The effect of pomegranate leaves powder on biological, biochemical and histological changes of induced obese rats

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Abstract: The present research was conducted on thirty male mature Wistar rats to study the effect of pomegranate leaves powder on Body weight Gain (BWG), Feed Intake (FI) and Feed Efficiency Ratio (FER), Liver Enzymes Activity, serum glucose levels, lipid profile and histological changes of liver in obese rats after 28 days were studied. Thirty male Wister Albino rats were distributed into five equal groups (6 rats each) as follows: group1: negative control, group 2: positive control was fed on high fat diet (obese rats), groups 3, 4 and 5 fed on high fat diet (obese rats) and treated with fed on 1,3 and 5% pomegranate leaves powder of the weight of rats respectively. The results showed that, obese rats (positive control group) had a significant (P<0.05) increase in body weight gain (BWG), feed Intake (FI) and feed efficiency ratio (FER). Compared to group 1 (negative control) by 142.28, 31.33 and 4.10% respectively. Rats were fed on pomegranate leaves powder at (1%, 3% and 5%) significantly (P<0.05) decreased of body weight gain (BWG), feed Intake (FI) feed efficiency ratio (FER), when compared to group 2 (positive control). Obese rats (positive control) had a significant (p<0.05) increase in levels of alanine aminotransferase(ALT) and aspartate aminotransferase (AST) enzymes, serum glucose level, Total cholesterol, Triglycerides, lipoprotein fraction and Atherogenic index comparing to group 1 (negative control group). On the other hand, Feed obese rats on pomegranate leaves powder at (1%, 3% and 5%) significantly (p<0.05) decreased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes, serum glucose level, Total cholesterol, Triglycerides, lipoprotein fraction and Atherogenic index when compared to group 2 (positive control). Histopathological examination of liver sections of rats of pomegranate leaves powder -treated groups showed slight vacuolization of hepatocytes, and focal cytoplasmic vacuolization of some hepatocytes was observed in liver hepatotoxic rats from1, 3 and 5% pomegranate leaves powder respectively.
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Key words: Pomegranate leaves powder, liver functions, lipid profile, phenolic compound histopathology, total cholesterol (TC), triglycerides (TG), atherogenic index (AI).

1. Introduction

Obesity is most common health problems become epidemic on a global scale, especially in the developed countries in the world including Europe, United States of America (USA), and Japan, presenting increase in the risk of morbidity and mortality. Obesity according to The World Health Organization (WHO) defines as an over fat accumulation which influence to human health. Obesity has also been defined as an increased of adipose tissue mass **(Sahib *et al.*, 2012).** Furthermore, obesity has an associated with many diseases like diabetes mellitus type-2, atherosclerotic, cardiovascular diseases, osteoarthritis, hypertension, and some cancers**(Yun 2010).** Obesity is an exceedly complex group of diseases and probably should be characterized as a syndrome. Its results from an imbalance between energy intake and expenditure. Has been reported various of dietary composition are important role in the regulation of metabolic process. So, dietary fat could promote body fat storage more effective than dietary carbohydrate. Thus, inhibition of digestion and absorption of dietary fat is a first key to treating obesity. This inhibition involve pancreatic lipase enzyme, the principle lipolytic enzyme synthesized and secreted by the pancreas. Pancreatic lipase is important enzyme in dietary triacylglycerol absorption, hydrolyzing triacylglycerol to monoacylglycerol and fatty acid. Substrates for lipase enzyme are long-chain triacylglycerol, which are separated from the aqueous medium by the surface phase. Thus, lipase enzyme must be adsorbed on the substrate lipid surface and the nature of the surface of the substrate is an key role for lipase activity **(Roh *et al.*, 2012).** Pomegranate (*Punicagr anatum* L.) is plant can found in Asia, including Himalayas, India, Mediterranean region, South East Asia, and the drier regions of USA. Pomegranate tree grows until over 12 feet, spiny branches, and the leaves are glossy. In India, the pomegranate used as Ayurvedic medicine and is used as treatment of parasite infections, blood tonic, and some gastrointestinal disorders like diarrhea, and peptic ulcer. In traditional Chinese, pomegranate have been used to treat diarrhea,

metabolic acidosis, and microbes infections. The parts of plants including barks, roots, flowers, and leaves have medicinal benefit as well **(Mohammad and Kashani, 2012).** Polyphenolic rich foods have attracted worldwide attention because of their cancer-preventive properties. The pomegranate leaf, like the peels, is rich in polyphenolic compounds including tannins (punicalin, pedunculagan, gallagic acid, ellagic acid and its esters of glucose) and flavonoids. Among the tannins, ellagic acid and punicalgins have aroused great interest, and in recent years most health advantages of the pomegranate have been linked to these tannins **(Lan*et al.*, 2009 and Bialonska *et al.*, 2010).**

