

دراسة التأثيرات المحتملة للerman كعلاج تغذويّ لفقر الدم الناجم عن نقص الحديد في الفئران

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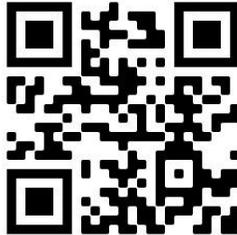
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Study the Potential Effects of Pomegranate as Nutraceutical for Iron Deficiency Anemia in Rats

دراسة التأثيرات المحتملة للبرمان كعلاج تغذوي لفقر الدم الناجم عن نقص الحديد في

الفئران

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ABSTRACT

Iron deficiency (ID) is thought to be the most prevalent underlying cause of anemia, accounting for half of all cases worldwide. Therefore this study aimed to assess the ability of pomegranate to treat iron deficiency anemia (IDA) induced by tannic acid (TA) in rats. 36 female albino rats were divided into two main groups; group I (6 rats) fed on basal diet and served as negative (-ve) control group. Group II, 30 rats fed on basal diet with the addition of 20 g tannic acid/kg diet for three weeks. After that; group II was divided into five equal subgroups (6 rats per each) and all of them fed on a basal diet with or without addition as follow: subgroup 1: fed on a basal diet only and served as positive (+ve) control group, subgroup 2 and 3: Received pomegranate (molasses and juice) at dose 1.17 and 2 ml/kg b.Wt., respectively. Subgroup 4: fed on a basal diet containing dried pomegranate peels (1.18 mg/kg body weight) and subgroup 5: Rats were fed on a basal diet containing dried pomegranate peels (0.6 mg/kg b.Wt.) and received a daily oral dose of pomegranate molasses and juice (0.6 and 1 ml/kg b.Wt.) respectively. At the end of the experiment; biological evaluation and hematologic indices for the diagnosis of IDA were estimated. The results indicated that TA induced a significant decrease in the Hb, Hct % and ferritin, while TIBC was increased. Also, AST, ALT and ALP were affected by TA. But, a supplemented diet with all forms of pomegranate reversed such changes and improved the anemic biomarkers. Moreover the best result was found in the group treated with the pomegranate forms mixture.

Key Words: Iron-binding capacity, tannic acid, ferritin, molasses, complete blood count.

INTRODUCTION

Anemia is a condition in which the level of hemoglobin in the blood falls below the age and sex-appropriate reference range (**Annasahebet *al.*, 2016**). Anemia is the most common form of micronutrient deficiency, affecting more than a quarter of the world's population. Anemia appears to be more prevalent in underdeveloped countries, with women of reproductive age and children being the most vulnerable (**Ghose and Yaya 2018**). Malnutrition and low micronutrient bioavailability are thought to be the most common reasons, which are mainly caused by poor dietary habits and insufficient intake of micronutrient-rich foods such fresh fruits and vegetables (**Ghose and Yaya 2018**). While the etiology of anemia is multifaceted, iron deficiency is thought to be the most frequent underlying cause, with iron deficiency accounting for half of all anemia cases worldwide (**Stoltzfus, 2003 and Beck *et al.*, 2014**). Furthermore, after hospitalization outside of the critical care unit, anemia is a prevalent and persistent finding in COVID-19 (**Hung *et al.*, 2020 and Bergamaschiet *al.*, 2021**). The issue should not be disregarded, and the pathophysiology of anemia should be researched and treated as soon as feasible. Due to high expenses, the risk of adverse effects, and a scarcity of blood, an issue that has gotten even worse during the COVID-19 pandemic. Correct identification and successful treatment of iron deficiency, in combination with developing therapeutic options, can hopefully lessen the clinical burden of anemia in COVID-19 (**Hung *et al.*, 2020 and Bergamaschiet *al.*, 2021**).

Tannins are a collection of chemicals found in plants that belong to the phenolic class of secondary metabolites. These chemicals, particularly tannic acid, have a wide range of physiological and functional roles (**Carbonaroet *al.*, 2001**). Tannins have been linked to a range of negative nutritional consequences. It has been shown that feeding these chemicals to growing animals causes a variety of physiological and biochemical effects. Growth suppression, negative nitrogen balances, lower intestinal absorption of carbohydrates and amino acids, reduced immunological response, and increased liver and protein catabolism are all manifestations of these consequences

(Marzoet *al.*, 2002). In addition, dietary TA or foods heavy in tannin impede or limit iron absorption from meals. (Afsanaet *al.*, 2004).

Iron supplementation and increased intake of iron-rich foods have been used as a technique to combat anemia among young women in countries with high anemia prevalence, such as Bangladesh and India. In resource-poor nations, inadequate dietary consumption and low iron bioavailability (particularly non-heme) are thought to be the main causes of low body iron reserves and anemia (Jamilet *al.*, 2008). Because dietary factors appear to be the primary cause of iron and other nutrient deficiencies, as well as the development of anemia (Beck *et al.*, 2014), food-based interventions, such as diet diversification by including seasonal fruits and vegetables, are frequently proposed as a long-term solution. Reduced ferrous (Fe²⁺) and oxidised ferric (Fe³⁺) forms of iron are both transition metals. Due to possible side effects such as nausea and constipation, ascorbates, citrates, and folic acid have not been very successful in making a major impact (Lopez *et al.*, 2015). Currently, studies are aiming to show that greens, herbs, fruits, and vegetables may be used as food and beverage to help with IDA control (Rao, 2014).

The seeds, skins, and arils of the pomegranate fruit can be split into three anatomical origins, each of which contains valuable compounds. Pomegranate juice is a valuable product that can be obtained from the arils or the complete fruit. Several scientists have reported on the high antioxidant activity of pomegranate and its components utilising a variety of in vitro assay techniques (Lathaet *al.*, 2004). Apart from water (85 %), peels contain sugars, pectin, organic acids, phenolics, and flavonoids, primarily anthocyanins, while arils contain sugars, pectin, organic acids, phenolics, and flavonoids. Proteins, crude fibres, vitamins, minerals, pectin, sugars, polyphenols, and isoflavones are found in seeds, and the oil derived from them (12–20 %) is high in polyunsaturated fatty acids like linolenic and linoleic acids, as well as other lipids like punicic acid, oleic acid, stearic acid, and palmitic acid (Martoset *al.*, 2010). Pomegranate juice is said to be high in iron, phosphorus, copper, sodium,

magnesium, potassium, calcium, zinc, and manganese, among other minerals (Viladomiu et al., 2013).

Pomegranate molasses, which is concentrated pomegranate juice and is extensively consumed in the Middle East, may be higher in antioxidants than the juice (Chalfoun-Mounayaret et al., 2012). Furthermore, pomegranate peels were discovered to have a substantially higher quantity of phenolic and flavonoid components, both of which are potent natural antioxidants. (Konsoula, 2016). Overall, regular consumption of pomegranate fruit, juice, or its compounds added to other food products is good for one's health and may even prevent against or improve the course of diseases such as obesity, diabetes, cardiovascular disease, and even some cancer kinds (Kandyli and Kokkinomagoulos, 2020).

