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جامعة عين شمس، كلية التربية النوعية، القاهرة، مصر

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 Remedial Effects of Cydonia oblonga Miller (quince) Extract and Powder on Enhancing the Anemia and Levels of Antioxidant Enzymes in the Blood Serum of Rats

Dr. Rasha H.H. Ashkanani

 The effect of interaction the two styles of sharing and two styles of adaptive systems in e-learning environment on the development of personal knowledge management skills among postgraduate students

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Remedial Effects of Cydonia oblonga Miller (quince) Extract and Powder on Enhancing the Anemia and Levels of Antioxidant Enzymes in the Blood Serum of Rats

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Remedial Effects of Cydonia oblonga Miller (quince) **Extract and Powder on Enhancing the Anemia and** Levels of Antioxidant Enzymes in the Blood Serum of Rats

Dr. Rasha Haji Hasan Ashkanani

Abstract

This study aimed to investigate the protective effects of guince agueous extract (QAE) and compare it with the effect of quince powder (QP) in different proportions on strengthening the immune system and levels of antioxidant enzymes in the blood serum of rats at a dose of (5 and 10 ml orally / day of the extract and 10% and 20% of the powder in animal diet). METHOD: Thirty male albino rats (about 170±10 grams) were used in this study. Then they were divided into five equal groups (6 rats each). The first considered negative control .The groups that were fed the basic diet throughout the experiment, the other groups (24 rats), including the second and third group they were fed a basal diet + oral doses of 5 and 10 ml orally/day of QAE for (4 weeks). Then the fourth and fifth groups were fed a basal diet + 10% and 20% of QP, and a complete blood cell count was done.

Keywords: Quince, Antioxidant Enzymes, Hemoglobin, Red Blood Cells, White Blood Cells.

ملخص:

العنوان: التأثيرات العلاجية لمستخلص ومسحوق السفرجل في تحسين فقر الدم ومستويات الانز يمات المضادة للأكسدة في الفئر ان

المؤلفون: رُرَشًا حاجي حسن أشكناني على المؤلفون على المؤلفون على المؤلفون على المؤلفون على المؤلفون على المؤلفون المؤلفة المؤ مسحوق السفر جل (QP) بنسب مختلفة على تقوية الجهاز المناعي ومستويات الإنزيمات المضادة للأكسدة في مصل دُم الفُئران عند مستوى جرعة (٥ و ١٠ مل فمويّاً / يوم من المستخلص و ١٠٪ و · ٧٪ من المسحوق في الطعام). تم استخدام ثلاثونُ من الفئر إن البيضاء الذكور (١٧٠ ± ١٠ جرام) في هذه الدراسة. ثم تم تقسيمهم إلى خمس مجموعات متساوية (٦ فئران لكل مجموعة). اعتبرت الأولى مجموعة ضابطة سلبية " التي تم تغذيتها بالنظام الغذائي الاساسي طوال فترة التجربة" ، المجموعات الأخرى (٢٤ فأر)، بما في ذلك المجموعة الثانية والثّالثة تم تغذّيتها بنظام عذائي أساسى + جرعات فموية من المستخلص المائي قدرها ٥ و ١٠ مل / يوم لمدة (٤ أسابيع). ثم تم تغذية المجموعتين الرابعة والخامسة بنظام غذائي أساسي + ١٠٪ و ٢٠٪ من مسحوق السفر جلQP، وتم إجراء تعداد كامل لخلايا الدم.

الكلمات الدالة: السفرجل، الإنزيمات المضادة للأكسدة، الهيموجلوبين، خلايا الدم الحمراء، خلايا الدم البيضاء.