The nutritional and antioxidant characteristics of the pomegranate leaves have increased recent interest in their use as a beneficial source of secondary metabolites. They have even been developed into a series of commercial products such as green tea and other teas consumed in Chinese and also been included in nutrition supplement capsules in the USA. Some studies have focused on the effects of pomegranate leaf extracts (PLEs) on obesity. **(Lei *et al.*, 2007)**investigated the anti obesity effects of the pomegranate leaf in a mouse model of HFD-induced obesity. These mice were treated with PLE at a dose of 800 mg/kg for 5 wk. The oral administration of PLE at a dose of800 mg/kg decreased not only the body weight and various adipose pad weight percentages but also serum total cholesterol triacylglycerol, glucose levels, and the total cholesterol/HDL cholesterol ratio. The PLE also decreased the dyslipidemia of obesity and cardiovascular risk factors from a decrease in abdominal fat pad weight percentage compared with control mice. Food intake was also lower in PLE-treated obese mice, similar to sibutramine-treated obese mice. Tannic acid and ellagic acid are thought to be responsible for the activity **(Lei *et al.*, 2007).** The aim of this study was performed to investigate the effect of pomegranate leaves powder 1, 2 and 3% on Body weight Gain (BWG), Feed Intake (FI) and Feed Efficiency Ratio (FER), Liver Enzymes Activity, serum glucose levels, lipid profile and histological changes of liver in obese rats after 28 days.

2. Material and Methods

Animals:

A total number of thirty male albino rats of Wistar strain weighed 180-200g each, were obtained from the experimental Animal Unit of King Fahd Medical Research Center, King Abdul-Aziz University, Jeddah, Saudi Arabia.

Kits for Biochemical Analysis:

Commercial diagnostic kits for estimating serum lipid profile (total cholesterol, triglycerides and lipoprotein fractions) were obtained from Randox Laboratories, U.K. The kits for estimating liver function enzymes Serum aspartate aminotransferase (AST) activity and alanine aminotransferase (ALT) activity were obtained from Diamond Company, Hannover, Germany. Antioxidant enzymes commercial kits were purchased from Roche Diagnostic laboratories, Germany.

Methods:

Preparation of the Basal Diet:

The basal diet for rats was prepared using AIN-93 according to (Reeves *et al*., 1993). the basal diet consists of the following: Protein (Casein) 20%; Sucrose 10%; Corn Oil 4%; Choline chloride 0.2%; Vitamin mixture 1%; Salt mixture 3.5%; Fibers (Cellulose) 5% and the remainder is Corn Starch up to 100%.

Preparation of pomegranate leaves extract:

The leaves of pomegranate was separated, cleaned and dried in oven at 40°C, then powdered in a grinder, then stored in an airtight container at 5°C until further use.

Induction of obesity: Induction of obesity was induced by feeding the rats on basal diets supplemented with 10% animal lipids.

Experimental Design of Rats:

The experiment was performed on thirty male mature Wistar rats. Animals were distributed randomly into five equal groups, six rats each. Rats were housed in standard plastic cages at a room temperature (24± 2 °C), with fixed 12-hour lighting system. All rats were allowed to free access to basal diet and water for one week before starting the experiment for acclimatization. After acclimatization period, the rats were allocated in to the following groups:

Group (1):

Rats were fed on the basal diet only, kept as a negative control group (Cont -ve).

Group (2):

Rats were fed on the basal diet supplemented with 10% animal lipids, kept as a positive control group (Cont +ve). **Group (3):**

Obesity rats were fed on 1% pomegranate leaves powder of the weight of rats.

Group (4):

Obesity rats were fed on 3 % pomegranate leaves powder of the weight of rats.

