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Aziza H. Abd El zahir, and Mona M. Farouk.

Nutrition & Food Science Dept., Faculty of Home Economics, Menoufia Univ., Egypt

مجلة البحوث في مجالات التربية النوعية

معرف البحث الرقمي DOI: 10.21608/jedu.2022.132013.1634

المجلد التاسع العدد 44 . يناير 2023

الترقيم الدولي

P-ISSN: 1687-3424 E- ISSN: 2735-3346

موقع المجلة عبر بنك المعرفة المصري https://jedu.journals.ekb.eg/

موقع المجلة http://jrfse.minia.edu.eg/Hom

العنوان: كلية التربية النوعية . جامعة المنيا . جمهورية مصر العربية

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Abstract

Anemia is a pathological condition in which the blood's ability to carry oxygen is reduced due to a decrease in the production of red blood cells or hemoglobin, which causes fatigue and weakness. This study aimed to use lemon and orange peel as an aid in the treatment of anemia. Forty-eight male albino rats weighing 140±10 g were used in this study and divided into 8 groups. The group contains 6 rats. Infected rats were treated with lemon peel, orange peel and their mixture on infected mice for 28 days. The results showed that the anemic mice treated with a mixture of lemon and orange peel had an improvement in raising hemoglobin and an improvement in liver function, and the best results were for a mixture (5%) of lemon and orange peel. As conclusion, anemia rats treated with (5%) mixture of lemon peel and orange peel powder had improvement (Hg) and liver functions compared with (2.5%) lemon peel powder.

Key words: lemon peel, orange peel, Rats, anemia and Biochemical analysis.

Introduction:

Anemia is the inability of the human body's blood to carry oxygen, and it occurs due to strikes inside the blood fluid, in which the size and number of red blood cells drop below their normal level, and also the level of hemoglobin decreases **(Mahoney and Armsby 2021)**. Symptoms of this disease are tension, shortness of breath, a feeling of loss of consciousness, increased shivering and thirst, the shape of the person is pale and the color is abnormal in a large way, and the symptoms may be large, depending on the percentage of deficiency, and the lack of blood increases with surgical operations and bleeding **(Janz *et al.*, 2013)**. Anemia is explained as a lack of red blood cells in terms of size and number, and therefore the body inability to produce an adequate amount of blood, and severe or hemolytic anemia in which a large amount of blood is lost, and it is called pernicious anemia. This is due to the severe deficiency of iron that binds to hemoglobin, the component of blood responsible for carrying oxygen **(Ng, *et al.*, 2019).** In order to describe many types of anemia that are due to many defects in the blood of women, such as a congenital defect, in which the red cell is not fully matured enough, and the hypertrophies that occur for red blood cells, which lead to the inability to form hemoglobin, and there is also a defect that may occur in the formation of hemoglobin This results from genetic factors and impaired hemoglobin formation, and there are other types, including sickle cell anemia and severe loss of red blood cells **(Mukherjee and Ghosh, 2012).** State, the body cannot produce sufficiency of red blood cells in the required quantity in order to use them to carry oxygen **(Olagunju *et al.*, 2017)**. The amount of iron is required to form hemoglobin, which plays a major role in the various functions of the body **Gogoi *et al*, (2017) and Omotoso (2012) (Olagunju *et al.*, 2017)**. The amount of iron is low in the blood fluid in all parts of the body, and this condition is women who are in the fertile period because of the increased obliteration that occurs every month, as well as because of bleeding and also because of surgical operations because of bleeding from the gastrointestinal tract. In this case, solutions must be taken to raise hemoglobin **ISSN, (2016)**. Iron deficiency also increases in the case of pregnancy, because the blood volume

increases by a large percentage and after the severe bleeding that occurs with pregnancy and children's growth spurts and malnutrition such as the diet that lacks iron and some medicines that interfere with the representation of the hump in the body and there is another type such as the lack of some vitamins such as vitamin B12 and Vitamin C, as well as intestinal diseases such as intestinal worms and pinworms **(Harper *et al.*, 2015).** Bioavailability is achieved by eating foods that help absorb iron and also by reducing the intake of iron-inhibiting substances such as phytate and tannin. One of the important factors for iron absorption is vitamin C, which is got from lemon from citrus and oranges, and vitamin C prevents the formation of insoluble iron compounds **(James, 2001).** Orange and Lemon contains a large amount of vitamin C and phenolic compounds, especially in the peels and ascorbic acid content (vitamin C) which treatment of many diseases