Therefore, the present study aims to assess the potential effects of different forms of pomegranate (juice, molasses and peel) to treat iron deficiency anemia induced by tannic acid in experimental rats. For the diagnosis of IDA biological evaluation and biomarkers and hematologic indices were conducted.

Materials and methods

Plant materials and chemicals

Fresh pomegranate fruit (*Punicagranatum* L.) was purchased from a local market, Tanta city, Egypt. TA, casein (85%), vitamins mixture and salt mixture were obtained from El-Gomhoryia Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt. Bran, corn starch and corn oil were purchased from the local market.

Preparation of pomegranate juice, molasses and peels powder

Pomegranate fruits were washed and peeled manually. The separated peels dried at room temperature around 20–22 °C in a dark place for 3 weeks, then crushed to a fine powder by a metal hunthen electric mixer (blender), powder was sieved by manual sieve and kept in a dark glass container until use. On the other hand the arils were homogenized in the blender without water. The resultant filtered using muslin cloth to obtain clear pomegranate juice and administered to rats immediately as a fresh juice. Pomegranate molasses is a thick syrup made from

pomegranate juice. The juice is boiled for more than six hours in order to obtain a concentrated substance called “molasses.” according to (**Chalfoun-Mounayaret al., 2012**).

Determination of crude fiber, iron and vitamin C

Crude fiber, iron and vitamin C levels were determined according to the Association of Official Analytical Chemists (AOAC, 2000).

Induction of iron deficiency anemia

Rats were fed on a basal diet containing tannic acid for three weeks (20g tannic acid/kg diet) to induce iron deficiency anemia according to **Afsana et al., (2004)**. After 3 weeks, blood samples were obtained from the eyes of a random sample of rats to estimate Hemoglobin (Hb) to ensure the occurrence of IDA.

Animals

Thirty-six female albino rats, *Sprague Dawley* Strain, weighing (150 ± 10 g) were obtained from the laboratory animal house of the Faculty of Science, Tanta University, Tanta, Egypt. Rats were housed individually in wire cages at a room temperature maintained at 25 ± 2 °C and kept under healthy conditions. During the acclimatization period and the whole period of the experiment, rats were fed on a basal diet and water was given *ad libitum*. The basal diet had the following composition: 10% corn oil, 4% salt mixture, 1% vitamin mixture, 0.2% choline chloride, 14% casein (85% protein) and corn starch up to 100g (**Reeves et al., 1993**).

Experimental design

Rats (n = 36) were fed on a basal diet for one week before starting the experiment for adaptation. After this week, they were divided into two main groups: **The first main group:** (6 rats) fed on a basal diet and kept as a negative (-ve) control group. **The second main group:** (30 rats) was fed on basal diet containing 20g of tannic acid/kg diet for three weeks to induce iron deficiency anemia according to (**Afsana et al., 2004**). After this period, the rats were divided into equal five subgroups (6 rats each) as the following:

Subgroup (1): was fed on a basal diet and kept as a positive (+ve) control group.

Subgroup (2): was fed on basal diet and received a daily oral dose of pomegranate juice (PJ) (2 ml/kg body weight) by gastric tube.

Subgroup (3): was fed on a basal diet and received a daily oral dose of pomegranate molasses(PM) (1.17 ml/kg body weight) by gastric tube.

Subgroup (4): was fed on a basal diet supplemented with dried pomegranate peel (PP) (1.18 mg/kg body weight).

Subgroup (5): was fed on a basal diet supplemented with dried pomegranate peels (0.6 mg/kg body weight) and received a daily oral dose of pomegranate juice and molasses (1 and 0.6 ml/kg body weight) respectively by gastric tube(Mixture group).

Biological evaluation

During the experimental period (45 days), the quantities of diet which were consumed and leftover were recorded every day. In addition, the rat's weight was recorded weekly. The body weight gain (BWG%), feed intake(FI), feed efficiency ratio (FER) and also relative organs weight % (liver, spleen and heart) were calculated according to (**Chapman et al., 1959**)

Biochemical analysis

At the end of the experiment, the rats were fasted overnight before being sacrificed under ether anesthesia and the blood samples were collected from each rat in two tubes, one centrifuged to obtain the serum. Serum was carefully separated and transferred into dry clean Eppendorf tubes and kept frozen at -20°C for analysis as described by (**Schermer, 1967**). Complete blood count was performed on blood samples with anticoagulant using a hematological analyzer (Exigo Eos Vet, Sweden). Hemoglobin (Hb) and hematocrit (HCT) levels were measured according to **Drabkin, (1949)** and **McInory, (1954)**, respectively. Red blood cells and white blood cells were determined based on the method was adopted by **Fischbach, (1996)**.

Serum ferritin and total iron-binding capacity (TIBC) were measured according to **Yamanishi et al., (2003)** and **White et al., (1986)**, respectively. Liver enzymes aspartate transaminase (AST)

and alanine transaminase (ALT) were measured in the serum according to the method described by **Reitman and Frankel (1957)** and alkaline phosphatase (ALP) according to **Roy (1970)**.

Histopathological examination

The liver, spleen and heart were removed from each rat by careful dissection, cleaned from the adhesive matter by a saline solution, dried by filter paper and weighed. Only the spleen was kept in formalin solution (10%), for histopathological examination according to the method described by **Drury and Wallington (1980)**.

Statistical analysis

Data were expressed as mean \pm standard deviation. Values were statistically analyzed by one-way analysis of variance (ANOVA test) using the SPSS 10.1 software package. Differences were considered significant at P values ≤ 0.05 . (**Snedecor and Cochran, 1989**).

Results and discussion

Vitamin C, crude fiber and iron for pomegranate forms:

Pomegranate forms ;juice, molasses and peels powder were analyzed for their contents of crude fibers, iron and ascorbic acid (vitamin C). As shown in Table (1), pomegranate peel powder contained 9.9 % of crude fibers of dry matter. On the other hand ;it could be noticed that the iron content in molasses and peels powder recorded the highest content than that of juice (17.60 & 17.65 mg/kg respectively) vs. (10.34 mg/L). While vitamin C content was higher in juice and molasses than peels powder (72.62, 36.60 ppm. respectively) vs. (4.24 ppm). This results are in line with previous findings by **Rowayshedet al., (2013)** who stated that the pomegranate peels powder is considered a good source of crude fibers, ash and carbohydrates as (3.30, 11.22 and 80.50 %).