Group (5):

Obesity rats were fed on 5 % pomegranate leaves powder of the weight of rats.

The experimental will take 28 days. At the end of the experimental period, all rats were fasted overnight then sacrificed. Blood samples were immediately collected from the retro orbital plexus with capillary

tubes under mild ether anesthesia, into clean dried centrifuge tubes. The tubes were then centrifuged at 3000 rpm for 15 minutes. Clear serum samples were carefully separated using Pasteur pipettes, and frozen at - 20°C until biochemical analysis **(Margoni *et al.*, 2011).** The liver were removed by careful dissection and blotted free of adhering blood immediately after sacrificing the rats. The organs were washed with cold saline solution and dried between two filter papers then weighed and they saved for the histopathological examination.

Determination of Feed Intake (FI), Body Weight Gain (DWG) and Feed Efficiency Ratio (FER):

Daily feed intake (FI) per group was calculated throughout the experimental period (14 days). The biological values of different diets were assessed by the determination of body weight gain percent (BWG %) which was calculated at the end of the experimental period as well as feed efficiency ratio (FER) was calculated twice a week, according to the method of **(Chapman *et al.*, 1959)**. Using the following equations:

Body Weight Gain (BWG %) = $\frac{\text{Final body weight.- Initial body weight}}{\text{Initial body weight}}$ x 100.

Feed efficiency ratio was calculated as follows:

Feed efficiency ratio (FER) = $\frac{\text{Gain in body weight (g)}}{\text{Feed consumed (g)}}$

At the end of the experimental period, all rats were fasted overnight then sacrificed. Blood samples were immediately collected from the retro orbital plexus with capillary tubes under mild ether anesthesia, into clean dried centrifuge tubes. The tubes were then centrifuged at 3000 rpm for 15 minutes. Clear serum samples were carefully separated using Pasteur pipettes, and frozen at - 20° C until biochemical analysis **(Margoni *et al.*, 2011)**. The liver was removed by careful dissection and blotted free of adhering blood immediately after sacrificing the rats. The liver was washed with cold saline solution and dried between two filter papers then weighed and they saved for the histopathological examination.

Biochemical analysis:

Total Phenolic Content in pomegranate leaves powder were determined and identified by High-performance liquid chromatography (HPLC) according to the method reported by **(Mattila *et al,.* 2000).**

Total flavonoids and anthocyanins content were determined according to the method described by **(Liu *et al.*, 2009)**,

Serum cholesterol was determined according to the method described by **(Allain *et al.*, 1974)**, using a Spectrophotometer (Thermo Fisher Scientific Genesys, USA) adjusted at 500 nm wave length. The concentration of the sample was calculated from the following equation:

TC concentration (mmol/L)= $\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}}$ x 5.17.

Concentrations of serum triglycerides were determined according to the method described by **(Trinder, 1969)**, using a Spectrophotometer (Thermo Fisher Scientific Genesys, USA) at 500 nm wave length. The concentration of the sample was calculated from the following equation:

TG concentration (mmol/L)= $\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}}$ x 2.25.

Serum high density lipoprotein cholesterol was calorimetrically determined according to the method described by **(Lopes-Virella *et al.*, 1977)**, using a Spectrophotometer (Thermo Fisher Scientific Genesys, USA) at 500 nm wave length. The concentration of the sample was calculated from the following equation:-

HDL-c concentration (mmol/L)= $\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}}$ x 4.65.

Serum low density lipoproteins cholesterol was calorimetrically determined according to the method described by **(Fridewald*et al.*, 1972)**. The concentration of the sample was calculated from the following equation:

LDL-c concentration (mmol/L)= Total cholesterol – ($\frac{\text{TG}}{2.2}$ + HDL-c).

Serum very low density lipoproteins cholesterol was calorimetrically determined according to the method described by **(Fridewald *et al.*, 1972)**. The concentration of the sample was calculated from the following equation:

VLDL-c concentration (mmol/L) = $\frac{\text{Triglycerides}}{2.2}$

Determination of Serum aspartate aminotransferase (AST) activity and alanine aminotransferase (ALT) activity**:**

Serum aspartate aminotransferase (AST) activity and alanine aminotransferase (ALT) activity were

estimated enzymatically based on color reaction formation. The developed color was measured according to the method described by **(Bergmeyer *et al*., 1978)** using a Spectrophotometer (Thermo Fisher Scientific Genesys, USA) adjusted at 505 nm wave length. The concentration was calculated by matching the reading of optical density of concentration of the sample with that of the standard solution.