This work was conducted to study the effect of orange and lemon peels and their mixture powder on biochemical analysis of anemic rats.

Materials and Methods

Materials:

lemon and orange were obtained from local market, Menoufia Governorate, Egypt. **Basal diet of experimental anemia**

Rats were fed a diet high in vitamin C in the basal diet used for control negative and were in the basal diet positive control.

Casein, cellulose, choline chloride, and DL- Methionine

Experiment materials were purchased (Casein, cellulose, choline chloride powder, and DL- methionine powder) from Morgan, Cairo; Egypt.

Experimental animals

A total of 48 adult normal male albino rats Sprague Dawley strain weighing 140±10 g purchased from the Vaccine and Immunology Organization of the Ministry of Health from Helwan Farm in Cairo Governorate.

The chemical kits

Chemical kits used for determination the biochemical parameters Purchased from Al-Gomhoria Company for Chemical, Medical and Instruments, Cairo, Egypt.

Methods

Preparations the powder of orange and lemon peel.

Orange and lemon peel were purchased to in the Menoufia Governorate local market. In March 2022. Under running water, the lemon and orange peels were scrubbed thoroughly. Following the thin slicing of the lemon and orange peels, samples were collected, dried in an air oven at 60 degrees Celsius, and then ground in a grinder until a powder was produced. Before use, the powder was put into bags and frozen.

Preparation of extracts

The milled sample was combined with ethanol at a ratio of 1:2 (w/v) and allowed to stand for 24 hours in order to create the extract. After that, a filter paper was used to filter the mixture (Whatman No. 1). The residue was cleaned with ethanol, and the extracted material was vacuum-dried in a rotary evaporator and stored dry for further investigation. Pure ethanol was used to reconstitute the extracts before they were analysed for total vitamin C content

Experimental design

The experiment rats were forty-eight adult male albino rats, of the type Sprague Dawley, 2 months age, they weighing (140±10g) When the rats were fed on basal diet for a week, then were organized into groups, each group containing 6 rats prepared according to **AIN (1993)**. After this adaptation period, rats are divided into 8 groups, each group which consists of five rats as anemic rats follows: group (I): Rats were fed a basic diet as negative control. Group (2): Anemic rats induced by fed on tannic acid supplemented 28 days in the basal diet and used as a positive control group. Group (3): A group of anemic rats fed on lemon peel as powder by 2.5% of the weight of basal diet. Group (4): A group anemic of infected rats fed on a lemon peel as powder by 5% of basal diet. Group (5): A group anemic of infected rats fed on orange peel as powder

by 2.5 % of the weight of the rat. Group (6): A group anemic of infected rats fed on orange peel as powder by 5 % of the weight of the rat. Group (7): A group anemic of infected rats fed on mixture of the alemon and orange peel as powder by 2.5 % of the weight of the rat. Group (8): A group anemic of infected rats fed on mixture of the lemon and orange peel as powder by 5 % of the weight of the rat. The body weight and feed intake of the rats were calculated weekly during the experimental period, and their general behaviour was assessed. The experiment lasted for 28 days, and at the conclusion of that time, each rat was weighed separately before being put to sleep and having blood samples obtained.

Blood sampling:

Blood samples were taken after a 12-hour fast, initially from the retro orbital vein and, at the conclusion of each trial, from the hepatic portal vein. Then, for 30 minutes, coagulation takes place in a water bath at a temperature of 37 degrees Celsius. Blood was taken into tubes and maintained in a deep freezer until use after being allowed to coagulate in a water bath (37°C) for 30 minutes. Blood samples were then centrifuged at 4000 rpm for ten minutes to separate the blood serum **Schermer (1967)**.

