

# **Study the effect of using freeze-dried lemon peels and pulp powder treating anemia in female rats.**

**دراسة تأثير استخدام مساحيق قشر ولب الليمون المعامل بالتجفيد في علاج فقر الدم لدى إناث الفئران..**

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## **ABSTRACT**

Anemia is a condition in which the blood's ability to carry oxygen is reduced due to decreased production of red blood cells or hemoglobin, causing fatigue and weakness. This study aimed to investigate the use of freeze-dried lemon peel and pulp powder as an adjunctive treatment for anemia. Twenty female white mice, each weighing  $140 \pm 10$  g, were used in this study and divided into four groups of five mice each. Group 1 (negative control) received only the basal diet. Group 2 (positive control) received the basal diet supplemented with tannic acid to maintain anemia. Group 3 consisted of anemic mice fed the basal diet supplemented with 2.5% freeze-dried lemon pulp powder. Group 4 consisted of anemic mice fed the basal diet supplemented with 2.5% freeze-dried lemon peel powder. The mice were treated with freeze-dried lemon peel and pulp for two months. The results showed that anemic mice treated with freeze-dried lemon peel and pulp experienced improved body weight. Cholesterol levels in the positive control group were  $197.67 \pm 2.52$ , while in groups 3 and 4, they were  $192.33 \pm 1.53$  and  $187.67 \pm 2.52$ , respectively. Triglycerides in the positive control group were  $76.67 \pm 1.53$ , while they decreased to  $82.33 \pm 1.53$  and  $181.33 \pm 1.53$  in groups 3 and 4, respectively. Hemoglobin levels in the positive control group decreased to  $7.23 \pm 0.40$ , while they increased to

12.77  $\pm$  0.55 and 14.07  $\pm$  0.23 in groups 3 and 4, respectively. Iron levels also decreased, from 39.00  $\pm$  1.00 in the positive control group while they increased to 47.00  $\pm$  2.00 and 50.33  $\pm$  2.52 in groups 3 and 4 respectively. The best results were achieved using 2.5% freeze-dried lemon peel compared to 2.5% freeze-dried lemon pulp.

**Key words:** freeze-dried, lemon, Rats, anemia and Iron and Ferritin Estimation

## Introduction

Lemon peels are among the major by-products of citrus processing and are often discarded as waste. However, revalorizing these residues in appropriate forms can offer significant economic and environmental advantages for the food industry. Their rich nutritional and technological properties can provide consumers with health benefits while also reducing environmental pollution and adding commercial value (Marin et al., 2002). Owing to their abundance of bioactive compounds, citrus by-products can be utilized as functional components in food formulations. These compounds can act as natural additives, antioxidants, antimicrobials, colorants, flavor enhancers, or thickening agents (Ayala-Zavala et al., 2011). Consequently, incorporating orange and lemon peels as functional ingredients holds potential for use in various food products, such as bakery items, meat products, and dairy formulations.

Lemon represents one of the most extensively cultivated citrus fruits worldwide, reaching approximately 19 million metric tons in 2018 (Harikrishnan et al., 2021). Global citrus fruit production from 2021 to 2022 was estimated at about 48.8 million tons, with *Citrus reticulata* contributing around 70% of the total. During citrus processing, large

quantities of waste such as pomace, peel, and seeds are generated, and peel alone can constitute up to half of the total fruit's dry weight (**U.S. Department of Agriculture, 2021**).

In lemon juice manufacturing, the yield of juice typically ranges between 20% and 30%, leaving behind 50%–60% of solid waste material, mostly composed of peel (**Boluda-Aguilar et al., 2013 and M'hiri et al., 2018**). Peels are therefore the most substantial by-product, accounting for nearly 50–65% of the fruit's weight (**Chatha et al., 2011**). The disposal of such waste presents environmental challenges and incurs additional costs. To address these concerns, lemon peels have been applied in various fields including animal feed, fertilizer, pectin recovery, bioethanol generation, and essential oil extraction (**González-Molina et al., 2010; Bouzenna et al., 2016**). Similar to many herbal plants, lemon exhibits multiple biological effects such as anti-inflammatory, antimicrobial, antioxidant, immunomodulatory, and hepatoprotective activities (**Harikrishnan et al., 2020; Zhuo et al., 2021**). Its bioactive constituents—including dietary fiber, citric acid, ascorbic acid, minerals, flavonoids, carotenoids, limonoids, and essential oils—contribute to numerous health-promoting functions (**Puangkaew et al., 2004 and Lee et al., 2017**).

Anemia refers to a condition where the blood's capacity to transport oxygen is reduced due to a decrease in the number or size of red blood cells or a reduction in hemoglobin concentration (**Mahoney and Armsby, 2021**). Adequate iron intake is essential for hemoglobin formation, which supports vital physiological processes (**Gogoi et al., 2017 and Omotoso, 2012**). Enhancing iron bioavailability depends not only on consuming iron-rich foods but also on the intake of compounds that promote absorption while limiting inhibitors such as phytates and tannins. Vitamin C, abundant in citrus fruits like lemons and oranges, is a key enhancer of iron absorption as it prevents the formation of insoluble

iron complexes (**James, 2001**). Citrus peels, especially those of lemon and orange, contain substantial levels of vitamin C and phenolic compounds, both of which are valuable for treating and preventing various health disorders.

