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EFFECTS OF ALCOHOLIC EXTRACT OF POMEGRANATE PEEL (PUNICA GRANATUM) ON GHRELIN AND RESISTIN HORMONE LEVELS, LIPID PROFILE AND BODY WEIGHT IN EXPERIMENTAL MICE

Noor Faris Talal Alhamdany*

Department of Chemistry / College of Education for Women /University of Mosul.

***Corresponding Author: Noor Faris Talal Alhamdany**

Department of Chemistry / College of Education for Women /University of Mosul.

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ABSTRACT

This study included extraction of phenolic compounds from pomegranate peels using a continuous extraction apparatus (Soxhlet) with 70 % Ethanol. Qualitative Screening of the Pomegranate Peel Ethanolic extract revealed a high content of Polyphenols, Tannins, Flavonoids, and Anthocyanins. Additionally, in this Study four groups (I, II, III, and IV) of male Experimental Mice, each group of (15) mice. Groups II, III, and IV were orally administered the Pomegranate Peel polyphenol extract at doses of 300, 400, and 500 µg/10 g of body weight, respectively, for a duration of four weeks. Group I served as the control group (untreated with the aforementioned extract). Subsequently, blood samples were collected from the groups to perform the necessary analyses. The findings of this study demonstrated that the ethanolic extract of pomegranate peels led to a significant decrease ($P \leq 0.05$) in the mean values of both Body Weight and body mass index (BMI) in the treated mice groups compared to the control group. Furthermore, the results showed a significant decrease ($P \leq 0.05$) in the Biochemical Parameters, including glucose, Total Cholesterol (TC): Low-Density Lipoprotein Cholesterol (LDL-c): and Triglycerides (TG). Conversely, a significant increase ($P \leq 0.05$) was observed in High-Density Lipoprotein Cholesterol (HDL-c) in the serum of the mice groups treated with the extract compared to the control group. Additionally, this study showed the effect of the Pomegranate Peel Ethanolic Extract on the serum levels of ghrelin and resistin hormones in the mice groups, The Results indicated a significant decrease ($P \leq 0.05$) in the levels of both hormones compared to the control group.

KEYWORDS: Pomegranate Peels, Ghrelin, Resistin, Insulin resistance, Body Weight.

INTRODUCTION

The pomegranate tree (Punica granatum) belongs to the family Punicaceae and is commonly known as pomegranate, It is native to Western Asia and is cultivated in most Arab regions, particularly the Mediterranean basin, the Levant and Iraq (Feng et al., 2022): The tree reaches a height of up to 6 meters, with drooping branches and its branches and leaves tend to have a reddish hue, Its flowers are bright red aesthetically appealing and known as "Golnar" which means red in Persian, The fruit is spherical in shape with a leathery pericarp (Enas et al., 2022). Pomegranate peels constitute 26–30% of the total fruit weight, where they have been utilized in traditional medicine for various therapeutic purposes, including the treatment of gastrointestinal disorders and dysentery, as anti-inflammatory agents for conditions such as gingivitis, and as antimicrobial and antibacterial agents (Yaxian. et al., 2022): Pomegranate peels contain a variety of chemical constituents of nutritional and medicinal importance, including dietary fiber, minerals, vitamins, and fatty acids, hey are also characterized by their content of phenolic compounds and polyphenols, as well as several bioactive compounds, including Flavonoids, Anthocyanins, Tannins, in addition to Gallic acid and Ellagic acid (Cava. Et al., 2023) (Hanafy et al., 2021): Pomegranate peels contain a higher concentration of antioxidants than the pulp these antioxidants act as natural antioxidants that protect cells from free radicals and oxidative stress, thereby reducing the risk of developing certain diseases, such as cancer (Azmat et al., 2024): furthermore, they contribute to maintaining bone

and cartilage health, treating bronchitis, protecting the heart, and reducing the risk of cardiovascular diseases and atherosclerosis (Chaves et al., 2020). Additionally, they function to lower both glucose and low-density lipoprotein (LDL) levels (Siddiqui.et al., 2024).

Ghrelin was first discovered in 1999; it is a peptide hormone commonly known as the 'hunger hormone' and consists of 28 amino acids. It is primarily secreted by the stomach, with trace amounts also released by the hypothalamus and pituitary gland. Ghrelin plays a vital role in regulating energy balance, body weight, and insulin resistance (Jiao & Luo., 2022).