Body weight gain (BWG), feed intake (FI), and feed efficiency ratio (FER):

The experiment lasted for 28 days, during which time the rat's weights were measured weekly, the amount of food consumed was noted, and the efficiency ratios (FER) of the food intake were also computed. **Chapman *et al.*, (1959)** as follow:

$$\text{FER \%} = \frac{\text{Body weight gain (g)}}{\text{Food intake (g)}} \times 100$$

Determination of vitamin C

By titrating the sample's aqueous extract with a solution of 2,6-dichlorophenol-indophenol dye to a pale pink endpoint, vitamin C content was evaluated **(AOAC, 2005).**

Biochemical analysis:

Liver functions

Determination of serum aspartate alanine aminotransferase (ALT) and aminotransferase (AST) were carried out according to the method of **Hafkenscheid (1979), Clinica Chimica (1980)**, **and Moss (1982**), respectively.

Kidney functions

Determination of Enzymatic determination of serum urea and serum creatinin was performed in accordance with **(Henry (1974)** and **Patton & Crouch 1977)**.

Determination of (Globulin, Alb and (Alb /Glo) (g/dl)

Albumin/Globulin (A/G) ratio test measures the total amount of protein in your blood.

Determination of Blood Hb test:

The results of Hb is generated by highly automated electronic and pneumatic multichannel analyzers based on aperture – impedances and/or laser beam cell sizing and counting according to **Jacobs *et al.*, (2001).**

Determination of Atherogenic, index of plasma useful predictor cardiovascular risk **Zephy, (2015).**

Statistical analysis:

Statistical analyzes **(SPSS, 1988)** were conducted to f present work result. Each analysis was performed with the Student-Newman-Keuls test. The differences between treatments ($P \leq 0.05$) were taken into account and were significant. Biological results were analyzed by using SPSS software. Biological results were analyzed by one way ANOVA.

RESULTS AND DISCUSSION

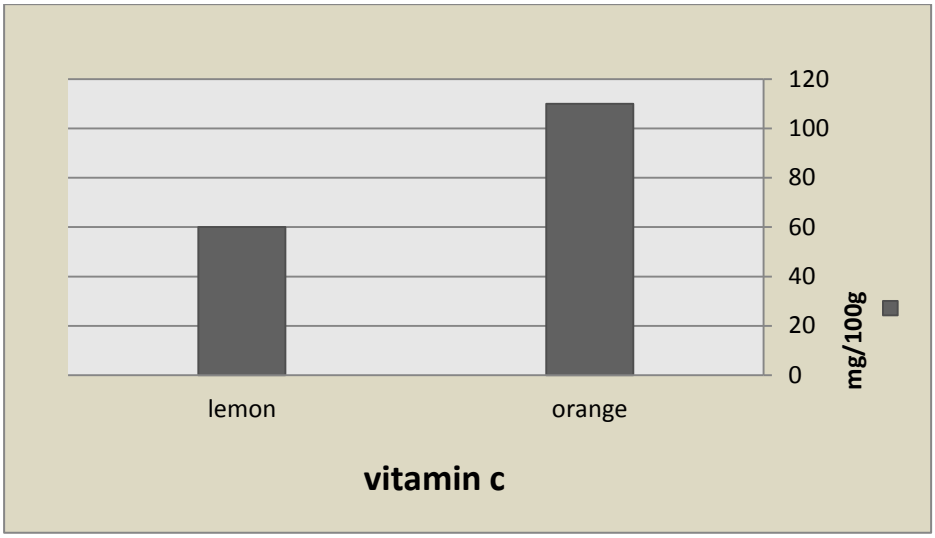

Figure 1: show the Vitamin C content of orange and lemon peel

The vitamin C content in the orange and lemon, was quantified (Figure 1). From the figure, it was observed that the vitamin C content was found much higher in orange than lemon.