Lemon is rich in numerous beneficial substances including vitamin C, sugars, calcium, phosphorus, iron, vitamins B1 and B2, niacin, citric, malic, and quinic acids, as well as flavonoids such as hesperidin and naringin, and compounds like coumarin. It is characterized by a high potassium and low sodium content, making it advantageous for human health (**Gao and Mao, 2014**). The nutritional profile of lemon supports tissue formation, intercellular structure maintenance, and normal physiological functions (**Zeng et al., 2018**). Additionally, lemon consumption promotes hydration, protects against cardiovascular diseases, alleviates heat and phlegm, and provides antibacterial, anti-inflammatory, and anti-aging effects (**Zhu et al., 2019**). While lemon pulp and juice are commonly used, other components such as leaves and seeds also hold value—for instance, lemon leaves serve as seasonings, while seeds yield edible oil (**Wang et al., 2019**). Despite these uses, lemon peels remain underutilized in food processing and are often discarded during industrial production. Identifying practical applications for lemon peels, particularly in functional food development, can enhance their economic value and promote comprehensive agricultural utilization (**Li et al., 2020**).

In light of these findings, the current research aims to investigate the effect of freeze-dried lemon pulp and peel powder on anemia, and to evaluate its potential role as an anti-anemic agent in experimental mice. It also seeks to determine the effect of feeding freeze-dried lemon peel and pulp on body weight, cholesterol levels, liver and kidney function, iron and ferritin levels, and their impact on arterial stiffness and hemoglobin.

## **2. Materials and Methods**

### **2.1. Materials**

Fresh lemon fruits were purchased from local vendors in Benha, Qalyubia Governorate, Egypt. Adult female albino rats of comparable age and body weight were obtained from the Experimental Animal Facility of the Food Technology Research Institute, Giza, Egypt.

### **2.2. Methods**

#### **2.2.1. Preparation of Freeze-Dried Lemon Pulp and Peel Powders**

The collected lemons were washed thoroughly with clean water, peeled carefully, and the pulp was separated from the outer rind. Seeds were manually removed from the pulp, which was then cut into small uniform pieces. The peels were also chopped into similar-sized fragments. Both pulp and peel portions were transferred into separate polyethylene bags and stored at  $-40 \pm 2$  °C for 48 hours to ensure complete freezing. After freezing, the samples were placed in a ZIRBUS laboratory freeze dryer (Model VaCo5-D, S/N: COM98754, Volt: 110/220 V) for 72 hours. The dried pulp and peel were then finely milled using to obtain lemon pulp and peel powders. The resulting powders were stored in airtight containers at ambient temperature ( $33 \pm 2$  °C) until required for experimental use.

#### **2.2.3. Biological Assay**

##### **2.2.3.1. Experimental Animals**

Twenty healthy adult female *Sprague-Dawley* albino rats, weighing  $140 \pm 10$  g, were procured from the Institute of Medical Insect Research, Giza, Egypt. The animals were maintained on a basal diet formulated

according to **Khalil et al., (2018)** with slight modifications. Each rat was housed individually in stainless steel cages equipped with wire-mesh floors under hygienic and controlled conditions (temperature 25–30 °C, relative humidity about 50%, and a 12 h light/12 h dark cycle). The rats were acclimatized to the experimental environment and diets for one week prior to the start of the experiment, which lasted for eight weeks in total. Throughout the entire period, animals were allowed unrestricted access to food and deionized water.

### **2.2.3.2. Induction of Anemia**

Following the adaptation phase, baseline hemoglobin (Hb) concentrations were measured in all rats. The animals were then fed a high-fiber diet (20%) for two weeks, followed by an iron-deficient diet for an additional two weeks to induce anemia, as outlined by **Bhalekar et al., (2016)** Blood samples were collected from the orbital vein to confirm anemia by measuring hemoglobin levels.

### **2.2.3.3. Experimental Design**

After anemia induction, the rats were divided into four groups (five animals per group) as follows:

- Group 1: (Negative Control): Received only the basal diet.
- Group 2: (Positive Control): Received the basal diet containing tannic acid to maintain anemia.
- Group 3: Anemic rats fed the basal diet supplemented with 2.5% freeze-dried lemon pulp powder.
- Group 4: Anemic rats fed the basal diet supplemented with 2.5% freeze-dried lemon peel powder.

Body weights were recorded weekly, and daily food intake was monitored. At the end of the study period (28 days), biological evaluation was performed by calculating body weight gain (BWG) and feed efficiency ratio (FER) according to **Zwain , (2020)**

$$\text{BWG} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}}$$

$$\text{FER} = \frac{\text{Body weight gain (g)}}{\text{Feed intake (g)}}$$

At the conclusion of the trial, rats were fasted overnight, anesthetized, and sacrificed. Blood samples were collected—some into plain tubes for serum separation and others into EDTA-treated tubes for hematological analysis. Serum was obtained by centrifugation at 3000 rpm for 15 min, transferred into labeled vials, and stored at  $-20^{\circ}\text{C}$  for subsequent biochemical tests.