The Atherogenic index was determined according to the method described by **(Mertz, 1980).**

Serum glucose was measured by enzymatic GOD / POD kits according to the method by **(Trinder, 1969).**

Histopathological Examination:

Specimens from the halves of liver was taken immediately after weighed the organ of the rats and immersed in 10% neutral buffered formalin. The fixed specimens were then trimmed, washed, and dehydrated in ascending grades of alcohol, then cleared in xylene, and stained with Hematoxylin and Eosin (H&E) and examined microscopically according to **(Bancroft and Gamble, 2008)**.

Statistical Analysis:

Statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) for Windows, version 21 (SPSS Inc., Chicago, IL, USA). The obtained data were presented as means ± standard error (SE). Statistical analysis of variance between mean values of different groups was performed using one way ANOVA test followed by the least significant difference (LSD) test to determine the variance between all treatments. Differences were considered significant at $P<0.05$.

3. Results and Discussion

Phenolic Compounds of pomegranate leaves powder:

The quantification of total phenolic, falavonoids, anthocyanin and tannins content of pomegranate leaves (mg/100g sample) were presented in Table 1, it was recorded 15.8, 25.11, 90, 64 and 128.2 respectively.

Identification of phenolic compound in pomegranate leaves powder were determined by HPLC and presented in Table (2). It's clear to mention that the highest amounts with significant difference of phenolic compounds in pomegranate leaves was ellagic acid, while, the lowest one was cinnamic acid. The mean values were 23.47 and 0.10 mg /100g dry weight. On the other hand, pomegranate leaves contain different amount of phenolic compounds with significant difference such as gallic acid, caffeic acid, coumaric acid, chlorogenic acid, catechin and fetulic acid. The mean values were 18.82, 15.26, 12.15, 7.60, 2.31 and 0.40mg/100g dry extract, respectively. While, phloridzin and rutin not detected in pomegranate leaves at their condition.

Table 1. Total phenols, Flavonoids**, anthocyanins and tannins content of pomegranate leaves.**

Total Poyphenol	Total Flavonoids	Total Anthocyanin	Tannins
15.80 ± 2.1	25.11 ± 1.14	90.64 ± 1.50	128.2 ± 1.49

Mean ± SE of triplicate measurement.

Table 2. Identification of Phenolic compound of pomegranate leaves

Compounds	Phenolic compound (mg / 100g dry weight)
Ellagic acid	23.47
Gallic acid	18.82
Caffic Acid	15.26
Coumatic acid	12.23
Chlorogenicacd	7.60
Catechin	3.32
Feurolic acid	0.40
Cinnamic acid	0.10

Effect of pomegranate leaves powder on Body weight Gain (BWG), Feed Intake (FT) and Feed Efficiency Ratio (FER):

Effect of pomegranate leaves powder on Body weight Gain (BWG), Feed Intake (FI) and Feed Efficiency Ratio (FER) in obese rats are presented in Table (3). Group 2 (positive control) had a significant ($p<0.05$) increase in body weight gain (BWG), feed Intake (FI) and feed efficiency ratio (FER) compared to the normal rats group 1 (negative control) by 142.28, 31.33 and 4.10% respectively. Rats were fed on pomegranate leaves powder at (1%, 3% and 5%) significantly ($p<0.05$) decreased of body weight gain (BWG), feed Intake (FT) feed efficiency ratio (FER). when compared to group 2 (positive control). The decreases in body weight gain (BWG) in obese rats were 10.94, 33.46 and 58.75 % for 1%, 3% and 5% pomegranate leaves powder respectively. While, the decreases in feed Intake (FI) in obese rats were 7.23, 21.74 and 23.84 % for 1%, 3% and 5% pomegranate leaves powder respectively. On the other hand, rats were fed with pomegranate leaves powder at 1%, 3% and 5% significantly ($p<0.05$) decreased of feed efficiency ratio (FER) by 3.80, 13.92 and 45.56 % respectively compared to group 2 (positive control). The obtained data are agreement with **(Sayed, 2014).** The reported that the rat fed on high fat diet had significant increase in food consumption, Body weight Gain (BWG) and Feed Efficiency Ratio (FER) compared with the other fed on basal diet (negative control).