Effect of lemon, orange peel and their mixture on body weight gain, feed intake and feed efficiency ratio of anemia rats:

Data presented at table (1) show the effecting of lemon, orange peel and their mixture on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of anemic rats. The obtained results showed that the body weight gain (BWG) % of negative control listed, the highest value when compared with positive control with significant difference. From anemic rats groups, it is clear to notice that the highest (BWG) % listed for 2.5 % lemon, while the lowest BWG% listed for 5% lemon and orange mixture with significant difference ($P \leq 0.05$), In case of feed intake, it could be notice that the feed intake (FI) % of negative control listed the highest value when compared with positive control with significant difference. While, 2.5 % lemon listed the highest FI while the lowest value recorded for 5% lemon and orange mixture with significant difference ($P \leq 0.05$). The mean values were on the other hand, feed efficiency ratio (FER)

of negative control recorded the highest value when compared with positive control with no significant difference (P≤0.05). The mean values were. In case of treated rat groups, it clear to mention that 2.5 % lemon recorded the highest FER while, the lowest value recorded for 5% lemon and orange mixture with significant difference. These results agreement with **Khalesia *et al.*, (2011)**, They said that results of an experiment are similar experiment revealed a significant (P < 0.01) difference except for the first week. However, the second week, significantly (P < 0.01) increase in body weight was observed by lemon peel and orange peel in the treated groups rich in essential oils, and any significant increase for the mixed groups compared with control group. Over the duration of the experiment, growth was better (p < 0.01) and better in the peripapillary amygdala than in the control groups. During the fifth week, meals fortified with lemon peel alone or lemon orange peel mixture had significantly (p < 0.01) body weights that were higher than that of the control groups. Moreover, body weights in the orange peel oil-supplemented group were significantly (p < 0.01) higher than that of the control group. Similar trend regarding in vivo weight improvement due to lemon oil or orange peel essential oil supplementation over the course of the trial **(Botsoglou *et al.*, 2002)**

Effect of lemon, orange peel and their mixture on liver functions level of anemia rats:

Data given in table (2) show the effect of lemon, orange peel and their mixture on liver functions (AST and ALT) of anemia rats. The obtained results indicated that the AST liver enzyme of positive group control recorded the highest value when compared with negative control group with significant difference (P<0.05). While, the highest AST liver enzyme of treated group recorded for group fed on 2.5 % lemon peel but, the lowest value recorded for group fed on 5% lemon and orange peel mixture with significant difference (P<0.05). In case of ALT liver enzyme of positive control rats group recorded the highest value when compared with negative control group with significant difference (P<0.05). While, the highest ALT liver enzyme of treated group recorded for group fed on 2.5 % lemon peel but, the lowest value recorded for group fed on 5% lemon and orange peel mixture with

significant difference (P<0.05). These results are in agreement with **Al-Bashri (2013)**, who reported that administration of low and medium concentration of lemon peel prevents the increase of serum levels of ALT significantly ($p < 0.05$) in medium and high concentration of lemon group.

Effect of lemon, orange peel and their mixture on Globulin, Alb and (Alb /Glob) of anemia rats:

Data given in table (3) showed the effect of lemon, orange peel and their mixture on, alb and (alb /glob) levels of anemia rats. The obtained results indicated that the globulin level of positive control rats group recorded the highest value when compared with negative control group with significant difference (P<0.05). While, the highest globulin level of treated group recorded for group fed on 2.5 % lemon peel but, the lowest value recorded for group fed on 5% mixture lemon and orange peel with significant difference (P<0.05). On the other hand, the Alb level of positive control rats group recorded the highest value when compared with negative control group with significant difference (P<0.05). While, the highest Alb level of treated group recorded for group fed on 5 % mixture lemon and orange peel but, the lowest value recorded for group fed on 2.5% lemon peel with significant difference (P<0.05). In case of Alb /Glob, the level of positive control rats group recorded the less value when compared with negative control group with significant difference (P<0.05). While, the highest level of treated group recorded for group fed on 5% lemon peel, the lowest value recorded for group fed on 2.5% lemon peel with significant difference (P<0.05) these results according to **Adil *et al.*, (2016) and (Alya, 2015).**