#### **2.2.4. Biochemical Analyses**

**2.2.4.1 Serum Cholesterol:** Determined by the method of **Gagel et al., (2022)**.

**2.2.4.2 High-Density Lipoprotein (HDL) Cholesterol:** Measured following . **Wang et al., (2024)**.

**2.2.4.3 Low-Density Lipoprotein (LDL) Cholesterol:** Estimated using the procedure of . **Ferhatbegović et al., (2022)**.

**2.2.4.4 Triglycerides (TG):** Quantified as described by. **Jiang et al., (2024)**.

**2.2.4.5 Liver Function Tests** Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to **Srinivasan et al., (2005)**.

**2.2.4.6 Kidney Function Tests** Serum urea and creatinine concentrations were measured following the enzymatic methods of **Menshawy et al., (2019)**.

**2.2.4.7 Iron and Ferritin Estimation** Serum iron and ferritin concentrations were determined using commercial diagnostic kits (Sigma Diagnostics, St. Louis, USA), according to the procedure of **Cavell (1986)**.

**2.2.4.8 Hemoglobin Determination** Hemoglobin levels were analyzed using an automated multichannel hematology analyzer based on aperture impedance and/or laser cell sizing techniques, following **Jacobs et al. (2001)**.

**2.2.4.9 Atherogenic Index of Plasma** The atherogenic index, a predictor of cardiovascular risk, was calculated as described by **Zephy (2015)**.

## **2.2.5. Statistical Analysis**

All numerical results were expressed as mean  $\pm$  standard deviation (SD). Statistical comparisons among treatment groups were performed using one-way ANOVA in Microsoft Excel. Differences were considered statistically significant at  $P \leq 0.05$ , following the method of **Ahmed et al., (2020)**.



### 3 RESULTS AND DISCUSSION

#### 3.1 Effect of feeding with freeze-dried lemon peel and pulp powder for 8 weeks on body weight gain, feed intake and feed efficiency ratio of anemic rats.

Table 1 presents the influence of an eight-week dietary supplementation with freeze-dried lemon pulp and peel powders on body weight gain, feed intake, and feed efficiency ratio in anemic rats. The findings revealed that the positive control group, which received only the basal diet and drinking water, exhibited a significantly higher ( $P \leq 0.05$ ) body weight gain compared to the negative control group. Similarly, the feed efficiency ratio (FER) in the positive control rats was significantly greater ( $P \leq 0.05$ ) than that of the negative controls.

Interestingly, the body weight gain (BWG) of the negative control group was found to be the greatest when compared with the positive control group, and this difference was statistically significant. Among the anemic groups, the data clearly indicate that the negative control animals showed the highest BWG values relative to the positive control rats ( $P \leq 0.05$ ). Regarding feed intake (FI), the negative control group demonstrated a significantly greater value than the positive control group.

Rats receiving diets enriched with freeze-dried lemon pulp or peel powders displayed lower mean body weight values than those of the positive control. Feed intake levels were also significantly higher in the positive control animals compared with the negative group ( $19.23 \pm 0.28$  vs.  $18.12 \pm 0.26$  g/day, respectively). In contrast, the anemic groups supplemented with lemon pulp or peel powders consumed significantly

less feed ( $17.55 \pm 0.24$  and  $17.9 \pm 0.86$  g/day, respectively) than both control groups.

These observations align with those of **Prockop and Kivirikko (1995)**, who noted that lemon peel is a natural source of pectin—a soluble dietary fiber known to assist in weight reduction. Similarly, **Aprikian et al. (2001)** reported that incorporating lemon peel into high-fat diets can reduce both body weight and food consumption in rats. Earlier studies by **Utpala et al. (2006)** and **Kolanjiappan et al. (2012)** also indicated that anemia induction is commonly accompanied by reduced appetite and weight loss, likely resulting from diminished oxygen delivery to tissues and subsequent declines in metabolic activity.

**Table 1 Effect of feeding with freeze-dried lemon peel and pulp powder for 8 weeks on body weight gain, feed intake and feed efficiency ratio of anemic rats.**

Groups	Initial Weight(g)	Final Weight (g)	B.W.G	Feed Intake (g/day/rat)	Feed efficiency ratio
G1	172.33±2.5	200.5±1.99	28.17±1.02	19.23±0.28	1.46±0.02
G2	160.33±1.53	185.6±1.84	25.27±95	18.12±0.26	1.40±0.03
G3	161.55±2.00	185.2±0.87	23.65±1.0	17.55±0.24	1.34±0.02
G4	162.80±2.08	185.7± 1.17	22.9±1.16	17.9 ±0.86	1.27±0.02
L.S.D *0.05	2.3	2.7	1.6	1.3	0.15

Value  $\pm$  SD with the same latter in the same row are not significantly different ( $P \leq 0.05$ ) \*: Least significant differences

Group (I): Rats were fed on basal diet as negative control.

Group (2): Rats were anemic by feeding on tannic acid added in the basal diet and were used as positive control.

Group (3): A group of anemic rats were fed on freeze-dried lemon pulp powder at 2.5% of the basal diet weight.