Table 3. Effect of pomegranate leaves powder on Body weight Gain (BWG), Feed Intake (FT) and Feed Efficiency Ratio (FER).

Treatments	Body weight Gain (BWG)	Feed Intake (FI)	Feed Efficiency Ratio (FER)
Group 1 Control (-)	$23.56^{d} \pm 0.65$	$19.47^{d} \pm 1.28$	$0.056^{c} \pm 0.006$
Group2 Control (+)	$57.08^{a} \pm 0.72$	$25.57^{a} \pm 1.28$	$0.079^{a} \pm 0.001$
Group 3	$50.83^{b} \pm 0.38$	$23.72^{b} \pm 1.47$	$0.076^{b} \pm 0.003$
Group 4	$37.98^{c} \pm 0.42$	$20.01^{c} \pm 1.31$	$0.068^{c} \pm 0.004$
group5	$23.54^{d} \pm 0.13$	$19.43^{d} \pm 1.26$	$0.043^{d} \pm 0.002$

Data are presented as means ± standard deviation, (n = 6 for each group).
Values with different superscripts within the column are significantly different at $p< 0.05$.
Group 3= obese rate were fed with 1% pomegranate leaves powder, Group 4= obese rate were fed with 3% pomegranate leaves powder, Group 5 =obese rate were fed with 5% pomegranate leaves powder.

Effect of pomegranate leaves powder on Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) in obese rats:

Effect of pomegranate leaves powder on Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) in obese rats are presented in Table (4). Group 2 (positive control) had a significant ($p<0.05$) increase in levels of ALT and AST enzymes comparing to group 1 (negative control) by 196.95% and 309.30 % respectively. On the other hand, rats were fed on pomegranate leaves powder at (1%, 3% and 5%) significantly ($p<0.05$) decreased of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) compared to group 2 (positive control). The decreases in alanine aminotransferase (ALT), in obese rats were 15.03, 35.11 and 52.36 % for 1%, 3% and 5% pomegranate leaves powder respectively. While, the decreases in aspartate aminotransferase (AST)in obese rats were 35.20, 49.19 and 54.81 % for 1%, 3% and 5% pomegranate leaves powder respectively. Serum Alanine Aminotransferase enzyme (ALT) and Aspartate Aminotransferase (AST) are a sensitive indicators of liver damages. **(Al-Mamary*et al.*, 2002).**

Table 4. Effect of pomegranate leaves powder on Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) in obese rats.

Treatments	ALT (U/L)	AST (U/L)
Group 1 Control (-)	$32.15^{e} \pm 1.90$	$20.97^{e} \pm 1.28$
Group2 Control (+)	$95.47^{a} \pm 2.13$	$85.83^{a} \pm 1.13$
Group 3	$81.12^{b} \pm 1.26$	$55.61^{b} \pm 1.25$
Group 4	$61.95^{c} \pm 2.16$	$43.15^{c} \pm 1.47$
Group 5	$45.48^{d} \pm 3.15$	$30.20^{d} \pm 1.70$

Data are presented as means ± standard deviation, (n = 6 for each group).
Values with different superscripts within the column are significantly different at $P< 0.05$.
Group 3= obese rate were fed with 1% pomegranate leaves powder, Group 4= obese rate were fed with 3% pomegranate leaves powder, Group 5 =obese rate were fed with 5% pomegranate leaves powder.

Effect of pomegranate leaves powder on glucose, Total cholesterol and Triglycerides in obese rats:

Effect of different ratio of pomegranate leaves powder on the serum glucose levels Total cholesterol and Triglycerides in obese rats is illustrated in Table (5).

Effect of different ratio of pomegranate leaves powder on the serum glucose levels in obese rats. The group 2 (positive control) had a significant ($p<0.05$) increase in serum glucose level compared to group 1 (negative control) by 143.15 %. Rats were fed on pomegranate leaves powder at (1%, 3% and 5%) significantly ($p<0.05$) decreased serum glucose level when compared to group 2 (positive control group). The decreases in serum glucose level in obese rats were 28.80, 39.25 and 48.55 % for 1%, 3% and 5% pomegranate leaves powder respectively.