Effect of lemon, orange peel and their mixture on kidney functions level of anemia rats:

Data given in table (4) showed the effect of lemon, orange peel and their mixture on the kidney functions (uric acid, urea and creatinine) level of anemia rats. The obtained results indicated that the creatinine level of positive control rats group recorded the highest value when compared with negative control group with significant difference (P<0.05). While, the highest creatinine level of treated group recorded for group fed on 2.5% lemon, peel but, the lowest value recorded for group fed on 5% mixture with

significant difference (P<0.05). On the other hand, the urea level of positive control rats group recorded the highest value when compared with negative control group with significant difference (P<0.05). While, the highest urea level of treated group recorded for group fed on 2.5% orange peel but, the lowest value recorded for group fed on 5% mixture with significant difference (P<0.05). In case of uric acid, the level of positive control rats group recorded the highest value when compared with negative control group with significant difference (P<0.05). While, the highest uric acid level of treated group recorded for group fed on 2.5%, lemon peel but, the lowest value recorded for group fed on lemon and orange 5% mixture with significant difference (P<0.05). The current study, the levels of creatinine, urea and uric acid were significantly decreased by lemon and orange peel treatment as compared with control negative groups in line with those of **Adil *et al.*, (2016).** Administration also decreased the elevated levels of kidney function creatinine urea & uric acid in parallel with this finding, **Hermenean *et al.*, (2013)** indicated the ability of lemon to protect the kidney against CCl4-induced renal toxicity in male rats

Effect of lemon, orange peel and their mixture on hemoglobin of anemia rats:

Data presented in Table (5) show the effect of lemon, orange peel and their mixture on the hemoglobin level of anemia rats. The obtained results indicated that the hemoglobin level of negative control rats recorded the highest value when compared with positive control group significant difference (P<0.05). While, the lowest hemoglobin level of treated group recorded for group fed on 2.5% orange peel but, the highest value recorded for group fed on 5% mixture with significant difference (P<0.05). Lemon and orange in the current study, the levels of hemoglobin were significantly increased by lemon and orange peel treatment and these results are in line with those of **Kandemir *et al.* (2017).**

Effect of orange, lemon peel and their mixture on Atherogenic index of anemia rats

Data presented in table (6) show the effect of lemon, orange peel and their mixture on the atherogenic index level of anemia rats. The obtained results indicated that the atherogenic index level of

positive control rats group recorded the highest value when compared with negative control group with significant difference (P<0.05). While, the lowest Atherogenic index level of treated group recorded for group fed on 2.5 % mixture but, the highest value recorded for group fed on 2.5% lemon peel with significantly (P<0.05). The current study, the level of atherogenic index were significantly decreased by lemon and orange peel which are in line with those of **Shahid, (2019).**

Table (1): Effect of orange, lemon peel and their mixture on body weight gain, feed intake and feed efficiency ratio of anemia rats

Parameters	BWG (g)	FI (g/day)	FER (%)
	M ± SD	M ± SD	M ± SD
Control group (-)	27.33^{a}±0.57	20.23^{a}±0.28	0.048^{a}±0.05
Control group (+)	24.66^{b}±0.57	19.16^{b}±0.28	0.046^{b}±0.01
anemic rats fed on lemon peel powder (2.5%)	22.83^{c}±0.28	18.5^{c}±0.00	0.044^{b}±0.01
anemic rats fed on lemon peel powder (5%)	22.16^{c}±0.57	17.5^{d}±0.05	0.045^{b}±0.005
anemic rats fed on orange peel powder (2.5%)	20.66^{d}±0.28	16.5^{e}±0.05	0.045^{b}±0.00
anemic rats fed on orange peel powder (5%)	19.36^{de}±0.32	15.6^{f}±0.05	0.044^{b}±000
anemic rats fed on mixture of lemon and orange peel powder (2.5%)	18.5^{de}±0.00	15.3^{f}±0.00	0.043^{b}±0.00
anemic rats fed on mixture of lemon and orange peel powder (5%)	$17.5.40^{e}$±0.05	14.5^{J}±0.43	0.043^{b}±0.00

values in the same column with letters are significantly different.

