLDL-c} = \text{Triglycerides}/5$$

The activity of aspartate aminotransferases (AST) and alanine aminotransferases (ALT) enzymes were assigned by the method of **Bergmeyer and Harder, (1986)**.

Blood was collected by tail venous puncture every week at the end of the experimental. Hemoglobin was determined according to **Drabkin, (1949)**. Hematocrit was measured using a heparinized tube according to **Mc-Inory procedure (1954)**. Using the serum samples obtained on the final day of the experiment, serum and total iron binding capacity (TIBC) were determined by means of commercial assay kits (Sigma Diagnostic, St. Louis) according to **Cavill *et al.*, (1986)**. Hemoglobin regeneration efficiency (HRE) were calculated according to the method and equations of **Miller (1982)** as follow:

$$\text{Hemoglobin Regeneration Efficiency (HRE)} = \frac{\{\text{Hb-Fe (mol)}\} \text{ at the end of each period} - \{\text{Hb-Fe (mol)}\} \text{ at the beginning of each period}}{\text{mol Fe consumed}}$$

Statistical analysis according to: **Snedecor and Cochran (1986)**.

Results

Effect of different levels of molina on feed intake (FI), body weight gain (BWG), and feed efficiency ratio (FER) in anemic rats.

According to the FI results showed that there were significant differences ($p < 0.05$) between anemic rats fed on 10 and 5% of plant and (15% and control group).

Anemic group fed on 10% was higher significantly ($p \leq 0.05$) than other groups followed by 5%, 15% and control group.

Table 1: Effect of different levels of molina on feed intake (FI), body weight gain (BWG), and feed efficiency ratio (FER) in anemic rats.

Groups	Anemic control group	Anemic group fed on 5% molina powder	Anemic group fed on 10% molina powder	Anemic group fed on 15% Molina powder
FI (g/day)	10.07±0.03 ^c	11.10±0.83 ^b	12.29±1.42 ^a	10.97±1.05 ^c
BWG (g/day)	0.95 ±0.12 ^c	1.06±0.07 ^b	1.48±0.27 ^a	1.01±0.17 ^b
FER	0.094±0.014 ^b	0.095±0.002 ^b	0.120±0.012 ^a	0.092±0.008 ^b

Values are mean±SD. Values in the same row sharing the same superscript letters are not statistically significantly different

Effect of different levels of molina on serum lipids parameters (mg/dl) in anemic rats.

Administration of molina powder of molina at 10% and 15% caused significant decreases in serum levels of total cholesterolTc, LDL-c and VLDL-c compared to control group (Table 2). Serum HDL-c levels increased significantly by the fed of molina at 15%. Anemic rats that were given molina at 10 and 15 % showed significantly lower levels of VLDL-c compared to control group.

Table 2: Effect of different levels of molina on serum lipids parameters (mg/dl)in anemic rats.

Groups	Anemic control group	Anemic group fed on 5% molina powder	Anemic group fed on 10% molina powder	Anemic group fed on 15% molina powder
TC	160.20±9.23 ^a	135.00±4.08 ^b	120.60±4.39 ^c	110.33±5.03 ^d
TG	112.60±6.95 ^a	90.00±4.08 ^b	83.60±4.67 ^c	82.67±2.52 ^c
HDL	28.36±5.57 ^c	34.85±3.06 ^b	32.40±1.82 ^b	40.67±5.51 ^a
LDL- c	109.32±9.83 ^a	82.15±6.84 ^b	66.48±6.22 ^c	53.13±5.76 ^d
VLDL- c	22.52±1.39 ^a	18.00±0.82 ^b	16.72±0.93 ^b	16.53±0.50 ^b

Values are mean±SD. Values in the same row sharing the same superscript letters are not statistically significantly different

Effect of different levels of molina on AST and ALT parameters (mg/dl) in anemic rats.

Concerning of lipid profiles results in table (2) showed that there were significant decreased in values of TC, TG, LDL and VLDL when the ratio of substitution increased, the values of mean \pm SD were 160.20 \pm 9.23, 135.00 \pm 4.08, 120.60 \pm 4.39 and 110.33 \pm 5.03 for TC, 112.60 \pm 6.95, 90.00 \pm 4.08, 83.60 \pm 4.67 and 82.67 \pm 2.52 for TG, 28.36 \pm 5.57, 34.85 \pm 3.06, 32.40 \pm 1.82 and 40.67 \pm 5.51 for HDL, 109.32 \pm 9.83, 82.15 \pm 6.84, 66.48 \pm 6.22 and 53.13 \pm 5.76 for LDL-c, 22.52 \pm 1.39, 18.00 \pm 0.82, 16.72 \pm 0.93 and 16.53 \pm 0.50 for VLDL-c, respectively. while serum HDL was increased significantly (p<0.05) when substitution increased, with 28.36 \pm 5.57, 34.85 \pm 3.06, 32.40 \pm 1.82 and 40.67 \pm 5.51 respectively.

Table 3: Effect of different levels of molina on AST and ALT parameters (mg/dl) in anemic rats.