These results are in agreement with that of **(Jafri *et al.*, 2000).** Who found that oral intake of aqueous ethanolic extracts of *Punicagr anatum* leaves led to significant blood glucose lowering effect in normal glucose fed hyperglycaemic and alloxan induced diabetic rats. Also **(Enas and Khalil, 2004),** found that the diabetic rats treated with aqueous of pomegranate leaves for 4 weeks displayed significantly lowered blood glucose level and augmentation in insulin level.

Effect of pomegranate leaves powder on Total cholesterol and Triglycerides in obese rats are illustrated in Table (5). Results of biochemical analyses revealed that group 2 (positive control) had a significant (p<0.05) increased in total cholesterol by 128.76 % and triglycerides by 96.77 % compared to normal rats (negative control). Rats were fed with pomegranate leaves powder at 1%, 3% and 5% significantly (p<0.05) decreased of serum total cholesterol by 36.56, 50.49 and 53.86 % respectively compared to group 2 (positive control).

Obese rats were fed with 1%, 3% and 5% pomegranate leaves powder significantly (p<0.05) reduced serum triglyceride levels by 31.72, 45.98 and 49.63 % respectively when compared to group 2 (positive control).

Effect of pomegranate leaves extract and pomegranate leaves powder on lipoprotein fraction in obese rats:

Effect of pomegranate leaves powder on high density lipoprotein cholesterol HDLc low density lipoprotein cholesterol LDL_c and very low density lipoprotein cholesterol $VLDL_c$ in obese rats are presented in Table (6). The group 2) positive control) had a significant (p< 0.05) increase in HDL-c, LDL_c and $VLDL_c$ compared to the normal rats (negative control) by 31.40 %, 252.01 and 87.00 respectively. On the other hand, rats were fed on pomegranate leaves powder at (1%, 3% and 5%) significantly (p<0.05) decreased of high density lipoprotein cholesterol HDLc low density lipoprotein cholesterol LDL_c and very low density lipoprotein cholesterol $VLDL_c$ compared to group 2 (positive control). The decreases in high density lipoprotein cholesterol HDLc in obese rats were 5.33, 8.37 and 10.94 % for 1%, 3% and 5% pomegranate leaves powder respectively. While, the decreases in low density lipoprotein cholesterol LDL_c in obese rats were 50.55, 75.84 and 81.65 % for 1%, 3% and 5% pomegranate leaves powder respectively. On the other hand, rats were fed on pomegranate leaves powder at (1%, 3% and 5%) decreased of very low density lipoprotein cholesterol $VLDL_c$ by 25.43, 27.87 and 36.38% respectively. These results are in agreement with the finding of **(Aviram *et al.*, 2008).** They reported that serum total lipids, triglycerides, total cholesterol, LDL-c and VLDL-c were significantly higher in rats fed on high fat diet (positive control) group.

Table 5. Effect of pomegranate leaves powder on glucose, Total cholesterol and Triglycerides in obese rats.

Treatments	Glucose level (mg/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)
Group 1 Control (-)	92.82^{e} ± 3,83	68.35^{d} ± 4.74	64.11^{d} ± 3.24
Group2 Control (+)	225.70^{a} ± 5.31	156.36^{a} ± 5.18	126.15^{a} ± 2.61
Group 3	160.13^{b} ± 13	99.20^{b} ± 4.23	86.13^{b} ± 4.38
Group 4	137.11^{c} ± 2.56	77.41^{c} ± 4.26	68.15^{c} ± 2.15
Group 5	116.12^{d} ± 1.83	72.15^{c} ± 2.77	64.80^{d} ± 3.72

Data are presented as means ± standard deviation, (n = 6 for each group).
Values with different superscripts within the column are significantly different at P< 0.05.
Group 3= obese rate were fed with 1% pomegranate leaves powder, Group 4= obese rate were fed with 3% pomegranate leaves powder, Group 5 =obese rate were fed with 5% pomegranate leaves powder.

Table 6. Effect of pomegranate leaves extract and pomegranate leaves powder on lipoprotein fraction in obese rats.