Resistin was first discovered in mice in 2001 and was named for its ability to induce insulin resistance in injected mice. It is a peptide hormone consisting of 108 amino acids in humans and 114 amino acids in mice. In mice, it is primarily secreted by white adipose tissue, whereas in humans, it is predominantly secreted by peripheral blood mononuclear cells, the heart, and other tissues, with minor amounts secreted by adipose tissue. Elevated resistin levels in humans are associated with an increased risk of developing insulin resistance, obesity, and cardiovascular diseases (Jamaluddin. et al., 2012).

MATERIALS AND METHODS

Pomegranate fruits were purchased from local markets. The peels were isolated, thoroughly cleaned of impurities, and shade-dried at ambient room temperature. In this study, white BALB/c mice obtained from the animal house of the College of Veterinary Medicine, University of Mosul, were utilized. Adult male mice weighing between 27 and 30 g were isolated and provided with water and a standard commercial pellet diet, all animals were maintained under uniform conditions of temperature and light.

Extraction of polyphenol from Pomegranate peels

The ethanolic extract of pomegranate peels was prepared according to the method followed by (Sharma and Janmeda, 2017). 50 g of pomegranate peels were taken and ground with an electric grinder. The powder was placed with 500 mL of 70% ethyl alcohol, and the extraction process was performed in a Soxhlet continuous extraction apparatus for 6 hours. The extract was filtered using filter paper and concentrated using a rotary evaporator under reduced pressure. A dry powder was obtained and preserved.

Experimental design

Experimental mice were divided into three groups, each group comprising 15 mice and each was weighed before and after treatment with the ethanolic extract of pomegranate peels for four weeks.

First group (I): A group of mice left without treatment and considered a control group.

Second group (II): This group comprises mice that were orally administered the alcoholic extract of pomegranate peels at a dose of 300 µg/10 g of body weight.

Third group (III): This group comprises mice that were orally administered the alcoholic extract of pomegranate peels at a dose of 400 µg/10 g of body weight.

Fourth group (IV): This group comprises mice that were orally administered the alcoholic extract of pomegranate peels at a dose of 500 µg/10 g of body weight.

Estimation of body mass index for experimental mice

The body mass index (BMI) of the experimental mice was calculated according to the method of the researchers (Kasim and Thanoo, 2014): as in the following equation.

$$BMI = \frac{Body\ Weight\ (gm)}{Length\ (cm)^2}$$

Collection and Preservation of Samples

Mice were fasted for 12 hours after the last administration of the alcoholic extract, then anesthetized with ether for a few seconds. Blood was subsequently collected from the orbital sinus puncture using specialized capillary tubes (Atta et al., 1983): The blood was collected into small tubes called Eppendorf tubes, which were then placed in a water bath at 37°C for 10 minutes. Serum was separated using a Microfuge 18 centrifuge designated for Eppendorf tubes at 3000 ×g for 15 minutes. The serum was drawn using a micropipette and directly stored in the freezer until the estimation of the biochemical parameters for this study.

Biochemical parameters

Determination of glucose level

Serum glucose levels in the experimental mice groups were estimated using commercial kits from the French company Biolabo, based on the enzymatic method of the researchers (Ashwood and Burtis, 1999): according to the following equation.

$$Glucose\ Concentration\ (mg/100ml) = \frac{A_{sample}}{A_{standard}} \times Standard\ Concentration(100mg/100ml)$$

Determination of total cholesterol level

Total serum cholesterol levels in the experimental mouse groups were estimated using commercial assay kits (Bio labo, France): based on the enzymatic method of Burtis and Ashwood (1999): according to the following equation.

$$\text{Total Cholesterol (mg/100ml)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{Standard Concentration}(200mg/100ml)$$

Determination of Triglyceride level

Serum triglyceride levels in the experimental mouse groups were estimated using commercial assay kits (Bio labo, France): based on the enzymatic method of Burtis

and Ashwood (1999): according to the following equation:

$$Triglyceride\ Concentration\ (mg/100ml) = \frac{A_{sample}}{A_{standard}} \times Standard\ Concentration(200mg/100ml)$$

Determination of HighDensity Lipoprotein – Cholesterol level

Serum high-density lipoprotein cholesterol (HDL-c) levels were estimated using commercial assay kits (Biolabo, France): based on the enzymatic method of Friedewald et al. (1972): according to the following equation.

$$HDL\text{-}c\ Concentration\ (mg/100ml) = \frac{A_{sample}}{A_{standard}} \times Standard\ Concentration(100mg/100ml)$$