Introduction

Plant medicines are integral therapy that uses a lot of plants to avoid disorders in various countries all over the world as therapeutic agents in traditional medicines (Kumar et al.,2012). But there is not enough review of the literature for their probable toxic and side effects, so we need more searches about this point (Monfared, 2013). Accumulated findings suggest that eating fruit and vegetables is beneficial against CVD (Rissanen et al.,2003). Positive effects of fruits and vegetables have been attributed to dietary fibers, antioxidants, and especially phenolic compounds (Joshipura et al.,1999). Fibers polyphenols are capable of improving the parameters.(Alvarez-Parrilla et al.,2010) Anemia is widespread nutritional deficiency disease and secular as a big problem which that affects developed countries. health According to the reports of WHO, one-third of the universal populations more than two billion are anemic because of the imbalance in their feed intake from nutritious (Shubham et al., 2020). There are some kinds of anemia such as: - Deficiency production of red cells (aplastic anemia). - Deficiency in hemoglobin synthesis (iron deficiency anemia). -Deficiency of maturation (megaloblastic anemia). - Genetic deficiency of hemoglobin maturation (thalassemia). - Synthesis of abnormal hemoglobin (hemoglobin opathies). - Physical loss of red cells (hemolytic anemia's) (El-kenawy, 2019) and (Abbas, 2020) . Cydonia oblonga or quince from Rosaceae family is a fruit tree cultivated in many parts of Europe, Mediterranean, North Africa, and Asia and its origin is attributed to Middle East, especially to Iran and Caucasus. (Entesar Hanan et al., 2023). It is largely cultivated in Iran so that Iran supplies about 75% of the world production, thus, it is recognized as an important dietary (nutrition) source and is traditionally used as a gastric tonic, ant inflammatory, and ulcer healing antidiarrheal, especially within the gut, suitable for uterine and hemorrhoid bleeding, antiemetic, and astringent. (Andreia et al., 2008). The preventive effect against anemia, as eating quince powder or extract because of its nutritional value and contains bioactive substances that work to enhance and improve vital blood

components.(National Center for Biotechnology Information, 2023). The antioxidant theory, which states that eating fruits with antioxidant properties can reduce oxidative stress associated with many diseases.(Aminjan et al., 2019)

Quince as an appropriate natural source of antioxidants including flavonoids and phenolic derivatives could be considered as a complementary medicine for anemia in addition to convenient treatment.(Daniela et al., 2010) Quince's phytochemicals are mainly pectin, malic acid, and tannins. It is rich in Vitamins B, C, and carotene. (Anwar Umar et al.,2015)

Materials and methods

Material:

Plants: Quince fruit was obtained, imported from Iran, and was chemically analyzed at the Institute of Food Technology, Giza, Egypt.

Rats and diets: Forty-two albino male rats weighing 170 ±10 grams were used in this study.

Chemicals: Serum biochemical analysis kits were purchased from Sigma.

Methods:

Preparation of Quince's extract: Quince fruits were cleaned very well and they were cut into approximate 10x10 mm-size pieces including the peel, pulp and seeds. 500g of the fruits were soaked in 1 liter of distilled water, in a beaker with the volume completed to 1 liter. The beaker was enveloped with foil and left about 12h at 4 o C. Then, filtered by a coarse screen . The extract was collected in the flask (1liter) and concentrated in a bath of water at 30oC till the volume of 500ml. prepared extract was equal (one gram from the fruit in two ml of extract). The extract was stored at 4° C, then for given to rats a maximum of two days. Calculated doses which giving to the rats were prepared by dilution using distilled water daily (Elhassan and Yagi, 2010).

Preparation of Quince's powder: Ripe quince obtained from the local market (Iranian import) was used. The surface of the quince was disinfected with a chlorinated water solution (100 ppm). One kilogram of quince, cut into thin slices. The slides were placed in trays and dried in a forced-air incubator at 55 and 60°C for 48 h. The dried samples were ground into fine flour using a blender, stored in transparent polyethylene bags and kept at a temperature of 4°C before chemical analysis and use.

Experimental design of biological study

Thirty albino rats weighing 170 ± 10 grams. All animals kept under controlled conditions of light (12 h of light and 12 h of darkness) with the ambient temperature of 22±2°C and relative humidity of 40 - 60% and free access to water and food in the cages. All animals will be allowed free access to basal diet. The basal diet composition will be as follows: Casein (20%), sucrose (50%), Corn Starch (15%), powdered cellulose (5%), corn oil (5%), mineral mix (3.5%), vitamin mix (1%), DL- Methionine (0.3%), Choline bitartrate (0.2%) according to AIN-93 guidelines (Reeves et al., 1993). Then they were divided into five equal groups (6 rats each). The first considered negative control. The groups that were fed the basal diet throughout the experiment. the other groups (24 rats), including the second and third groups they were fed a basal diet + oral doses of 5 and 10 ml orally/day of QAE for (4 weeks). Then the fourth and fifth groups were fed a basal diet + 10% and 20% of QP (4 weeks). The body weight of rats will be measured at the beginning of experimental period and after 7 days intervals. At the end of the experimental period , rats were fasted overnight, then anaesthetized & incised longitudinally and blood samples were collected from the aorta, blood samples will be collected and centrifuged at 3000 rpm to obtain the blood serum which will store at (-20°C) for biochemical analysis.