Treatments	HDL_c(g/dl)	LDL_c (g/dl)	$VLDL_c$ (g/dl)
Group 1 Control (-)	33.47^{d} ± 2.60	25.62^{c} ± 1.74	14.39e ± 0.78
Group2 Control (+)	43.98^{a} ± 4.50	89.48^{a} ± 1.77	26.91^{a} ± 0.73
Group 3	41.50^{b} ± 5.11	44.24^{b} ± 1.75	20.25^{b} ± 0.21
Group 4	40.14^{bc} ± 4.47	21.62^{c} ± 1.75	19.41^{c} ± 0.60
Group 5	39.17^{d} ± 4.28	16.42^{d} ± 1.87	17.12^{d} ± 0.51

Data are presented as means ± standard deviation, (n = 6 for each group).
Values with different superscripts within the column are significantly different at P< 0.05.
Group 3= obese rate were fed with 1% pomegranate leaves powder, Group 4= obese rate were fed with 3% pomegranate leaves powder, Group 5 =obese rate were fed with 5% pomegranate leaves powder.

Effect of pomegranate leaves powder on atherogenic index:

Effect of different ratio of pomegranate leaves powder on Atherogenic index (AI) in obese rats are shown in Table 7. Group 2 (positive control) had a significant (P<0.05) increase in Atherogenic index (AI) in obese rats compared to the group 1 (negative control) by 70.51 %. Rats were fed on pomegranate leaves powder at (1%, 3% and 5%) significantly (P<0.05) decreased atherogenic index (AI) compared

to group 2 (positive control). The decreases in atherogenic index (AI) in obese rats were 27.03, 41.89 and 48.11 % for 1%, 3% and 5% pomegranate leaves powder respectively. These results are in agreement with the finding of **(Aviram *et al.*, 2008)**. They described how pomegranate powder inhibited atherogenic modification of LDL including its retention oxidation and aggregation. The antiatherogenicity capability of pomegranate powder is related to 3 components of atherosclerosis plasma lipoproteins, arterial macrophages and blood platelets.

Table 7. Effect of pomegranate leaves powder on Atherogenic index (AI) in obese rats

Treatments	Atherogenic index (AI)
Group 1 Control (-)	**$2.17^{c} \pm 1.42$**
Group2 Control (+)	**$3.70^{a} \pm 1.15$**
Group 3	**$2.70^{b} \pm 1.30$**
Group 4	**$2.15^{c} \pm 1.63$**
Group 5	**$1.92^{d} \pm 1.85$**

Data are presented as means ± standard deviation, (n = 6 for each group).
Values with different superscripts within the column are significantly different at P< 0.05.
Group 3= obese rate were fed with 1% pomegranate leaves powder, Group 4= obese rate were fed with 3% pomegranate leaves powder, Group 5 =obese rate were fed with 5% pomegranate leaves powder.

Histopathological changes:

Microscopically, liver of healthy rat from control (-) group 1 showing the normal histological of hepatic lobule (Fig.1). Meanwhile, examined liver of hepatotoxic rat from control (+) group 2 showing congestion of central vein and hepatic sinusoids (Fig. 2).

Liver of hepatotoxic rats from pomegranate leaves powder 1% diet group 3 showing slight vacuolization of hepatocytes (Fig. 3). Also, liver of hepatotoxic rats from pomegranate leaves powder 3% diet group 4 showing slight vacuolization of hepatocytes (Fig. 4). On the other hand, focal cytoplasmic vacuolization of some hepatocytes was observed in liver hepatotoxic rats from pomegranate leaves powder 1% group 5 (Fig. 5).