Determination of LowDensity Lipoprotein – Cholesterol level

Serum low-density lipoprotein cholesterol (LDL-c) levels were calculated using the following equation according to (Burtis and Ashwood, 1999).

$$LDL - c = (Total\ Cholesterol) - (HDL - c) - \left(\frac{T.G}{5}\right)$$

Determination of Very LowDensity Lipoprotein – Cholesterol level

Serum very low-density lipoprotein cholesterol (LDL-c) levels were calculated using the following equation according to Burtis and Ashwood (1999).

$$\mathbf{VLDL - c = \frac{T.G}{5}}$$

Determination of Ghrelin hormone level in blood serum by enzyme linked immunosorbent assay (ELISA)

Plasma ghrelin levels in the experimental mice groups were determined by enzyme-linked immunosorbent assay (ELISA) using a Mice Ghrelin ELISA Kit (Sunlong Biotech,)

Determination of Resistin hormone level in blood serum by enzyme linked immunosorbent assay (ELISA)

Serum resistin levels in the experimental mice groups were determined by enzyme-linked immunosorbent assay (ELISA) using a commercial Mice Resistin ELISA Kit (Sunlong Biotech)

Statistical Analysis

Data were analyzed using SPSS software. Significant differences among the mouse groups were evaluated using Duncan's Multiple Range Test (Bruning & Kintz, 1977): In addition, an unpaired Student's t-test was used to determine statistical significance between the variables, and differences were considered significant at ($P \leq 0.0$).

RESULTS AND DISCUSSION

Effects of Pomegranate Peel Alcoholic Extract on Body Weight and Mean Body Mass Index (BMI) in Experimental Mice Groups.

The results presented in Table (1) showed a significant decrease ($P \leq 0.05$) in the mean body weight of the mice groups (II, III, and IV) treated with the alcoholic extract of pomegranate peel for four weeks at the doses specified in the table, compared to the control group (I). The weight reduction may be attributed to the presence of polyphenols and flavonoids in pomegranate peels, which limit body weight gain and fat accumulation through the regulation of obesity-related hormones (Ramzy, 2019).

Additionally, the results in Table 1 showed that treatment with the alcoholic extract of pomegranate peel led to a significant decrease ($P \leq 0.05$) in the mean body mass index (BMI) of the three experimental mice groups (II, III, and IV) compared to the control group (I): this reduction may be attributed to the effect of the mentioned extract in decreasing the mean body weight of the mice groups. (Al-Darrji et al. 2015) indicated that there is a correlation between body weight and BMI, where obesity rates increase with higher BMI values. These results align with the findings of researchers who reported that pomegranate peels improve lipid profiles.

Table (1): Effect of treatment with pomegranate peel alcoholic extract on the "mean body weight and mean Body Mass Index (BMI)" of the mice groups.

Parameters / Treatments	+Dose Conc. (10g/µg)	Wt (gm)	BMI (g/cm²)
Group I (control)	—	29.11±0.27 D	0.43±0.008 D
Group II	300	23.88±0.30 C	0.37±0.003 C
Group III	400	22.07±0.34 b	0.32±0.008 B
Group IV	500	19.82±030 a	0.27±0.009 A

values followed by different letters vertically indicate a significant difference at ($P \leq 0.05$).
+ values are expressed as Mean ± Standard Error.

Effect of pomegranate peel Alcoholic Extract on serum glucose levels in Experimental Mice groups.

The results in Table (2) showed that oral administration of the pomegranate peel alcoholic extract at doses of 300, 400, and 500 µg/10 g of body weight led to a statistically significant decrease ($P \leq 0.05$) in mean serum glucose levels in the three mouse groups (II, III, and IV) compared to the control group (I): This decrease may be attributed to the fact that the pomegranate peel extract, which is rich in antioxidants, works to lower blood sugar levels by stimulating pancreatic beta cells to increase insulin secretion, thereby regulating blood sugar levels, studies have demonstrated that this effect increases proportionally with the concentration of the extract, research has also indicated that natural antioxidants, particularly phenolic compounds, influence blood glucose levels through the mechanism of inhibiting glucose absorption in the intestines or via its uptake by peripheral tissues (Scalbert, et.al.,2005).

Table (2): Effect of treatment with pomegranate peel alcoholic extract on the mean levels of glucose, total cholesterol, triglycerides, HDL-C, LDL-C, and VLDL-C in experimental mice groups.