Group (4): A group of anemic rats were fed on freeze-dried lemon peel powder at 2.5% of the basal diet.

### **3.2 Effect of feeding with freeze-dried lemon peel and pulp powder for 8 weeks on organs weight to anemic rats.**

Table 2 illustrates the impact of the experimental diets on organ weights among the treated rat groups. The results indicated that the positive control animals, which were provided with the basal diet and tap water, exhibited significantly ( $P \leq 0.05$ ) higher organ weights compared to the negative control rats maintained on the same diet. Likewise, anemic rats receiving diets supplemented with 2.5% freeze-dried lemon pulp powder or 2.5% freeze-dried lemon peel powder also showed significantly greater organ weights ( $P \leq 0.05$ ) than those of the negative control group. In general, increasing the dietary fiber content led to a marked ( $P \leq 0.05$ ) reduction in overall organ mass. When comparing the heart, liver, and kidney weights, rats fed the diets containing freeze-dried lemon pulp or peel powder exhibited noticeable variations relative to the positive control group. As summarized in Table 2, the liver, kidney, and heart weights in the anemic rats were significantly elevated compared to those of the negative controls. These findings are consistent with the observations of **Aprikian et al. (2001)**, who demonstrated that lemon peel supplementation can help protect organs such as the liver and spleen against damage and toxic effects. Although the mean relative weights of the liver and heart declined slightly in the groups treated with freeze-dried lemon pulp and peel powders compared with the other anemic groups, these reductions were not statistically significant.

**Table 2 Effect of feeding with freeze-dried lemon peel and pulp powder for 8 weeks on organs weight to anemic rats.**

Groups	Organs weight (g)		
	Liver	Kidney	Heart
G1	6.79 ± 0.25	1.37 ± 0.12	0.55 ± 0.02
G2	7.30 ± 0.42	1.55 ± 0.14	0.80 ± 0.05
G3	7.01 ± 0.56	1.23 ± 0.04	0.63 ± 0.03
G4	7.02 ± 0.41	1.56 ± 0.12	0.59 ± 0.02
L.S.D*0.05	1.63	1.2	0.18

Value ± SD with the same latter in the same row are not significantly different ( $P \leq 0.05$ ) \*: Least significant differences.

### **3.3Effect of feeding with freeze-dried lemon peel and pulp powder for 8 weeks on total cholesterol and triglycerides of anemic rats.**

As presented in Table 3, feeding anemic rats with diets supplemented by freeze-dried lemon pulp or peel powder for eight weeks produced clear effects on serum cholesterol and triglyceride concentrations. The positive control rats that consumed only the basal diet exhibited significantly ( $P \leq 0.05$ ) higher total cholesterol and triglyceride levels than the negative control group. However, a notable reduction ( $P \leq 0.05$ ) in both parameters occurred as the inclusion level of freeze-dried lemon pulp or peel powder increased. In agreement with these findings, **Heba Abdelhaliem and Hanady Sheha (2018)** found that Treatment with lemon peels significantly decreased body weight, total serum cholesterol, triglycerides, low density lipoprotein (LDL-C), very low density lipoprotein (VLDL-C) and increased the level of high density lipoproteins (HDL-C). The level 10% recorded the best results followed by 5 and 2.5% Plasma calcium was the high affected by increasing the level of

lemon peels. Finally, it can be concluded that lemon peels improved lipid profile and the other biochemical parameters without increasing the using level above 10%. The positive control group of anemic rats showed significant changes ( $P<0.05$ ) in increased levels of total cholesterol, liver lipids and decreased TG values, while all other groups showed significant changes in decreased levels of cholesterol, liver lipids, and triglycerides according to the data in Table 3. Consistent with the positive control group, TGs were enhanced when administered lemon peels and pulp freeze-dried powder accordingly, hesperidin, a flavonoid that helps lower cholesterol and triglycerides, is found in lemon peels and pulp. These results are consistent with **Tiwari et al., (2017)** found that lemon's cholesterol-lowering ability is attributed to the limonene found in its peels. Furthermore, the polymethoxylated flavones found in citrus peels can lower cholesterol more effectively and safely than some prescription drugs. On the other hand, by lowering triglyceride and cholesterol levels, lemon peel powders have a significant anti-inflammatory effect. **Aina et. al, (2012)** reported that lemon peels are a source of health-promoting carbohydrates. Peels also contain healthy polymethoxylated flavones (PMF), which are plant pigment compounds, present in all citrus fruits. Several authors found that the PMF compounds in citrus peels have the potential to lower cholesterol when included in our diet as well as LDL cholesterol without the side effects of mainstream cholesterol drugs lemon peel and pulp contain hesperidin, a flavonoid that helps lower cholesterol and triglycerides. Lemon peel is also a source of pectin, a natural fiber that helps reduce cholesterol levels and in a joint study with the US department of Agriculture identified a class of compounds isolated from lemon peels that shows promise in animal studies as a potent natural alternative for lowering LDL cholesterol, without the possible side effects, such as liver disease and muscle weakness, of

conventional cholesterol lowering drugs. **Lindahl and Hook, (2002)** and **Archibald, (2005)** reported that lemon peels had a powerful antioxidant as alpha, delta and gamma tocotrienols and other constituents which had been shown to lower total cholesterol and triglyceride levels. They outlined the potential dietary benefits of lemon peels pectin and fiber. **Liu, (2017)** stated that lemon peel's hydroxycinnamic acids inhibited human LDL oxidation in vitro. The present investigation was designed to evaluate anti hypercholesterolemic activity of lemon peels powders on albino induced hypercholesterolemic rats as well as their effect on their other biological parameters. **Fadl E. El-Deeb et al., (2023)**. A study showed that feeding mice spinach and lentils increased iron and zinc levels and reduced triglycerides and cholesterol, due to their high phenolic compound content.