Table (1): Vitamin C, crude fiber and iron content in pomegranate forms

Vitamin C, crude fiber and iron content			
Sample names	Vit C (Ppm)	Crude fibers(%)	Iron (Fe)
Juice	72.62	--	10.34 mg/L
Molasses	36.60	--	17.60 mg/kg
Powder	4.24	9.9	17.65 mg/kg

Biological effects

Effect of different pomegranate forms on feed intake (FI), percentage of body weight gain (BWG %) and feed efficiency ratio (FER) for anemic rats:

As shown in table (2). When compared to normal rats (9.87 and 19.6 g/day, respectively), FI results revealed a significant decrease in the (+ve) control group. The FI increased significantly in all experimental groups treated with pomegranate juice, molasses, powder, or mixture, ranging from 14.74 g/day to 18.62 g/day. The group treated with the mixture had the greatest mean value of FI (18.62 g/day), followed by the group treated with molasses (17.60 g/day).

Furthermore, when compared to normal rats (-ve), the BWG % reported a significant drop in the positive (+ve) control group (2.24 and 25.37 %, respectively). BWG % reductions were considerable in all treatment groups, ranging from (17.84 to 7.81 %). The group that was given the mixture had the best performance in terms of BWG % (17.84 %). From these results; obvious that anemic rats gradually dropped in body weight gain.

When compared to normal rats (-ve), the FER data showed a considerable decrease in the (+ve) control group (0.07 % and 0.6 %, respectively). The FER of all treated groups improved significantly, ranging from (0.25 % to 0.32 %). The best result was obtained by the group that was given the mixture (0.32 %).

These findings support **Chin et al., (1994)**'s findings that pomegranate is a growth factor for rats, as evidenced by increased

weight gain and improved feed efficiency. **El-deab, (2019)** also discovered that groups supplemented with (2 and 4ml) PJ had higher FER than the control group. The FER was also raised by feeding 0.5 ml PM and 10% PP.

Table (2): Effect of different pomegranate forms on (FI), (BWG %) and (FER) for anemic rats

	FI g /day	BWG%	FER
	Mean \pm SD	Mean \pm SD	Mean \pm SD
(-ve) control	19.60 ^a \pm 2.00	25.37 ^a \pm 0.14	0.60 ^a \pm 0.03
(+ve) control	9.87 ^e \pm 1.63	2.24 ^f \pm 0.21	0.07 ^e \pm 0.01
Juice	16.65 ^c \pm 0.90	12.86 ^d \pm 0.94	0.25 ^c \pm 0.05
Molasses	17.60 ^{bc} \pm 0.75	14.74 ^c \pm 1.63	0.27 ^{bc} \pm 0.09
Peels Powder	14.74 ^d \pm 1.00	7.81 ^e \pm 0.90	0.16 ^d \pm 0.03
Mixture	18.62 ^{ab} \pm 0.85	17.84 ^b \pm 2.25	0.32 ^b \pm 0.04

- Each value represents the mean \pm SD. Means with the different superscript letters in the same column were significantly different ($P \leq 0.05$).

Effect of different pomegranate forms on relative organs weight % (liver, heart and spleen) for anemic rats

The findings of relative liver weight (%) in table (3) showed that the (-ve) control group's mean value was lower than the (+ve) control group's, with a significant difference between them. The relative liver weight (%) of all treated groups decreased significantly, ranging from 3.47 to 4.84 of the (+ve) control group. The group treated with the mixture had the best results, with only a minor difference from the (-ve) group.

In terms of relative heart weight (%), the (-ve) control group was less than the (+ve) control group, with a significant difference. In addition, the treated groups experienced a significant reduction in relative heart weight (%) that varied from 0.5 to 0.41 of the (+ve) control group. The groups that were given the molasses and mixture had the best results.

The mean value of the (-ve) control group was less than that of the (+ve) control group, with a significant difference between them, according to the results of relative spleen weight (%). The

relative spleen weight of all treatment groups decreased significantly, ranging from 0.41 to 0.36 percent of the (+ve) control group. The best result was recorded for the group treated with the mixture which was 0.36 %.

The present results partially agreed with **Nakamura et al., (2001)** who found that the liver weight of iron deficient anemic rats decreased whereas the heart weight increased. Similarly, in rats given 0.5g/kg of tannic acid, the relative weight of the liver was considerably lower than in control rats. According to **Peretz et al., (2006)**, iron deficient anemic rats showed a considerable reduction in liver weights.. But, these results are supported by **Dong et al., (2005)** who found that iron deficient rats' absolute heart weight and heart size were increased. Furthermore, **Wei et al., (2015)** found that the liver and spleen index in the pomegranate peels and seeds extract group was considerably lower than the CCl4 group.

Table (3): Effect of different pomegranate forms on relative organs weight % (liver, heart and spleen) for anemic rats:

	Relative organs weight%		
	Liver	Heart	Spleen
	Mean ± SD	Mean ± SD	Mean ± SD
(-ve) control	2.58 ^d ± 0.26	0.29 ^d ± 0.02	0.26 ^c ± 0.01
(+ve) control	4.84 ^a ± 0.35	0.59 ^a ± 0.05	0.54 ^a ± 0.07
Juice	4.16 ^b ± 0.20	0.46 ^b ± 0.02	0.40 ^b ± 0.05
Molasses	4.06 ^b ± 0.22	0.40 ^c ± 0.03	0.40 ^b ± 0.04
Peels Powder	4.25 ^b ± 0.43	0.50 ^b ± 0.04	0.41 ^b ± 0.05
Mixture	3.47 ^c ± 0.28	0.41 ^c ± 0.05	0.36 ^b ± 0.03

- Each value represents the mean ± SD. Means with the different superscript letters in the same column were significantly different (P≤0.05).

Hematological parameters

Effect of different pomegranate forms on hemoglobin (Hb), hematocrit (HCT%), serum ferritin and total iron-binding capacity (TIBC) for anemic rats

Table (4) revealed that the (+ve) control group's mean Hb value was lower than the (-ve) control group's, with a significant difference between them. When compared to the (+ve) control

group, the treated groups demonstrated a significant increase in Hb ranging from 11.94 to 13.90. The group that received the mixture had the best results, followed by the group that received pomegranate molasses.

In terms of (HCT%), (+ve) control group was lower than (-ve) control group, with a significant difference between them. HCT increased significantly in the experimental groups treated with pomegranate pieces, ranging from 40.14 to 48.66 in the (+ve) control group. The group treated with the mixture had the best results, with no significant difference between them and the (-ve) normal control group.

Also, table (4) cleared that serum ferritin of (+ve) control group recorded less than (-ve) control group, with significant difference between them. All experimental groups treated with pomegranate as powder, juice, molasses and mixture had significant increase in serum ferritin ranged from 3.35 to 3.67 of (+ve) control group. The best result was recorded for the group treated with the mixture followed by the group treated with pomegranate molasses and recorded the nearest values of serum ferritin for normal control group.

In contrast to (TIBC), (+ve) control group was found to be more than (-ve) control group, with a significant difference between them. The TIBC of the experimental groups treated with pomegranate forms decreased significantly, ranging from 206.52 to 245.62 of the (+ve) control group. The groups treated with the mixture, as given in table (4), had the best results, with 206.52 g/dl.