Groups	Anemic control group	Anemic group fed on 5% molina powder	Anemic group fed on 10% molina powder	Anemic group fed on 15% molina powder
AST	108.2 \pm 9.76 ^a	93.33 \pm 5.50 ^b	86.00 \pm 1.01 ^c	46.01 \pm 1.01 ^c
ALT	63.80 \pm 8.43 ^a	57.00 \pm 2.65 ^b	54.20 \pm 3.89 ^d	36.60 \pm 2.51 ^d

Values are mean \pm SD. Values in the same row sharing the same superscript letters are not statistically significantly different

Effect of different levels of molina on Serum iron concentration and hemoglobin indices in anemic rats.

Data shown in Table (4) serum iron were significantly ($P<0.05$) increased as affected by different levels of molina intake (5, 10 and 15%), the values of iron were 76.3 ± 2.1 , 80.25 ± 4.1 and 84.3 ± 2.12 $\mu\text{g/dl}$ respectively. In addition, all serum levels of hemoglobin were also elevated in the range of 12.35 ± 1.11 , 13.15 ± 2.36 and 14.25 ± 1.1 g/L respectively. While, ferritin was increased in the range of 57.10 ± 0.36 , 62.3 ± 0.1 and 65.12 ± 0.15 $\mu\text{g/L}$ respectively. Hemoglobin regeneration efficiency (HRE) was increased at the range of 0.15 ± 0.05 , 0.17 ± 0.02 and 0.19 ± 0.01 . Hematocrit was increased at the ranges of 31.11 ± 1.3 , 37.17 ± 2.11 and $42.15\pm 1.27\%$ in the experimental rats fed 5, 10 and 15% respectively. Hemoglobin regeneration efficiency (HRE) in the molina fed rats was significantly higher than that of the control rat group while, total iron binding capacity (TIBC) in the fruit-fed rats was significantly lower than that of the control rat group.

Table 4: Effect of different levels of molina on Serum iron concentration and hemoglobin indices in anemic rats.

Serum Profile	Anemic control group	Anemic group fed on 5% fruit powder	Anemic group fed on 10% fruit powder	Anemic group fed on 15% fruit powder
Serum Fe ($\mu\text{g/dl}$)	59.11 ± 4.55^d	76.30 ± 2.10^a	80.25 ± 4.10^b	84.30 ± 2.12^c
HRE ¹	0.12 ± 0.01^d	0.15 ± 0.05^c	0.17 ± 0.02^b	0.19 ± 0.01^a
Serum ferritin ($\mu\text{g/L}$)	49.55 ± 2.25^d	57.10 ± 0.36^c	62.30 ± 0.10^b	65.12 ± 0.15^a
TIBC ($\mu\text{g/dl}$) ²	345.10 ± 6.20^a	339.5 ± 33.50^b	330.6 ± 25.55^c	321.7 ± 10.50^d
Hemoglobin (g/L)	10.11 ± 1.10^c	12.35 ± 1.11^b	13.15 ± 2.36^b	14.25 ± 1.10^a
Hematocrite (%)	29.15 ± 0.15^d	31.11 ± 2.11^c	37.17 ± 2.11^b	42.15 ± 1.27^a

Values are mean \pm SD. Values in the same row sharing the same superscript letters are not statistically significantly different HRE¹: Hemoglobin regeneration efficiency, TIBC²: total iron-binding capacity.

Discussion

Anemia is considered as one of the most common index of malnutrition over the world and is caused by iron deficiency store or iron-deficiency erythropoiesis based on the screening criteria for iron-deficiency anemia (**Lin et al.,2003**).

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 Molina together with normal load of iron and calcium 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 signs of iron-deficiency anemia such as decreases in hemoglobin and serum iron concentrations, and increases in total iron binding capacity were observed (**Baynes and Bothwell 1990**).

Several studies have shown that molina contains considerable amounts of important compounds which may serve as antioxidants. For example, **Ahmed and Fatima, (2014)** reported that molina had high content of phenolics compounds (48.1mg/g), flavonoids (6.23 mg/g) and carotenoids (0.32 mg/g). Furthermore, **Ghule et al. (2016)** found that molina fruit extract contain considerable amounts of total phenolics compounds and have antioxidant activity and free radical-scavenging capacity. It is well-known from the literature that the main active compounds of molina fruit extract are inulin and fructooligosaccharides. Inulin is a polymer of fructose with β -(2-1) glycosidic linkages. As it is water soluble and not hydrolysed by human digestive enzymes, it behaves like a soluble fiber. It may increase the viscosity of the stomach content, which can slow down the rate of gastric emptying of water, nutrients and lipids, or it can cause alterations in hormone secretions, which affect lipid metabolism. The observed effect of Molina on food intake and body weight in this study was agreed with that reported by **Malik et al. (2017)** that the addition of oligofructose; a short chain fructans obtained from chicory inulin; might enhance satiety, thereby resulting in greater reductions in energy intake and protects against the body weight gain, fat mass development in normal and obese rats. The effect of molina fruit and seed on feed intake and body weight could be attributed to the presence of inulin-type fructans of molina fruit.