Table (2): Effect of lemon, orange peel and their mixture on liver functions level of anemia rats

Treatment/Parameter	AST (U/L)	ALT (U/L)
Control group (-)	$70^{e}\pm0.00$	$50.33^{J}\pm0.57$
Control group (+)	$171.33^{a}\pm1.50$	$90.50^{a}\pm0.50$
anemic rats fed on lemon peel powder (2.5%)	$89.66^{bc}\pm1.15$	$87.66^{a}\pm0.57$
anemic rats fed on lemon peel powder (5%)	$87.0^{c}\pm1.00$	$81.00^{b}\pm1.00$
anemic rats fed on orange peel powder (2.5%)	$85.0^{bc}\pm1.30$	$77.50^{c}\pm1.00$
anemic rats fed on orange peel powder (5%)	$78.66^{c}\pm3.78$	$73.33^{d}\pm1.50$
anemic rats fed on mixture of lemon and orange peel powder (2.5%)	$75.33^{d}\pm1.52$	$63.33^{e}\pm1.50$
anemic rats fed on mixture of lemon and orange peel powder (5%)	$74.66^{de}\pm0.57$	$57.00^{f}\pm1.00$

values in the same column with letters are significantly different**.**

Table (3): Effect of lemon, orange peel and their mixture on Globulin, alb and (alb /glob) of anemia rats

Treatment/Parameter	(Globulin) (g/dl)	(Alb) (g/dl)	(Alb /Glo) (g/dl)
Control group (-)	3.5 ± 0.5^{b}	5.66 ± 0.11^{e}	$1.75^{a}\pm0.15^{d}$
Control group (+)	5.40 ± 1.0^{a}	2.78 ± 1.8^{a}	0.17 ± 0.005^{b}
anemic rats fed on lemon peel powder (2.5%)	2.63 ± 0.05^{c}	4.00 ± 0.26^{b}	1.50 ± 0.45^{ab}
anemic rats fed on lemon peel powder (5%)	2.50 ± 0.10^{cd}	4.36 ± 0.15^{c}	2.06 ± 0.01^{ab}
anemic rats fed on orange peel powder (2.5%)	2.43 ± 0.05^{cd}	4.53 ± 0.11^{b}	2.24 ± 0.31^{ab}
anemic rats fed on orange peel powder (5%)	2.33 ± 0.05^{de}	4.63 ± 0.11^{c}	2.43 ± 0.14^{ab}
anemic rats fed on mixture of lemon and orange peel powder (2.5%)	2.13 ± 0.05^{ef}	4.80 ± 0.11^{d}	2.37 ± 0.005^{a}
anemic rats fed on mixture of lemon and orange peel powder (5%)	2.06 ± 0.05^{f}	5.06 ± 0.05^{e}	2.37 ± 0.005^{a}

values in the same column with letters are significantly different ($p < 0.05$).

Table (4): Effect of lemon, orange peel and their mixture on creatinine, urea and uric acid of anemia rats.