These findings are backed up by **Afsana (2004)**, who discovered that in week 3, Hb concentration was lower in the 10, 15, and 20 g TA/kg diet-fed groups than in the control group. These decreases were dose-dependent on TA. The Hct percent and Hb concentrations in week 3 were coordinated. In addition, **Ibrahim and Yehia (2011)** found that adding tannic acid to the basal diet reduced Hb and Hct percent concentrations considerably.

The lower Fe absorption caused by TA-added to the test meals for rats caused anemia, according to these findings. Where, according to **Afsana (2004)**, TA treatment reduced Fe absorption

in a dose-dependent manner, as evidenced by blood parameters, which showed that the rate of Fe absorption was significantly lower in the 10, 15, and 20 g TA/kg diet groups compared to the control group. The presence of numerous galloyl residues with a trihydroxybenzene structure, which bind Fe with high affinity, may have inhibited Fe absorption (**Bruneet *al.*, 1989; South and Miller, 1998**).In return, Experiments in human have shown that polyphenols strongly inhibit Fe absorption (**Cook *et al.*, 1995**).

Polyphenols, on the other hand, have been demonstrated in human studies to strongly impede Fe absorption (Cook *et al.*, 1995).

These findings were corroborated by **AbdElmonem (2014)**, who found that combining PM with Diazinon boosted Hb concentration and Hct percent. The enhanced change in hematological parameters in the group given PM as a protective agent could be owing to the fact that pomegranate is a good source of iron (which is needed to make hemoglobin) and phenolic compounds, which are antioxidants and free radical scavengers (**Rosenblatet *al.*, 2006**). Also, **Manthouet *al.*, (2017)** showed that drinking 0.5 liters of pomegranate juice each day for 14 days enhanced the number of red blood cells and hemoglobin content in healthy participants.

Table (4): Effect of different pomegranate forms on hemoglobin (Hb), hematocrit (HCT%), serum ferritin and total iron-binding capacity (TIBC) for anemic rats:

	Hb g/dL	HCT %	Ferritin (ng /ml)	TIBC (µg/dl)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
(-ve) control	15.34 ^a ±0.58	51.60 ^a ±2.11	3.90 ^a ±0.10	189.40 ^e ±12.98
(+ve) control	10.66 ^e ±0.34	33.66 ^e ±3.41	2.95 ^e ±0.20	268.88 ^a ±10.80
Juice	12.86 ^c ±0.21	45.38 ^c ±1.31	3.51 ^c ±0.088	223.52 ^c ±7.89
Molasses	13.32 ^c ±0.15	47.10 ^{bc} ±0.69	3.63 ^{bc} ±0.08	216.12 ^{cd} ±8.85
Peels Powder	11.94 ^d ±0.65	40.14 ^d ±3.83	3.35 ^d ±0.08	245.62 ^b ±12.10
Mixture	13.90 ^b ±0.23	48.66 ^{ab} ±1.15	3.67 ^b ±0.11	206.52 ^d ±12.44

- Each value represents the mean ± SD. Means with the different superscript letters in the same column were significantly different ($P \leq 0.05$).

Effect of different pomegranate forms on red blood cells (RBCs) count and white blood cells (WBCs) count for anemic rats

Table (5) demonstrated that the (+ve) control group's mean RBCs count was lower than the (-ve) control group's, with a significant difference between them. When compared to the (+ve) control group, the treated groups demonstrated a significant increase in RBCs ranging from 7.18 to 8.31. The group treated with the mixture had the best results, with only a minor difference from the regular control group.

Table (5) also revealed that the (+ve) control group's WBCs count was lower than the (-ve) control group's, with no difference between them. The WBCs count of the normal control group did not differ in any of the treated groups.

(Hemoglobin levels, hematocrit percent levels, and RBCs count) all increased significantly when pomegranate juice, peel, molasses, and their mixtures were consumed ($P < 0.05$). WBCs, on the other hand, did not vary appreciably. These findings are in line with those of **Abdelmonem (2014)**, who found that combining PM with Diazinon increased the quantity of RBCs and WBCs. Due to the verified functional features of PJ. **Manthouet et al., (2017)** discovered that two weeks of PJ supplementation resulted in an enhanced RBC count and hemoglobin concentration in healthy persons.

Table (5): Effect of different pomegranate forms on red blood cells (RBCs) count and white blood cells (WBCs) count for anemic rats:

	RBCs (10^3 / μ L)	WBCs (10^3 / μ L)
	Mean \pm SD	Mean \pm SD
(-ve) control	8.82 ^a \pm 0.22	6.28 ^a \pm 0.97
(+ve) control	5.93 ^d \pm 0.65	6.10 ^a \pm 0.66
Juice	7.28 ^c \pm 0.65	6.18 ^a \pm 0.80
Molasses	7.99 ^b \pm 0.30	6.52 ^a \pm 0.54
Peels Powder	7.18 ^c \pm 0.39	7.02 ^a \pm 0.46
Mixture	8.3 ^{ab} \pm 0.27	6.02 ^a \pm 0.72

- Each value represents the mean \pm SD. Means with the different superscript letters in the same column were significantly different ($P \leq 0.05$).

Effect of different pomegranate forms on serum AST, ALT and ALP for anemic rats

Table (6) showed that the mean value of the AST (U/L) of (+ve) was higher than the (-ve) control group, with significant difference between them. The treated groups demonstrated a significant drop in AST activity ranging from 160.02 to 99.7. The group treated with the mixture had the best results, followed by the molasses group.

Table (6) showed that the mean value of the ALT (U/L) of (+ve) control group showed more than that of the (-ve) control group, with significant difference between them. The ALT activity of the experimental groups treated with pomegranate forms

decreased significantly, ranging from 44.2 to 26.12 of the (+ve) control group. As indicated in table, the group treated with the mixture had the best results, followed by the group treated with molasses .

Table (6) also revealed that the (+ve) control group's ALP (U/L) was higher than the (-ve) control group's, with a significant difference between them. ALP levels in all treated groups decreased significantly, ranging from 295.82 to 169.40 times those in the (+ve) control group. The best result was recorded for the group treated with the mixture and recorded the nearest value of ALP for normal control group.

These findings are reinforced by **Kaur et al., (2006)**, who found that pomegranate treatment protects against liver injury by inhibiting blood levels of AST and ALT enzymes, which could be owing to pomegranate's high antioxidant and hepatoprotective effects. **Osman et al., (2012)** investigated the antioxidant impact of pomegranate peels and juice on diabetes mellitus in female rats produced by alloxan. The results revealed that the diabetes group's AST and ALT levels were much higher, but that following treatment with PP and PJ, the diabetic group's AST and ALT levels fell and became closer to the control level, especially the ALT value. This effect is due to the antioxidant content of PP and PJ. Additionally, **Al-Moraie et al., (2013)** confirmed that; giving hypercholesterolemic rats 1, 3, or 5 ml/kg b. wt. of pomegranate juice lowered blood AST and ALT considerably ($P < 0.05$) compared to the hypercholesterolemic rats.