Parameters / Treatments	+ Glucose (mg /dl)	Cholesterol (mg /dl)	T.G (mg /dl)	HDL _c (mg /dl)	LDL _c (mg /dl)	VLDL_c (mg /dl)
Group I (control)	118.28±1.89 c	113.56±2.72 c	117.55±2.65 d	43.64±0.85 a	46.42±2.11 D	23.50±0.53 d
Group II	105.08±1.44 b	103.49±1.83 b	108.006±1.57 c	52.38±1.10 b	29.39±1.34 C	21.59±0.31 c
Group III	97.32±2.08 a	96.01±1.98 a	90.06±1.91 b	58.46±0.95 c	20.55±1.60 B	18.25±0.39 b
Group IV	93.76±2.31 a	91.17±1.82 a	72.92±3.76 a	62.66±1.63 d	13.92±1.50 A	14.58±0.75 a

values followed by different letters vertically indicate a significant difference at (P≤0.05).
+ values are expressed as Mean ± Standard Error.

Effect of pomegranate peel Alcoholic Extract on serum Total Cholesterol levels in Experimental Mice groups.
The results in Table (2) showed that oral administration of the pomegranate peel alcoholic extract led to a statistically significant decrease (P≤0.05) in total cholesterol levels in mouse groups II, III, IV compared to the control group I, This decrease is attributed to the fact that the antioxidants present in the extract maintain the activity of the enzymes Cholesterol ester hydrolase and Cholesterol ester synthetase, which work to balance total cholesterol levels, The elevated activity of these two enzymes induces oxidative stress, while a deficiency in antioxidants leads to an increased production of free radicals, (Ramzy, 2019) indicated that pomegranate peels are a rich source of antioxidant phenolic compounds, particularly ellagic acid, which reduces cholesterol by maintaining the activity of the paraoxonase enzyme. A decrease in this enzyme elevates total cholesterol levels and increases the risk of atherosclerosis, (Ramzy, 2019) also indicated that ellagic acid and tannic acid act to prevent lipid peroxidation and scavenge free radicals. Furthermore, this decrease may be attributed to the extract containing compounds that inhibit the enzyme HMG-CoA reductase, which is responsible for cholesterol synthesis. Additionally, this reduction is ascribed to elevated insulin levels and decreased blood glucose, leading to lower lipid levels due to the role of insulin in inhibiting the lipase enzyme in adipocytes (Lei. et al, 2007) (Al-Zorri,2009).

Effect of pomegranate peel Alcoholic Extract on serum Triglyceride levels in Experimental Mice groups.
Treatment with the pomegranate peel alcoholic extract resulted in a statistically significant decrease (P≤0.05) in triglyceride levels in the administered mice groups (II, III, and IV) compared to the control group (I): as shown in Table (2): This decrease is attributed to the effect of the pomegranate peel extract in activating the lipoprotein lipase enzyme, which in turn hydrolyzes triglycerides into fatty acids that are absorbed by adipocytes, The decrease in TG levels may also be attributed to the antioxidant content of the alcoholic extract, which plays an important role in reducing TG levels. Furthermore, this reduction might be due to active compounds in the pomegranate peel extract that function by activating cholesteryl ester transfer protein (CETP) to transport triglycerides to VLDL-c molecules for degradation, thereby lowering triglyceride levels. This finding aligns with several studies, including (Al-Hamdany, N.F. and Al-Flayeh, K.A. 2019): who investigated the effect of sumac fruit extract treatment.

Effect of pomegranate peel Alcoholic Extract on serum HDL-c levels in Experimental Mice groups.
The results in Table (2) showed that administration of the pomegranate peel alcoholic extract led to a statistically significant increase (P≤0.05) in HDL-c levels in the treated mice groups (II, III, IV) compared to the control group (I): This increase is attributed to the antioxidant effect of the Pomegranate peel extract, which reduces oxidative stress and suppresses the activity of the hepatic lipase enzyme, thereby decreasing HDL-c degradation and subsequently elevating its serum levels. Furthermore, this elevation is ascribed to the influence of the pomegranate peel extract components in stimulating intestinal and hepatic cells to synthesize HDL-c particles, which is consistent with several studies, including (Al-Hamdany, N.F.and Al-Flayeh, K.A. 2019).