Treatment/Parameter	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
Control group (-)	1.91 ± 0.06^{c}	26.66 ± 0.57^{J}	1.87 ± 0.05^{d}
Control group (+)	4.33 ± 0.15^{a}	55.57 ± 1.10^{a}	3.80 ± 0.10^{a}
anemic rats fed on lemon peel powder (2.5%)	3.80 ± 0.10^{b}	48.00 ± 0.10^{b}	3.66 ± 0.05^{a}
anemic rats fed on lemon peel powder (5%)	3.43 ± 0.02^{c}	44.66 ± 2.08^{bc}	3.40 ± 0.10^{b}
anemic rats fed on orange peel powder (2.5%)	3.06 ± 0.05^{d}	41.50 ± 1.80^{cd}	3.23 ± 0.05^{b}
anemic rats fed on orange peel powder (5%)	2.76 ± 0.05^{de}	36.50 ± 3.50^{de}	2.56 ± 0.15^{c}
anemic rats fed on mixture of lemon and orange peel powder (2.5%)	2.53 ± 0.11^{ef}	35.33 ± 1.52^{ef}	2.5 ± 0.10^{c}
anemic rats fed on mixture of lemon and orange peel powder (5%)	$2.23\pm0.05^{f\,J}$	30.33 ± 0.57^{fJ}	2.06 ± 0.057^{d}

values in the same column with letters are significantly different.

Table (5): Effect of lemon, orange peel and their mixture on hemoglobin (HG) and of anemia rats

Treatment/Parameter	Hb (g/dl)
Control group (-)	15.4 ± 0.43^{a}
Control group (+)	6.73 ± 0.24^{f}
anemic rats fed on lemon peel powder (2.5%)	11.20 ± 0.10^{e}
anemic rats fed on lemon peel powder (5%)	12.26 ± 0.15^{d}
anemic rats fed on orange peel powder (2.5%)	$12.\ 6\pm0.15^{d}$
anemic rats fed on orange peel powder (5%)	13.20 ± 0.10^{c}
anemic rats fed on mixture of lemon and orange peel powder (2.5%)	13.5 ± 0.1^{bc}
anemic rats fed on mixture of lemon and orange peel powder (5%)	14.03 ± 0.15^{b}

values in the same column with letters are significantly different.

Table (6): Effect of lemon, orange peel and their mixture on atherogenic index of anemia rats

Treatment/Parameter	Atherogenic index
Control group (-)	0.32 ± 0.01^{c}
Control group (+)	4.30 ± 0.20^{a}
anemic rats fed on lemon peel powder (2.5%)	2.03 ± 0.06^{b}
anemic rats fed on lemon peel powder (5%)	1.55 ± 0.48^{b}
anemic rats fed on orange peel powder (2.5%)	0.84 ± 0.06^{c}
anemic rats fed on orange peel powder (5%)	0.73 ± 0.03^{c}
anemic rats fed on mixture of lemon and orange peel powder (2.5%)	0.63 ± 0.01^{c}
anemic rats fed on mixture of lemon and orange peel powder (5%)	0.51 ± 0.06^{c}

values in the same column with letters are significantly different.

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دراسة تأثير قشور الليمون والبرتقال على فقر الدم في فئران التجارب

عزيزه حافظ عبد الظاهر – منى فاروق شعلان

قسم التغذية وعلوم الأطعمة – كلية الاقتصاد المنزلى . جامعة المنوفية

الملخص العربى

الأنيميا هي حالة مرضية تنخفض فيها قدرة الدم على حمل الأكسجين بسبب انخفاض إنتاج خلايا الدم الحمراء أو الهيموجلوبين والتي تسبب في الإرهاق والوهن هدفت هذه الدراسة إلى استخدام قشر الليمون والبرتقال كعامل مساعد في علاج الأنيميا.

تم استخدام ثمانية وأربعين ذكور الجرذان البيضاء التي تزن 140±10 جرام في هذه الدراسة وقسمت إلى 8 مجموعات، كل مجموعة تحتوي على 6 فئران. عولجت الفئران المصابة بقشر الليمون وقشر البرتقال وخليطهما على لمدة 28 يومًا وأظهرت النتائج أن الفئران المصابة بالأنيميا المعاملة بخليط قشر الليمون والبرتقال حدث لها تحسنًا في رفع الهيموجلوبين وتحسن في وظائف الكبد وكانت أفضل النتائج لخليط قشر الليمون والبرتقال (5٪)

الكلمات الافتتاحية: قشر الليمون – بقشر البرتقال – الفئران – العامل المساعد في علاج الأنيميا التحاليل الكيميائية الحيوية.