**Table 3 Effect of feeding with freeze-dried lemon peel and pulp powder for 8 weeks on total cholesterol and triglycerides of anemic rats.**

Groups	Parameters	
	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)
G1	194.00±2.00	76.67±1.53
G2	197.67 ± 2.52	87.67 ± 1.53
G3	192.33 ± 1.53	82.33 ± 1.53
G4	187.67 ± 2.52	81.33 ± 1.53
L.S.D*0.05	3.94	3.4

Value ± SD with the same latter in the same row are not significantly different (P≤0.05) \*: Least significant differences.

### **3.4 Effect of feeding with freeze-dried lemon peel and pulp powder for 8 weeks on HDL and LDL levels (mg/dl) of anemic rats.**

Table 4 summarizes the influence of feeding anemic rats with diets enriched in freeze-dried lemon pulp and peel powders for eight weeks on lipid parameters, including HDL, LDL, triglycerides, and lipoproteins (mg/dL). The data revealed that the positive control group exhibited significantly ( $P \leq 0.05$ ) elevated concentrations of triglycerides, total cholesterol, LDL, and VLDL compared to the negative control group, whereas HDL levels displayed an opposite pattern.

These findings corroborate those of **Sohn et al. (2016)**, who demonstrated that lemon peel supplementation improves serum lipid balance and maintains normal lipid homeostasis. Similarly, **Kay and Truswell (1977)** earlier reported a pronounced hypocholesterolemic effect in rats fed a high-cholesterol diet containing 7.5% lemon peel. In the present study, the rats receiving diets supplemented with freeze-dried lemon pulp and peel exhibited significantly lower ( $P \leq 0.05$ ) triglycerides, total cholesterol, LDL-C, and VLDL-C than the positive control rats. Prolonged feeding with the lemon-based diets further reduced these lipid fractions, although their values remained slightly higher ( $P \leq 0.05$ ) than those recorded in the negative controls.

Comparable outcomes were observed by **Hussein (2012)** and **Amira Abdel-Gawad (2012)**, who noted considerable reductions in serum triglycerides, total cholesterol, and LDL-C in mice consuming bread fortified with 20% dietary fiber, resulting in decreases of 42.4%, 38.4%, 65.1%, and 54.2%, respectively, relative to controls. Likewise, **Syed Ahmed (2014)** found that progressive inclusion of pomegranate peel powder in baked products led to significant declines in total lipids,

triglycerides, cholesterol, and LDL-C, accompanied by a rise in HDL-C. Extending the feeding period beyond four weeks with fiber-enriched bread diets further normalized lipid parameters such as triglycerides, total cholesterol, LDL, and VLDL.

In the current work, both lemon peel and pulp freeze-dried powders exerted favorable effects on blood lipid metabolism in anemic rats. Animals in the anemic control group displayed elevated LDL and reduced HDL levels, while supplementation with lemon-derived powders reversed this trend by decreasing LDL and enhancing HDL concentrations. Notably, the combination of lemon pulp and peel powders produced a synergistic improvement in lipid regulation within the positive control group.

These findings are consistent with **Osfor et al. (2013)**, who reported that citrus flavonoids possess lipid-lowering activity, partly due to their antioxidant potential, which limits oxidized LDL uptake by macrophages and prevents LDL aggregation and oxidation. Additionally, studies using diets fortified with 10–20% orange powder showed substantial reductions in total cholesterol, triglycerides, and LDL-C among hypercholesterolemic mice. Incorporation of orange pulp and peel into the diet has also been associated with improved glucose regulation, decreased LDL-C and total cholesterol, and reduced risk of atherosclerosis and cardiovascular disease.



**Table 4 Effect of feeding with freeze-dried lemon peel and pulp powder for 8 weeks on HDL and LDL levels (mg/dl) of anemic rats.**

Groups	Parameters	
	HDL (mg/dl)	LDL (mg/dl)
G1	48.22± 1.53	115.33± 2.52
G2	39.00 ± 1.00	143.33± 5.64
G3	42.00 ± 1.00	125.67± 2.06
G4	43.67 ± 1.53	124.33± 2.04
L.S.D*0.05	3.3	3.9

Value ± SD with the same latter in the same row are not significantly different ( $P \leq 0.05$ ) \*: Least significant differences.