Effect of pomegranate peel Alcoholic Extract on serum LDL-c and VLDL-c levels in Experimental Mice groups
The results in Table (2) showed that administration of the pomegranate peel alcoholic extract led to a statistically significant increase (P≤0.05) in high-density lipoprotein cholesterol (HDL-c) levels in the treated mice groups (II, III, IV) compared to the control group (I): This significant decrease is attributed to the effect of the pomegranate peel extract on fatty acid metabolism, reducing VLDL-c synthesis in the liver and decreasing LDL-c formation in the bloodstream, Additionally the flavones present in pomegranate peels act to remove LDL-c from the bloodstream by increasing its receptors in the liver and its binding to Apolipoprotein B (Viswanathan et al., 2004).

Additionally, the results showed that administration of the pomegranate peel alcoholic extract led to a statistically significant decrease (P≤0.05) in very low-density lipoprotein cholesterol (VLDL-c) levels in the treated mouse groups (II, III, IV) compared to the control group (I). This reduction is attributed to the extract's effect in increasing the activity of lipoprotein-degrading enzymes, particularly triglycerides, thereby breaking down VLDL-c particles and reducing their levels in the blood serum (Viswanathan et al., 2004).

Effect of pomegranate peel Alcoholic Extract on serum resistin hormone level in Experimental Mice groups.
The results in Table (3) showed that the groups treated with the pomegranate peel extract for four weeks at doses of (300,400, 500) µg/10 g body weight via oral gavage exhibited a statistically significant decrease (P≤0.05) in the mean level of the resistin hormone in the treated mice groups (II, III, IV) compared to the control group (I): Studies have shown that resistin is an adipose tissue–specific hormone associated with increased insulin resistance, diabetes, and obesity. They also indicated that serum resistin levels are closely correlated with glucose and lipid levels, and changes in resistin gene expression and its circulatory levels in cases of obesity are mediated by insulin and glucose (Michael et al., 2004): this decrease in resistin levels may be attributed to the effect of the used extract, which acts to improve insulin sensitivity owing to its antioxidant content (Yaxian et al., 2022).

Table (3): Effect of treatment with pomegranate peel alcoholic extract on mean serum resistin and ghrelin levels in experimental mice groups.

Parameters / Treatments	+Dose Conc. 10g)/µg(	Resistin levels (ng/ml)	Ghrelin levels (ng/ml)
Group I (control)	—	19.78±0.43 d	0.96±0.036 a
Group II	300	18.19±0.23 c	1.13±0.033 b
Group III	400	15.92±0.28 b	1.53±0.035 c
Group IV	500	11.50±0.43 a	1.83±0.034 d

values followed by different letters vertically indicate a significant difference at (P≤0.05).
+ values are expressed as Mean ± Standard Error.

Effect of pomegranate peel Alcoholic Extract on serum Ghrelin hormone level in Experimental Mice groups.
The results in Table (3) showed that the groups treated with the pomegranate peel extract for four weeks at doses of (300,400, 500) µg/10 g body weight via oral gavage exhibited a statistically significant increase (P≤0.05) in the mean level of the ghrelin hormone in the treated mouse groups (II, III, IV) compared to the control group (I): This increase may be attributed to the effect of phenolic compounds present in the extract, which inhibit the enzyme ghrelin O-acyltransferase (GOAT)—the enzyme responsible for converting inactive ghrelin into its active form—thereby leading to the accumulation of un acylated ghrelin in the blood, Additionally, researchers have noted that ghrelin levels are inversely correlated with BMI in both obese and normal-weight individuals (Budak et al., 2006): attributing this increase to a loss of body weight and fat mass. Furthermore, this elevation may be ascribed to the gastric cells responsible for secreting this hormone increasing its production when oxidative stress is reduced by the antioxidant effects of phenolic compounds (Hanafy et al., 2021).

Ghrelin (Hunger Hormone) is a biomarker that signals the brain to induce hunger, classified as the only appetite-stimulating hormone secreted by the gastrointestinal tract into the bloodstream. Elevated levels of ghrelin increase the sensation of hunger, representing a natural physiological response to weight loss and depleted energy stores (Perreault et al., 2004) (Mitoiu, et al., 2024).

In conclusion this study demonstrates that treatment with the alcoholic Extract of pomegranate peel reveals a strong correlation between glucose levels and the resistin hormone, thereby enhancing cellular insulin sensitivity and increasing the body's energy expenditure efficiency. Furthermore, the findings indicate an inverse relationship between Resistin and Ghrelin levels, wherein the

elevation of ghrelin serves as a defensive physiological response to the reduction in total lipids and body weight.

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