Biochemical analysis:

Hematological tests: were completed using Beckman coulter LH750 Germany/ U.S.A. - Determination of total leucocyte count (WBC): WBC (total and differential) was determined according to **(KodaKimble et al., 2001).**

- Determination of differential count of white blood cells: WBC leukocytes are divided into two groups, Leukocytes are a part of the body's defense system; they respond immediately to foreign invades by going to the site of involvement. The differential count of white blood cells was determined according to (Mathy and Koepke, 1974)
- Hemoglobin, (Hb): Hemoglobin was determined in whole blood according to (Lewis and Dacie, 1965)
- Red Blood Corpuscles count (RBC): R.B. Cs corpuscles were determined according to Lubsandorzhiev, (2006).
 Platelet Count Determination: Serum PLT was determined according to Daly, (2011).
- Determinations of hematocrits (HCT): Serum hematocrits were determined as % according to Purves et al.,(2004).

Statistical analysis

Data are presented as means ± SD and the analysis was conducted using SPSS program, Version 16.0 (2007). The Dunk'test multiple range post-hoc test was used to conduct the statistical analysis using SPSS, PC statistical software (Verion 16.0 SPSS Inc., Chicago, USA). One-way analysis of alteration was functioned to analysis the data (ANOVA). At P under 0.05, the values were considered substantially different (Snedecor & Cochran, 1980).

Results:

TABLE (1): CHEMICAL ANALYSIS OF QUINCE(CYDONIA OBLONGA)/100 G.

Nutrients	Amounts	
Water (g)	76.8	
Protein (g)	0.4	
Energy (Kcal)	53.5	
Lipids (g)	0.1	
Total carbohydrates (g)	14.1	
Fiber (g)	8.6	
Calcium (mg)	10.1	
Phosphorus (mg)	15.2	

Magnesium (mg)	8,2
Potassium (mg)	182
Iron (mg)	0.7
Sodium (mg)	3.6
Cupper (µg)	131
Vitamin C (mg)	15.7
Vitamin A (IU)	36.8

Haemoglobin and Haematocrit of rats which received different levels of quince aqueous extract (QAE)and quince powder (QP).

Table (2) illustrates the effect of QAE and QP on haemoglobin and haematocrit levels of healthy rats. Tabulated data showed that there were significantly increase in mean values of HB and HCT of all treatments. It could be noticed that the highest values for HB and HCT were recorded for group 5 (rats received 20% QP) by the percent of (22.14and 43.44% respectively).

Table (2) Haemoglobin and Haematocrit of rats which received different levels of quince aqueous extract (QAE)and quince powder (QP).

Groups	HB (g/dl) Mean ± SD	% change of control negative	HCT (%) Mean ± SD	% change of control negative
Control (-)	10.7 ± 0.1e		27.3 ± 0. 1e	
QAE (5 ml)	10.92 ± 0.11 d	2.05	30.63 ± 0.1 d	12.19
QAE (10 ml)	11. 14 ± 0.08 c	4.11	$33.7 \pm 0.03 c$	23.44
QP (10 %)	12.88 ± 0.11 b	20.3	35.3 ± 0.1 b	29.30
QP (20 %)	13.07 ± 0.016 a	22.14	39.16 ± 0.18 a	43.44
LSD (p ≤ 0.05)	0.157		0.216	

Values are expressed as mean \pm SD. Significance at p<0.05.

Values which don't share the same letter in each column are significantly different.

Platelet (PLT), White Blood Cells (WBCs) and Red Blood Cells (RBCs) (cm) of rats which received different levels of quince aqueous extract (QAE)and quince powder (QP).