### **3.5 Effect of feeding with freeze-dried lemon peel and pulp powder for 8 weeks on GPT/ALT and GOT/AST enzymes of anemic rats.**

Table 5 presents the influence of administering freeze-dried lemon pulp and peel powders for eight weeks on serum levels of glutamic pyruvic aminotransferase (GPT/ALT) and glutamic oxaloacetic aminotransferase (GOT/AST) in anemic rats. According to the results, rats in the positive control group showed a significant elevation ( $P \leq 0.05$ ) in both GPT/ALT and GOT/AST enzyme activities compared to the negative controls at the conclusion of the study period. In contrast, animals supplemented with the lemon pulp and peel powders exhibited a marked decline in these enzymatic activities relative to both the positive and control groups. Serum transaminases such as AST and ALT are widely recognized as key biochemical indicators of hepatic injury. The data in Table 5 further demonstrate that negative control rats exhibited significantly lower ( $P \leq 0.05$ ) GOT/AST and GPT/ALT values than their positive counterparts. Conversely, those fed with lemon pulp or peel

powder showed substantial reductions in these enzymes, with the degree of suppression becoming more pronounced as the inclusion level of the lemon powders increased. These outcomes align with the findings of **Al-Bashri (2013)**, who observed that low to moderate doses of lemon peel supplementation effectively limited serum ALT elevation. Similarly, **Heba Abdelhaliem and Hanady Sheha (2018)** noted that adding 10% lemon peel to the diet of rats yielded the greatest reductions in AST and ALT levels compared with other treatments. **Sayed Ahmed (2014)** also reported improved hepatic performance in rats fed high-fat diets when pomegranate peel powder was incorporated as a fiber source. In the current study, GPT/ALT activity reached 56.00 U/L in the positive control rats, a value significantly higher ( $P \leq 0.05$ ) than that of the negative group (24.33 U/L), reflecting the impact of elevated cholesterol intake. Although rats fed diets enriched with freeze-dried orange pulp and peel powders displayed lower GPT/ALT levels, their values remained higher than those of the negative controls. Likewise, GOT/AST activity ranged from 45.7 to 80.8 U/L across the treated groups—still exceeding that of the negative group. **Amira Abdel-Gawad (2012)** found similar trends in hypercholesterolemic rats, where dietary inclusion of 15% dried red cabbage fiber reduced serum ALT and AST activities compared to untreated animals. AST is primarily localized in hepatocytes, cardiac muscle, skeletal tissue, and erythrocytes. When hepatic cells are compromised, transaminases leak into the bloodstream, serving as diagnostic indicators of hepatocellular damage (**Haram et al., 2011**). The present study observed a clear rise in serum AST activity indicative of hepatic stress; however, supplementation appeared to mitigate this effect. This observation is consistent with evidence linking iron-deficiency anemia (IDA) to altered hepatic enzyme function and metabolic disturbances in the liver. Furthermore, **Saha et al., (2011)** reported that

treatment with glibenclamide normalized hepatic enzyme activities, confirming its protective potential. Phytochemical profiling of herbs such as saffron, ginger, and cumin revealed compounds including fucosterol, campesterols, flavonoids, triterpenoids, cucurbitacins, saponins, and polyphenolics—many of which exhibit hepatoprotective and antioxidant properties. Correspondingly, **Sowhagya et al., (2018)** and **Kumar et al., (2016)** emphasized that both *in vivo* and *in vitro* investigations support the antioxidant and liver-protective efficacy of these bioactive components, although comprehensive evaluation of their effects on anti-tubercular drug-induced hepatotoxicity in rats remains limited.

**Table 5 Effect of feeding with freeze-dried lemon peel and pulp powder for 8 weeks on GPT/ALT and GOT/AST enzymes of anemic rats.**

Groups	Parameters	
	GPT/ALT( U/L)	GOT/AST (U/L)
G1	24.33±2.08	30.67 ± 2.08
G2	55.33 ± 3.21	51.00 ± 5.57
G3	23.33 ± 4.04	25.33 ± 3.06
G4	23.00 ± 2.00	28.00 ± 2.65
L.S.D*0.05	2.92	2.98

Value ± SD with the same latter in the same row are not significantly different (P≤0.05) \*: Least significant differences.

### **3.6 Effect of feeding with freeze-dried lemon peel and pulp powder for 8 weeks on urea and creatinine (mg/dl) of anemic rats.**

Urea represents the terminal compound produced during the breakdown of proteins in the body. Elevated concentrations of blood urea are commonly linked to impaired renal performance, but such increases can also arise from dehydration, gastrointestinal hemorrhage, cardiac failure, excessive dietary protein, or diminished renal perfusion. This condition is medically referred to as azotemia. Conversely, reduced urea levels may signal hepatic dysfunction (**Massy et al., 2016**).

As presented in Table 6, both serum urea and creatinine concentrations were determined in rats fed the basal diet (negative and positive controls) and those receiving diets enriched with freeze-dried lemon peel or pulp powders. The positive control group of anemic rats exhibited a marked rise ( $P \leq 0.05$ ) in serum urea compared with the negative group, recording  $57.00 \pm 3.00$  mg/dL versus  $25.00 \pm 2.00$  mg/dL, respectively. Dietary supplementation with lemon pulp and peel powders effectively reduced urea accumulation by the end of the feeding period. The average urea values in groups G3 and G4 ( $19.33 \pm 4.51$  mg/dL and  $19.65 \pm 4.73$  mg/dL, respectively) were significantly lower than those of the positive control rats ( $57.00 \pm 3.00$  mg/dL).