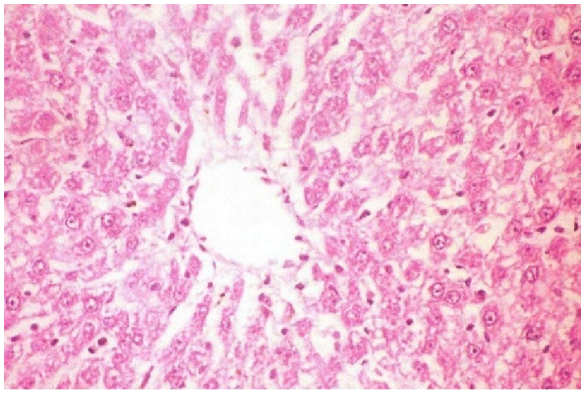

Fig. 2. Liver of hepatotoxic rat from control (+) group 2 showed kupffer cell activation, cytoplasmic vacuolization of hepatocytes and atrophy of some hepatocytes

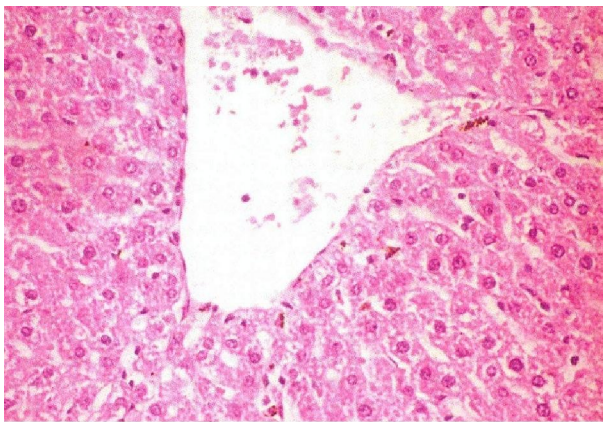

Fig. 1. Liver of healthy rat from control (-) group 1 revealed the normal histology of hepatic Lobule

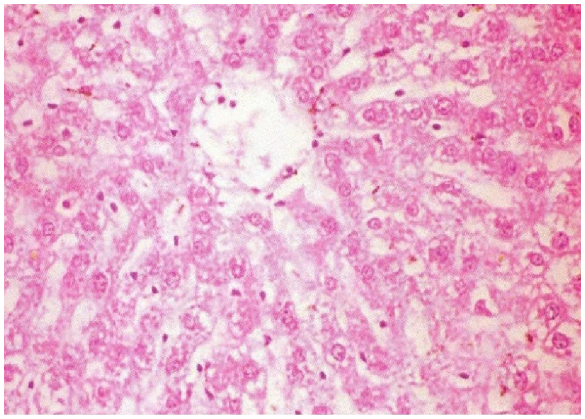

Fig. 3. Liver of hepatotoxic rat from 1% pomegranate leaves powder rat from group 3 showing slight cytoplasmic vacuolization of hepatocytes

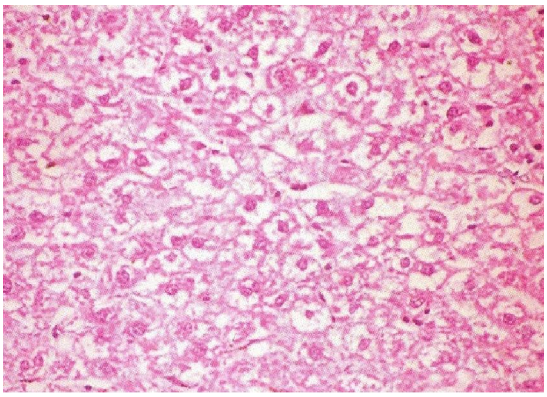

Fig. 4. Liver of hepatotoxic rat from 3% pomegranate leaves powder rat from group 4 showing focal cytoplasmic vacuolization of some hepatocytes

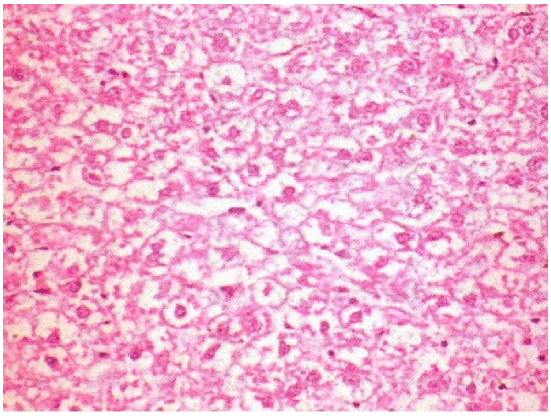

Fig. 5. Liver of hepatotoxic rat from 5% pomegranate leaves powder rat from group 5 showing focal cytoplasmic vacuolization of some hepatocytes

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