Table (3) illustrates the effect of **different dosages** on (**PLT**), (**WBCs**) and (**RBCs**) (cm) of healthy rats. Data illustrated that there were significant increases in values of **PLT** and **RBCs** of all treated groups compared to the control .The highest value

was for group 5 (rats received 20% QP) by the percent of the increase (27.01 and 77.17 % respectively) compared with the control, For **WBCs**, it could be observed a significant decrease in the mean values of all treated groups. The lowest value was for group 2 (rats received 5ml **QAE**) compared with other groups.

Table (3): Platelet (PLT), White Blood Cells (WBCs) and Red Blood Cells (RBCs) (cm) of rats which received different levels of quince aqueous extract (QAE) and quince powder (QP).

Groups	PLT (103cm) Mean ± SD	% change of control negative	WBC (103cm) Mean ± SD	% change of control negative	RBC(106cml) Mean ± SD	% change of control negative
Control (-)	609.66 ±1.3 e		10.86 ± 0.01 a		2.76 ± 0.41 d	
QAE (5 ml)	641.66 ± 2.11 d	5.24	10.13 ± 0.05 b	-9.32	3.33 ± 0.01 c	20.65
QAE (10 ml)	662.33 ± 3.66 c	8.63	9.71 ± 0.06 c	-8.94	$3.86 \pm 0.04 b$	39.85
QP (10%)	715.66 ± 3.07 b	17.3	8.31 ± 0.04 e	-7.65	4.11 ± 0.11 b	48.91
QP (20 %)	774.33 ± 3.33 a	27.01	$8.05 \pm 0.06 d$	-7.41	4.89 ± 0.13 a	77.17
LSD (p ≤ 0.05)	4.87		0.166		0.423	

Values are expressed as mean \pm SD. Significance at p<0.05.

Values which don't share the same letter in each column are significantly different.

Superoxide Dismutase (SOD u /ml) and Catalase (CAT ng/ml) of rats received different levels of quince aqueous extract (QAE) and quince powder (QP).

Table (4) showed the effect of **QAE** and **QP** on **SOD** and **CAT** of healthy rats. For (**SOD**) data showed increases in mean values of all treatments compared with control without any significant differences except for group (5) which was increased by the percent of 28.88 %) the lowest value was for the group (2) which was increased by the percent of **7.65**%). For **CAT** was significantly higher means of **CAT** of all treated groups compared with the negative control. The highest value was for the group (5) by the percent of the increase (**71.48**%) compared with the negative control, and the lowest value was for the group (2) by the percent (**17.8**%).

Table (4) Superoxide Dismutase (SOD u/ml) and Catalase (CAT ng/ml) of rats which received different levels of quince aqueous extract (QAE)and quince powder (QP).

Groups	SOD (u /ml) Mean ± SD	% change of control negative	CAT (ng/ml) Mean ± SD	% change of control negative
Control (-)	123.6 ± 0.4 b		2.35 ± 0.1 e	
QAE (5 ml)	133.06 ± 0.3 b	7.65	2.77 ± 0.05 d	17.8
QAE (10 ml)	139. 81 ± 2.75 b	13.11	3.23 ± 0.13 c	37.44
QP (10 %)	144.35 ± 3.27 b	16.78	$3.86 \pm 0.07 b$	64.25
QP (20 %)	159. 3 ± 5.76 a	28.88	4.03 ± 0.06 a	71.48
LSD (p ≤ 0.05)	31.07		0.213	

Values are expressed as mean \pm SD. Significance at p<0.05. Values which don't share the same letter in each column are significantly different.

Glutathione (GSH ng /ml) and Glutathione Peroxidase (GPx ng/ml) of rats which received different levels of quince aqueous extract (QAE)and quince powder (QP).

Table (5) showed the effect of QAE and QP on the GSH and GPx of healthy rats. The obtained data illustrated a significantly higher in means of GSH and GPx of all treatments except group (2) of GSH which showed a significant decrease. The highest value was for group 5 (QP 20 %)) by the percent of the increase (25.2and 19.72% respectively) .The lowest value was for group 2 rats received 5 ml QAE by the percent of (4.93and 7.02% respectively).

Table (5) Glutathione (GSH ng /ml) and Glutathione Peroxidase (GPx ng/ml) of rats which received different levels of quince aqueous extract (QAE)and quince powder (QP).