These outcomes align with **Hassan et al. (2003)**, who reported that long-term intake of small quantities of lemon peel enhanced renal efficiency. Regarding creatinine, positive control animals displayed significantly higher levels ( $0.92 \pm 0.02$  mg/dL) compared to the negative controls ( $0.76 \pm 0.03$  mg/dL). However, groups G3 and G4 showed no statistically significant variation in creatinine concentration, supporting the observations of **Adil et al. (2016)**, who found that lemon supplementation

lowered creatinine, urea, and uric acid levels in parallel with improved kidney function. Similarly, **Hermenean et al. (2013)** demonstrated the nephroprotective role of lemon against CCl<sub>4</sub>-induced toxicity in male rats. In addition, **Aziza and Mona (2022)** confirmed that both lemon and orange peel treatments produced significant decreases in creatinine, urea, and uric acid when compared to untreated control animals.

**Table 6 Effect of feeding with freeze-dried lemon peel and pulp powder for 8 weeks on urea and creatinine (mg/dl) of anemic rats.**

Groups	Parameters	
	Urea (mg/dl)	Creatinin (mg/dl)
G1	25.00± 2.00	0.84± 0.02
G2	57.00 ± 3.00	1.87 ± 0.11
G3	19.33 ± 4.51	0.82 ± 0.04
G4	19.65± 4.73	0.89 ± 0.04
L.S.D*0.05	3.6	0.03

Value ± SD with the same latter in the same row are not significantly different (P≤0.05) \*: Least significant differences.

### **3.7Effect of feeding with freeze-dried lemon peel and pulp powder for 8 weeks on IRON (mg/dl) and FERRITINE enzymes of anemic rats.**

Impaired absorption of dietary iron is often attributed to the presence of certain food constituents that interfere with its uptake, which in turn contributes to the widespread incidence of iron deficiency (**Hasan et al., 2018**). According to the data presented in **Table 7**, feeding anemic rats

with diets containing freeze-dried lemon pulp and peel powders for eight weeks produced a marked impact on serum iron (mg/dL) and ferritin levels. Rats in the positive control group exhibited significant reductions in both iron and ferritin concentrations compared with those in the negative control group, recording values of  $39.00 \pm 1.00$  mg/dL and  $23.33 \pm 1.53$  ng/mL, respectively.

By contrast, supplementation with lemon pulp and peel powders effectively restored iron status. At the end of the experimental period, groups receiving lemon pulp powder and lemon peel powder showed substantial increases in both parameters relative to the positive control. The serum iron concentrations reached  $47.00 \pm 2.00$  mg/dL and  $50.33 \pm 2.52$  mg/dL, while ferritin levels rose to  $43.00 \pm 2.65$  ng/mL and  $54.67 \pm 2.52$  ng/mL, respectively.

These outcomes agree with the findings of **Kim et al., (202)**, who noted that the administration of ginger and saffron extracts mitigated anemia and enhanced serum hemoglobin, hematocrit, and iron indices in a dose-dependent manner. Similarly, **Bochowski et al., (2019)** observed that rats supplemented with lactoferrin displayed significantly higher hemoglobin and hematocrit levels than those on a standard diet, with a strong positive association between serum iron concentration, absorption efficiency, and the hemoglobin regeneration efficiency ratio.

Furthermore, **Salma Nasr El-Deen et al., (2024)** emphasized that fruits and vegetables are rich sources of biologically active polyphenolic compounds with potent antioxidant capacities. These phytochemicals not only contribute to improved iron metabolism but also exert beneficial effects on glucose regulation, lipid profiles, and the enzymatic activities of the liver and kidneys.

**Table 7 Effect of feeding with freeze-dried lemon peel and pulp powder for 8 weeks on IRON (mg/dl) and FERRITINE enzymes of anemic rats.**

Groups	Parameters	
	IRON	FERRITINE
G1	56.33 ± 1.53	73.00 ± 2.00
G2	39.00 ± 1.00	23.33 ± 1.53
G3	47.00 ± 2.00	43.00 ± 2.65
G4	50.33 ± 2.52	54.67 ± 2.52
L.S.D*0.05	2.7	2.4

Value ± SD with the same latter in the same row are not significantly different (P≤0.05) \*: Least significant differences.

### **3.8Effect of feeding with freeze-dried lemon peel and pulp powder for 8 weeks on hemoglobin (HG) and Atherogenic index of anemic rats.**

Table 8 presents the influence of feeding anemic rats with freeze-dried lemon pulp and peel powders for eight consecutive weeks on their hemoglobin (Hb) concentrations. The findings revealed that rats in the negative control group exhibited the highest hemoglobin values compared to the anemic (positive control) group, showing a statistically significant difference (P < 0.05). Nevertheless, supplementing the diets of anemic rats with either lemon pulp or peel powders led to a notable elevation in hemoglobin levels relative to the untreated positive control. Specifically, hemoglobin concentrations were recorded as 12.77 ± 0.55 g/dL for the lemon pulp group and 14.07 ± 0.23 g/dL for the lemon peel group.