Similar results were reported by **Abou Zaid et al., (2016)** who reported that; groups administered with PM findings revealed a substantial decrease in AST and ALT activity. Also, **EL-Beltagi et al., (2020)** discovered that oral treatment of PJ, SP, and their combination (PJSP) resulted in a significant reduction in plasma levels of AST and ALT when compared to a group of rats treated for liver injury. Furthermore, **Wei et al. (2020)** discovered that extracts of pomegranate peels (EPP) protected rats from CCl₄-induced liver function damage. When compared to the CCl₄ group, EPP treatment for 4 weeks dramatically lowered serum levels of ALT and AST. Pomegranate peel extract was discovered to have a moderate therapeutic effect against hepatic abnormalities

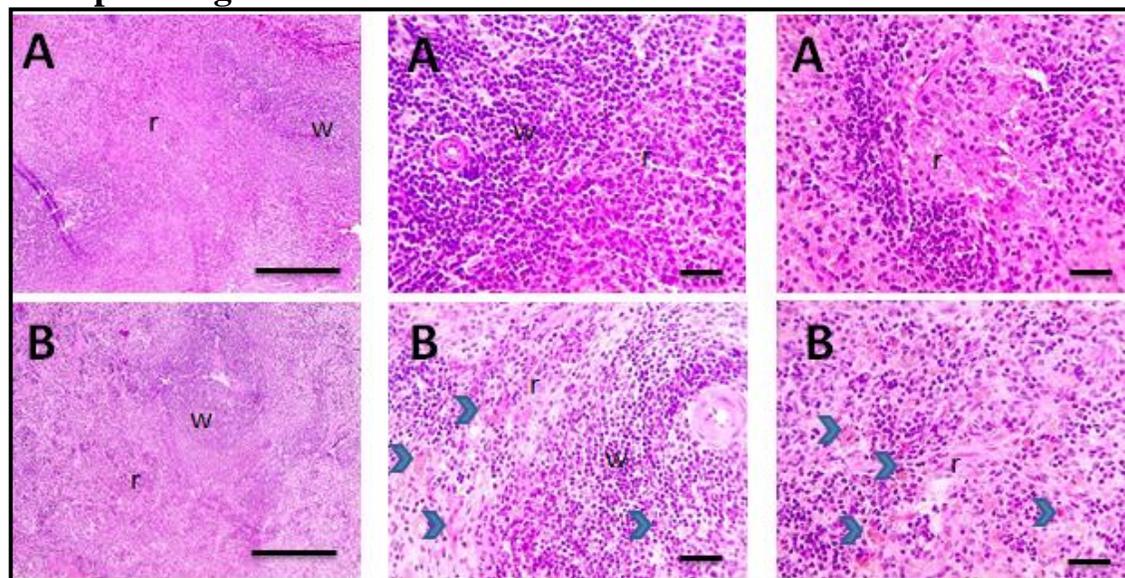
in male rats, according to **Faddladdeen, (2021)**. It may be recommended for diabetic patients who are experiencing early changes in their liver functions.

Table (6): Effect of different pomegranate forms on serum AST, ALT and ALP for anemic rats:

	AST U/L	ALT U/L	ALP U/L
	Mean \pm SD	Mean \pm SD	Mean \pm SD
(-ve) control	89.76 ^f \pm 4.63	21.58 ^e \pm 2.48	142.34 ^f \pm 13.72
(+ve) control	198.00 ^a \pm 7.54	52.14 ^a \pm 3.10	349.96 ^a \pm 15.64
Juice	123.47 ^c \pm 3.24	38.00 ^c \pm 2.05	248.46 ^c \pm 14.45
Molasses	114.74 ^d \pm 7.45	35.54 ^c \pm 3.31	196.16 ^d \pm 9.35
Peels Powder	160.02 ^b \pm 9.29	44.20 ^b \pm 1.70	295.82 ^b \pm 5.91
Mixture	99.70 ^e \pm 4.07	26.12 ^d \pm 2.10	169.40 ^e \pm 12.85

- Each value represents the mean \pm SD. Means with the different superscript letters in the same column were significantly different ($P \leq 0.05$).

Histopathological Examination:



Figure(1): Microscopic pictures of H&E stained splenic sections from the negative control group (A) showing well-defined white pulps (w) and normal red pulp (r) containing megakaryocytes and

hematopoietic centers. Normal lymphocytes density appears in lymphoid follicles and red pulp in the normal group (A). Splenic sections from the diseased group or (+ve) (B) showed marked depletion of lymphocytes from white and red pulps with the presence of many hemosiderin-laden macrophages in white and red pulps (arrowheads). Low magnification X:100 bar 100 and high magnification X:400 bar 50.