Groups	GSH (ng /ml) Mean ± SD	% change of control negative	GPx (ng /ml) Mean ± SD	% change of control negative
Control (-)	107.06 ± 0.3 d		119.7 ± 0. 53 d	
QAE (5 ml)	112.34 ± 0.3 e	4.93	128.11 ± 2.23 d	7.02
QAE (10 ml)	122.07 ± 0.66 c	14.02	133.6 ± 0.33 c	11.61
QP (10 %)	128.2 ± 0. 19 b	19.74	139. 17 ± 0.63 b	16.26
QP (20 %)	134 .04 ± 0.61a	25.2	143.31 ± 0.28 a	19.72
LSD (p ≤ 0.05)	0.526		2.542	

Values are expressed as mean \pm SD. Significance at p<0.05.

Values which don't share the same letter in each column are significantly different.

Discussion

In this study, supplemented rats with quince for 28 days caused some improvement in most haematological parameters. Also, significant increases were observed for RBCs and platelets count. Trivial increase was observed for HB. These results was found to be in the same line with (Kawahara and lizuka ,2011) who found that oral dosages (250 and 500 mg/kg/rat) of ethanol extract of quince for 3 weeks increased haemoglobin levels significantly. And this may be due to the amounts of vitamin c in fruit (Magalhães et al., 2009). Quince was also found to have strong anti-inflammatory and antioxidant capacity (Essafi et al., 2012)

This study paves the way for further studies on the cardiovascular effect of quince consumption as a beneficial nutraceutical in humans.(**Zubair et al.,2015**)

Quince's widespread therapeutic use and valuable phytochemical composition (Abliz et al. 2014). The fruit contains vitamin C and various minerals such as sodium, calcium, phosphorus, potassium, and nitrogen (Rop et al., 2011)

COM seeds contain triterpenes, sterols, and tannins that are reported to be responsible for their ant anemic activity which has antioxidative activity, in quince is significantly higher than that of an apple (Sut Set al., 2019)

Furthermore, it was reported that different doses of Cydonia oblonga fruit for four weeks led to a significant decrease in the number of PLT. The same observation also applies herbal medicines (Cheesbrough, to some **2005**). Significant increases in the number of PLT and megakaryocytes were observed in mice that received an aqueous extract of Cydonia oblonga fruit for 1 week (Deutsch and Tomer, 2006). Quince also contains many phenolic compounds such as caffeoylquinic acid, procyanidin-B2, oligomeric procyanidin, polymeric procyanidin, etc., essential oils such as furfural, limonene, linalool, fumifolol, toluene, beta ionone, terpineol, etc. together and These compounds give quince its unique aroma. (Ashraf et al., 2016)

Ripe quince fruit contains a good concentration of vitamin C. 100 grams of fruit provides 15 mg or 25% of the recommended daily amount of vitamin C. Fruits rich in this vitamin help remove harmful oxygen-free radicals from the body. Vitamin C helps boost immunity, enhance iron absorption in the body, and reduce viral attacks and inflammatory conditions. (Kiefer et al., 2004)

It is a good source of minerals such as copper (130 mcg or 14% of the recommended daily amount), iron and copper needed for blood formation, potassium and magnesium as well as B complex vitamins such as thiamin, riboflavin and pyridoxine (vitamin B6).(Harries and Dye:2006)

Quinces are rich in antioxidants, which help reduce inflammation and protect cells from damage caused by free radicals. Polyphenols like quercetin and kaempferol found in quinces have anti-inflammatory properties that may help prevent chronic diseases. (Zohalinezhad et al.,2016)

Supplementation with ethanolic extract (E.X) of quince fruit leads to an improvement in Hb, RBCs and PCV. Improvement in PLT count was also observed. (Shubham et al.,2020). The improvement in most blood parameters in the quince-treated groups may be due to the antioxidant contents in quince extract. Nutritional supplements also improved hemoglobin levels in rats. These results obtained support the main use of quince fruits except in cases of anemia (Oloyede et al., 2015).

Conclusion: The quince fruit used in this study contains effective and improved CBC and antioxidant enzymes. The results obtained from previous studies supported that this fruit contains a lot of bioactive compounds capable of enhancing blood parameters. Therefore, we recommend consuming more of this fruit in our daily diet.

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