These outcomes align with the observations of **Aziza and Mona (2022)**, who reported that anemic rats receiving a combination of lemon and orange peel showed enhanced hemoglobin levels and improved hepatic function, with the 5% peel mixture producing the most favorable effects. In summary, dietary administration of a 5% blend of lemon and orange peel powders appears effective in restoring hemoglobin concentration and supporting liver function in anemic conditions compared with untreated counterparts.

Table 8 illustrates the effect of the same dietary treatments on atherosclerotic development in anemic rats over the same period. The results demonstrated that the negative control group displayed significantly lower atherosclerosis indices than the positive control ( $P < 0.05$ ). Furthermore, dietary supplementation with lemon pulp and peel powders produced a marked decline in atherosclerosis levels when compared with the positive control. The mean values for the groups receiving lemon pulp and peel powders were  $2.20 \pm 0.26$  and  $1.87 \pm 0.11$ , respectively, confirming the protective influence of these citrus-derived supplements against vascular complications associated with anemia.



**Table 8 Effect of feeding with freeze-dried lemon peel and pulp powder for 8 weeks on hemoglobin (HG) and Atherogenic index of anemic rats.**

Groups	Parameters	
	hemoglobin (HG)	Atherogenic index
G1	16.30±0.82	0.55± 0.04
G2	7.23±0.40	3.85± 0.35
G3	12.77±0.55	2.20± 0.26
G4	14.07±0.23	1.87± 0.11
L.S.D*0.05	2.22	1.6

Value ± SD with the same latter in the same row are not significantly different (P≤0.05) \*: Least significant differences.

#### **4Conclusion**

The administration of freeze-dried lemon peel and pulp powders proved effective in alleviating anemia symptoms in experimental rats. Both forms of the lemon powders contributed to lowering triglyceride and LDL-cholesterol levels, enhancing overall lipid metabolism, and supporting healthier liver function and histological structure. Prolonged dietary inclusion of these powders appeared to elevate iron and ferritin concentrations, which may be linked to reduced overall caloric intake and improved feelings of fullness. Among the two, the freeze-dried lemon peel powder being richer in soluble dietary fiber seemed to exert a more pronounced influence on body weight regulation than the pulp powder.

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### الملخص

فقر الدم هو حالة تقل فيها قدرة الدم على حمل الأكسجين بسبب انخفاض إنتاج خلايا الدم الحمراء أو الهيموغلوبين، مما يسبب التعب والضعف. هدفت هذه الدراسة إلى التحقيق في استخدام مساحيق قشرو لب الليمون المجفف كعلاج لفقر الدم. تم استخدام عشرين فأرة بيضاء أنثى، تزن كل منها  $140 \pm 10$  جم، في هذه الدراسة وتم تقسيمها إلى أربع مجموعات من خمسة فئران لكل منها. تلقت المجموعة 1 (مجموعة التحكم السلبية) النظام الغذائي الأساسي فقط. تلقت المجموعة 2 (مجموعة التحكم الإيجابية) النظام الغذائي الأساسي مضافاً إليه حمض التانيك للحفاظ على فقر الدم. تتكون المجموعة 3 من الفئران المصابة بفقر الدم والتي تتغذى على النظام الغذائي الأساسي المضاف إليه 2.5% من مسحوق لب الليمون المجفف بالتجميد. تتكون المجموعة 4 من الفئران المصابة بفقر الدم والتي تتغذى على النظام الغذائي الأساسي المضاف إليه 2.5% من مسحوق قشر الليمون المجفف بالتجميد. عولجت الفئران بقشر الليمون المجفف بالتجميد ولها لمدة شهرين. أظهرت النتائج أن الفئران المصابة بفقر الدم، والتي عولجت بقشر الليمون المجفف بالتجميد ولها، شهدت تحسناً في وزن الجسم. بلغت مستويات الكوليسترول في المجموعة الضابطة الإيجابية  $197.67 \pm 2.52$ ، بينما بلغت في المجموعتين الثالثة والرابعة 3 و4  $192.33 \pm 1.53$  و  $187.67 \pm 2.52$  على التوالي. بلغت الدهون الثلاثية في المجموعة الضابطة الإيجابية  $76.67 \pm 1.53$ ، بينما انخفضت إلى  $82.33 \pm 1.53$  و  $181.33 \pm 1.53$  في المجموعتين 3 و4 على التوالي. انخفضت مستويات الهيموغلوبين في المجموعة الضابطة الإيجابية إلى  $7.23 \pm 0.40$ ، بينما ارتفعت إلى  $12.77 \pm 0.55$  و  $14.07 \pm 0.23$  في المجموعتين 3 و4 على التوالي. وانخفضت مستويات الحديد أيضاً في المجموعة الضابطة الإيجابية حيث سجلت ثم ارتفعت في المجموعتين 3 و4 إلى  $39.00 \pm 1.00$  إلى  $47.00 \pm 2.00$  و  $50.33 \pm 2.52$  على التوالي. وتم تحقيق أفضل النتائج باستخدام 2.5% من قشر الليمون المجفف بالتجميد مقارنة بـ 2.5% من لب الليمون المجفف بالتجميد