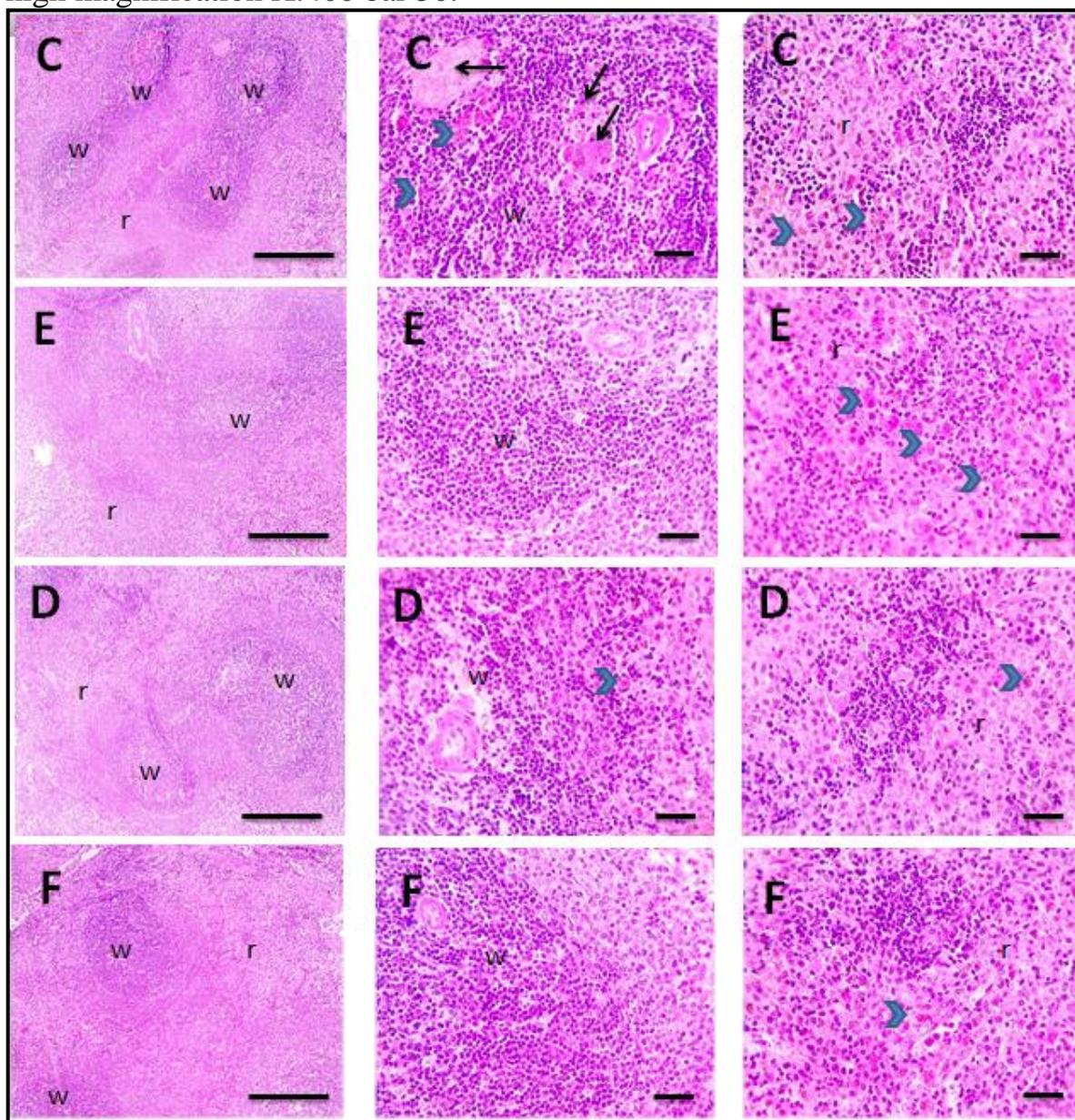


Figure (2): Microscopic pictures of H&E stained splenic sections from juice group (C) showing depletion of lymphocytes from white and red pulps with excess hemosiderosis characterized by the presence of free hemosiderin (arrows) and many hemosiderin laden macrophages (arrowheads) in white and red pulps. Splenic sections from powder group (E) show slight depletion of lymphocytes from white pulp with many hemosiderin laden macrophages (arrowheads) in red pulp. Splenic sections from molasses group (D) show slight depletion of lymphocytes from white pulp with few hemosiderin laden macrophages (arrowheads) in red pulp. Lymphocytes population re-increased in red pulp from molasses group (D). Splenic sections from mixture group (F) showing well-defined lymphoid follicles with normal lymphocytes density, few hemosiderin laden macrophages (arrowheads) in the red pulp, higher lymphocytes population in red pulp than in groups B, C, E. Low magnification X:100 bar 100 and high magnification X:400 bar 50.

In conclusion , foods high in tannin limit the absorption of iron from meals. Long-term use can lead to iron deficiency anemia.

Because of its prospective health-promoting and disease-preventing capabilities, pomegranate is one of the most researched and valued fruits. In comparison to fruits of the same and other classes, clinical research in human subjects and animal models have shown that pomegranate consumption improves the body's inherited defense against numerous infections, inflammatory, and non-inflammatory illnesses.

This study is conducted to evaluate the effect of Pomegranate (juice, molasses and peel) in the treatment of IDA. The results found that Pomegranate products appear to be effective treatment for IDA, where produces increase in hemoglobin concentration and serum ferritin levels.

References

- A.O.A.C. (2000) . Official Methods of Analysis, 17 Ed., *Association of Official Analytic Chemists International*. Arlington, Virginia, USA.
- AbdElmonem, H. A. (2014). Assessment the effect of pomegranate molasses against diazinon toxicity in male rats

- .IOSR Journal Of Environmental Science, Toxicology And Food Technology. Volume 8, Issue 2.*
- AbouZaid, O. A. R. , El Sonbaty, S. M. , Hamam, N. M. A. (2016). Biochemical role of pomegranate molasses in modulation and treatment of hyperlipidemia associated with hyperglycemia. *Radiation Microbiology Dept, National Center for Radiation Research and Technology (NCRRT).*
- Afsana, K.,Kazuki, Satoshi I. , Hiroshi, H.(2004). Effect of ingesting tannic acid on the absorption of iron, but not of zinc, copper and manganese by rats.*Biosci.Biotechnol.Biochem., 3:584- 592. doi.org/10.1271/bbb.68.584.*
- Al-Moraie, M. M. D., Arafat R. A., Al-Rasheedi A. A (2013).Effect of Pomegranate Juice on Lipid Profile and Antioxidant Enzymes in Hypercholesterolemic Rats.*Life Science Journal 10(3).2717-2728 (ISSN: 1097- 8135).*
- Annasaheb G S., Bhagwat S., Pande P. P., Deshpande P.R.(2016). Effect Of DadimSwarasa(Pomegranate Juice)on Pandu in Females w.s.r. to. Iron Deficiency Anaemia. *IJAAR 2 (9) 1226-1230.*
- Beck, K.L., Conlon, C.A., Kruger, R., Coad, J. (2014). Dietary determinants of and possible solutions to iron deficiency for young women living in industrialized countries: a review. *Nutrients; 6(9):3747–3776. doi.org/10.3390/nu6093747.*
- Bergamaschi, G. , de Andreis, F. B. , Aronico, N. , Lenti M. V., Barteselli, C., Merli, S., Pellegrino, I., (2021). Anemia in patients with Covid-19: pathogenesis and clinical significance. *ClinExp Med. 2021 Jan 8 : 1–8. doi: 10.1007/s10238-020-00679-4.*
- Brune, M.,Rossander, L., Hallberg, L. (1989). Iron absorption and phenolic compounds: importance of different phenolic structures. *Eur. J. Clin. Nutr., 43, 547–558 .*
- Carbonaro M., Grant G., Pustai A. (2001).Evaluation of polyphenol bioavailability in rat small intestine.*Eur. J. Nutr., 40 (2): 84-90. doi.org/10.1007/s003940170020*
- Chalfoun-Mounayar, A., Nemr. R., Yared P., Khairallah S.,Chahine R. (2012).Antioxidant and Weight Loss Effects of