In accordance with the present results, **Ghule et al., 2016** reported that molina fruit improve lipid profiles by lowering plasma total cholesterol and triglyceride concentrations . The hypocholesterolemic effect of fruit could be attributed to presence of isoflavones which prevent intestinal absorption of cholesterol by competition for its absorption sites . The potent hypercholesterolemic and hypotriglyceridemic effects of fruit could be due to the presence of inulin which behaves like a soluble fiber and possesses hypolipidemic effect . On the other hand, serum total cholesterol and triglyceride concentrations were not significantly affected by molina seed feeding. The difference in the cholesterolemic effect of similar dietary fibers among different studies may be due to the percentage of added dietary cholesterol, the presence or absence of cholic acid, the level of dietary fiber and species.

The observed elevation of ALT and AST in anemic rats. Moreover, **Clark et al. (2003)** reported that decrease the iron is commonly associated with long term elevations in liver enzymes. The reduction in the serum levels of aminotransferases as a result of Molina administration during the present study might probably be due in part to the presence of isoflavones, polyphenols and other antioxidants as mentioned before which aided in reducing the liver injury induced by anemia. The water soluble antioxidant properties of Molina was investigated by **Malik et al. (2017)** and evaluated in *vitro* and in *ex vivo* as protective activity against rat liver cell microsome lipid peroxidation. Moreover, reduced fat cells in the liver as a result of reducing body weight may also improve liver function. **Ahmed et al. (2003)** concluded that Molina has antihepatotoxic effect and significantly lowers serum levels of AST and ALT enzymes even in CCL4 intoxicated rats.

Several authors have reported that iron mal-absorption is mainly caused by some of the food constituents which can be inhibitors of iron absorption and may contribute to the high prevalence of iron deficiency found. Data indicate that Molina feeding prevented the development of anemia and improved hemoglobin, the hematocrit and both serum iron contents. The final hemoglobin concentration and hematocrit in the rats fed molina were significantly higher than those in the rats fed the control diet. Serum iron and HRE also significantly increased after

Molina fruit. It has been reported that there was a high positive correlation between serum iron concentration and iron absorption **Buchowski et al. (1989)** also reported a correlation between HRE and apparent absorption of iron. Feeding Molina -containing diet appears to increase in total iron binding capacity as shown in obtained results. It seems that the effect of the ingested doses of molina were enough to stimulate iron absorption in the experimental rat groups with significant ($P < 0.05$) different effect according to the ingested dose.

In the present study, Molina was used, which is a water-insoluble compound, as the iron source of the experimental diets. In this case Molina feeding is highly suggested to decrease the pH of the cecal contents and therefore increases the iron concentration in the soluble fraction of the cecal contents. The mechanism of iron absorption via not only the small intestine, but also via the large intestine has not yet been clarified (**Ohta et al.; 1997**). However, sufficient iron is absorbed via the large intestine for recovery from iron-deficiency anemia in rats (**Ebihara & Okano 1995**). Therefore, this study speculates that the effect of the tested molina in increasing the absorption of iron takes place in the large intestine in rats.

In conclusion, The observed improvements may be revealed to the presence of many antioxidant components found in molina fruit. On the basis of the present results, it could conclude that molina especially at 20% may have synergistic effect and its intake of be useful for treating obesity as it reduces feed intake and body weight, improves serum lipid profile, liver function and thyroid activity in obese rats.

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تأثير المستويات المختلفة من اليقطين على الحالة الصحية للفئران المصابة بالأنيميا

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الملخص العربى

تم تقييم الدور العلاجي الوقائي لليقطين للأنيميا المصاحبة بنقص الحديد كغذاء وظيفي تم تغذيته ثلاث مجاميع تجريبية علي الفئران بنظام غذائي بمستويات مختلفة من ثمره اليقطين لمدة اربع اسابيع مع وجود مجموعته الضابطة التي تعتمد في غذائها علي وجبة خالية من الحديد وقد لاحظ انه في نهاية التجربة ان مجموعة الفئران التي اعتمدت في غذائها علي وجبات غذائية مدعمة باليقطين

(4.55 ± 69.11 إلى 2.12 ± 83.3 ملجرام) وجود زياده في مستوي الحديد بمعدل الإضافة الي انه عند قياس مستوي الهيموجلوبين وجد زيادة نسبته (1.1 ± 11.11 إلى 1.1 ± 14.25 جرام)

والهيماتوكريت: (0.15 ± 39.15 إلى 1.27 ± 42.15 %)
والفيريتين: (2.25 ± 49.55 إلى 0.15 ± 65.12 ملجرام)

وذلك مقارنة مع مجموعة الضابطة. وقد وجد ان ١٠% من اليقطين يزيد امتصاص الحديد في الوجبات الغذائية اليومية ولذلك يمكن تدعيم الغذاء به لمنع وعلاج فقر الدم.

الكلمات المفتاحية: اليقطين , امتصاص الحديد فيتامين سي, انيميا.