- Pomegranate Molasses. *Journal of Applied Pharmaceutical Science* 02 (06); 2012: 45-50.
- Chapman, D. G., Castilla, R., Campbell, J. A. (1959). Evaluation of Protein in Food. I. A method for the determination of protein efficiency ration. *Can. J. Biochem. Physiol.*, 37: 679-686.
- Chin, S. F., Storkson, J. M., Albright, K. J., Cook, M. E., Pariza, M. W. (1994). Conjugated linoleic acid is a growth factor for rats as shown by enhanced weight gain and improved feed efficiency. *Journal of Nutrition*. 124: 694-701. doi: 10.1093/jn/124.5.694.
- Cook, J. D., Reddy, M. B., Hurrell, R. F. (1995). The effect of red and white wines on nonheme iron absorption in humans. *Am. J. Clin. Nutr.*, 61, 800-804. doi.org/10.1093/ajcn/61.4.800
- Dong, F., Zhang, X., Culver, B., Chew, H. G., Kelley, R. O., Ren, J. (2005). Dietary iron deficiency induces ventricular dilation, mitochondrial ultrastructural aberrations and cytochrome c release: involvement of nitric oxide synthase and protein tyrosine nitration. *Clin. Sci.*, 109(3): 277-286. doi: 10.1042/CS20040278.
- Drabkin, D. (1949). The standardization of hemoglobin measurements. *Am. J. Med. Sci.*, 21(7):710.
- Drury, R. A. B., Wallington, E. A. (1980). Carleton's histological technique, 5th edn. *Oxford University Press, Oxford New York Toronto*, pp 188-189, 237-240, 290-291.
- El-deab, S. M. (2019). Protective Effects of Pomegranate Juice, Peel and Their Molasses on Aging. *Journal for Education and Science Publications (MECSJ)*, ISSN:26169185. www.mecs.com.
- Faddladdeen, K. A. J. (2021). Ameliorating effect of pomegranate peel extract supplement against type 1 diabetes-induced hepatic changes in the rat: biochemical, morphological and ultrastructural microscopic studies. *Folia Morphol.* 80(1):149-157. doi:10.5603/FM.a2020.0034•Pubmed:32207851.
- Fischbach, F. T. (1996). A manual of Laboratory and Diagnostic Tests 5th Ed. P.P. 31-120. *Lippincott, Philadelphia New York*.

- Ghose, B. , Yaya, S. (2018). Fruit and vegetable consumption and anemia among adult non-pregnant women: Ghana Demographic and Health Survey. *PeerJ*. 2018; 6: e4414. doi10.7717/peerj.4414
- Gil, M., Tomas, F., Hess-Pierc, B. (2000).Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing, *J. Agric. Food Chem.*48 4581–4589.doi.org/10.1021/jf000404a
- Hung, I. F., Cheng V. C., Li, X., et al. (2020).SARS-CoV-2 shedding and seroconversion among passengers quarantined after disembarking a cruise ship: a case series [published online ahead of print, 2020 Jun 12] *Lancet Infect Dis*. 2020 ;S1473–3099(20):30364–9.doi: 10.1016/S1473-3099(20)30364-9.
- Ibrahim , A. El-R., Yehia, N. M. (2011). Biochemical and histological studies on the Ameliorative role of fenugreek Products in tannic acid-induced iron deficiency anemia in Albino rats . The Egyptian Society of Experimental Biology .*Egypt. J. Exp. Biol. (Zool.)*, 7(2): 185 – 196.
- Jamil, K. M., Rahman, A. S.,Bardhan, P. K., Khan, A. I., Chowdhury, F.,Sarker, S. A., Khan, A. M., Ahmed, T. (2008). Micronutrients and anaemia. *Journal of Health, Population and Nutrition*. 2008; 26(3) :340–355.doi: 10.3329/jhpn.v26i3.1900
- Kandyliis, P. ,Kokkinomagoulo, E. (2020).Food applications and potential health benefits of pomegranate and its derivatives.*Multidisciplinary Digital Publishing Institute.doi.org/10.3390/foods9020122*.
- Kaur, G., Jabbar, Z.,Athar, M., Alam, M. S. (2006).Punicagranatum (pomegranate) flower possesses potent antioxidant activity and abrogates Fe-NTA induced hepatotoxicity in mice. *Food and Chemical Toxicology*. 44(7): 984-993.doi.org/10.1016/j.fct.2005.12.001
- Konsoula, Z. (2016). Comparative efficacy of pomegranate juice, peel and seed extract in the stabilization of corn oil under accelerated conditions. *World Academy of Science, Engineering and Technology, International Journal of*

- Nutrition and Food Engineering*, 3(9), 556–563.
doi.org/10.5281/zenodo.1126219.
- Latha, M., Pari, L., Sitasawd, S., Bhonde, Scopariadulcis, R. (2004). A traditional antidiabetic plant, protects against streptozotocin induced oxidative stress and apoptosis in vitro and in vivo, *Biochem. Mol. Toxicol* 18(5)261–272. doi.org/10.1002/jbt.20035.
- Lopez, A., Cacoub, P., Macdougall, I.C., Biroulet, L.P. (2015). Iron deficiency anaemia. *The Lancet*, Volume 387, Issue 10021. Pages 907-916 [doi.org/10.1016/S0140-6736\(15\)60865-0](https://doi.org/10.1016/S0140-6736(15)60865-0).
- Manthou, E., Georgakouli, K., Deli, C. K., Sotiropoulos A., Fatouros, L. G., Kouretas, D., Haroutounian, S., Matthaiou, C. H., Koutedakis, Y., Jamurtas, A.Z. (2017). "Effect of pomegranate juice consumption on biochemical parameters and complete blood count," *Experimental and Therapeutic Medicine*, vol. 14, no. 2, pp. 1756–1762. doi.org/10.3892/etm.2017.4690
- Marzo, F., Urdaneta, E., Santidrian, S. (2002). Liver proteolytic activity in tannic acid-fed birds. *Poultry Sci.*, 81: 92–94. doi.org/10.1093/ps/81.1.92
- McInory, L. (1954). A micro hematocrit for determining the packed cell and hemoglobin concentration on capillary blood. *J. Clin. Path.*, (7):32.
- Nair, M. K., Augustine, L. F., Konapur, A. (2015). Food-based interventions to modify diet quality and diversity to address multiple micronutrient deficiency. *Frontiers in Public Health* volume 3, article 277. doi.org/10.3389/fpubh.2015.00277
- Nakamura, Y., Kaihara, A., Yoshii, K., Tsumura, Y., Ishimitsu, S., Tonogai, Y. (2001). Effects of the oral administration of green tea polyphenol and tannic acid on serum and hepatic lipid contents and fecal steroid excretion in rats. *J. Health Sci.*, 47(2): 107-117. doi.org/10.1248/jhs.47.107.
- Osman, H. F., Eshak, M. G., El-Sherbiny, E. M., Bayoumi, M. M. (2012). Biochemical and Genetical Evaluation of Pomegranate Impact on Diabetes Mellitus Induced by Alloxan in Female Rats, *Life Science Journal*, vol. 9(3): 1543-53.

- Peretz, G., Link, G., Pappo, O., Bruck, R., Ackerman, Z. (2006). Effect of hepatic iron concentration reduction on hepatic fibrosis and damage in rats with cholestatic liver disease. *World J. Gastroenterol.*, 12(2): 240-245. doi: 10.3748/wjg.v12.i2.240
- Rao, S. (2014). Potential of community based approach for prevention of anaemia among women of child bearing age from rural India. *Journal of Food and Nutrition Sciences*; 2: 270-6. doi: 10.11648/j.jfns.20140206.15
- Reeves, Ph. G., Nielsen, F. H., Fahey, G. C., (1993). AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet. *The Journal of Nutrition, Volume 123, Issue 11, Pages 1939–1951*, doi.org/10.1093/jn/123.11.1939.
- Reitman, S., Frankel, S. (1957). "A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases". *Am. J. Clin. Path.*, 28 : 56 .
- Rosenblat , M., Hayek, T., Aviram, M. (2006). Anti-oxidative effects of pomegranate juice (PJ) consumption by diabetic patients on serum and on macrophages, Atherosclerosis; 187(2), 2006, 363-71. doi.org/10.1016/j.atherosclerosis.2005.09.006.
- Rowayshed , G., Asraf, M. M. S., Mahmoud, M. M. (2013). Stability of carbamate pesticides residue in some vegetables throughout the household processing and cooking. *Middle East J. App. Sci. Curreweb.com*.
- Roy, A. V. (1970). "Colorimetric determination of serum alkaline phosphatase". *Clin. Chem.*, 16:431-432. doi.org/10.1093/clinchem/16.5.431
- Schermer, S. (1967). "The Blood Morphology of Laboratory Animal". Longmans, Printed in Great Britain, *Green and Co. Ltd, P. 350*.
- Snedecor, G. W. and Cochran, W. G. (1967): *Statistical Methods*. 6th Ed., Iowa State University Press. Ames. Iowa. USA .

- South, P. K., Miller, D. D. (1998). Iron binding by tannic acid: Effects of selected ligands. *Food Chem.*, 63(2): 167-172.[doi.org/10.1016/S0308-8146\(98\)00040-5](https://doi.org/10.1016/S0308-8146(98)00040-5).
- Stoltzfus, R. J. (2003). Iron deficiency: global prevalence and consequences. *Food and Nutrition Bulletin*.2003;24(4 Suppl):S99–S103.doi.org/10.1177/15648265030244S106.
- Viladomiu, M., Hontecillas, R., Lu, P., Bassaganya-Riera, J. (2013). Preventive and Prophylactic Mechanisms of Action of Pomegranate Bioactive Constituents. *Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine* ,Article ID 789764, 18 pages doi.org/10.1155/2013/789764.
- Martos, M.V., López, J.F., Álvarez, J.A. P. (2010) .Pomegranate and its many functional components as related to human health: A review. *Compr. Rev. Food Sci. Food Saf.*, 9, 635–654.
- Wei, X., Li, S., Li, T., Liu, L., Liu, Y., Wang, H., Zhou, Y., Liang, F., Yu, X., Zang, W., Zhao, M., Zhao, Z. (2020). Pomegranate peel extract ameliorates liver fibrosis induced by carbon tetrachloride in rats through suppressing p38MAPK/Nrf2 pathway. *Journal of Functional Foods*.65(2020).103712. doi.org/10.1016/j.jff.2019.103712.
- Wei, Xl., Fang, Rt., Yang, Yh., Bi, Xy., Ren, Gx., Luo, Al., Zhao, M., Zang, Wj. (2015). Protective effects of extracts from Pomegranate peels and seeds on liver fibrosis induced by carbon tetrachloride in rats. *BMC Complementary and Alternative Medicine*.[doi10.1186/s12906-015-0916-9](https://doi.org/10.1186/s12906-015-0916-9).
- White, D., Kramer, D., Johnson, G., Dick, F., Hamiton, H. (1986). Enzyme Immunoassay for the Quantitative Determination of Human Ferritin Concentration in Human Serum. *A. M. J. Clin. Path.*, 72: 346.CF.
- Yamanishi, H., Lyama, S., Yamaguchi, Y. et al., (2003). Total iron-binding capacity calculated from serum transferrin concentration or serum iron. *Clin. Chem.*, 49: 175-178.doi.org/10.1373/49.1.175

دراسة التأثيرات المحتملة للرمان كعلاج تغذوي لفقر الدم الناجم عن نقص الحديد في الفئران

المستخلص

يُعتقد أن نقص الحديد هو السبب الأساسي الأكثر شيوعًا للإصابة بالأنيميا حيث يُفترض أن نصف حالات فقر الدم العالمية تعزى إلى نقص الحديد (ID). تهدف هذه الدراسة لقياس قدرة الرمان (العصير، القشور، دبس الرمان وخليطهما) على علاج أو تحسين فقر الدم الناجم عن حمض التانيك (TA). تم تقسيم ستة و ثلاثين من إناث الجرذان إلى مجموعتين رئيسيتين؛ المجموعة الأولى تركت كمجموعة ضابطة سالبة (6 فئران) وتغذت على النظام الغذائي الأساسي والمجموعة الثانية (30 فأر) تغذت على النظام الغذائي مع إضافة 20 جم من حمض التانيك / كجم من وزن الوجبة لمدة ثلاثة أسابيع. تم تقسيم المجموعة الثانية إلى خمس مجموعات فرعية متساوية، كل منها يحتوي على 6 فئران. المجموعة الفرعية 1: تركت كمجموعة ضابطة موجبة (تتغذى على النظام الغذائي الأساسي)، المجموعتان الفرعيتان 2 و 3: تم تغذيتها على الرمان (دبس وعصير) بجرعة 1.17 و 2 مل / كجم وزن على التوالي، المجموعة الفرعية 4: تم تغذية الفئران على نظام غذائي أساسي يحتوي على قشر الرمان المجفف (1.18 ملجم / كجم من وزن الجسم) والمجموعة الفرعية 5: تم تغذية الفئران على نظام غذائي أساسي يحتوي على قشر الرمان المجفف (0.6 ملجم / كجم من وزن الجسم) وأعطيت جرعات فموية يومية من عصير الرمان ودبس الرمان (1 و 0,6 مل / كجم من وزن الجسم) على التوالي. في نهاية التجربة ؛ تم تقدير التقييم البيولوجي والمؤشرات الدموية لتشخيص الأنيميا . أشارت النتائج إلى أن حمض التانيك تسبب في انخفاض معنوي في الهيموجلوبين و نسبة الهيموتيكريت و الفريتين ، بينما زادت القدرة الكلية للحديد على الارتباط. أيضًا ، أثر حمض التانيك على إنزيمات الكبد. لكن النظام الغذائي المكمل بجميع أشكال الرمان عكس هذه التغييرات وحسن المؤشرات الحيوية لفقر الدم. علاوة على ذلك ، تم العثور على أفضل نتيجة في المجموعة المعالجة بخليط أشكال الرمان

الكلمات المفتاحية: القدرة الكلية للحديد على الارتباط - حمض التانيك - الفريتين - دبس الرمان - صورة الدم.