EFFECT OF POMEGRANATE (PUNICAGRANATUM L.) (ARILS, PEELS AND SEEDS) EXTRACTS ON FATTY LIVER IN RATS

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ABSTRACT

Objectives: This study aimed to investigate the ameliorative effect of pomegranate extracts (arils, peels, and seeds) on nonalcoholic fatty liver disease in rats. Methods: The nonalcoholic fatty liver disease was induced by methionine choline deficient (MCD) diet. Different pomegranate extracts (arils, peels, and seeds) were administered orally at dose 5ml/kg/b.wt./day. Rats were divided into 2 groups. Group I healthy group were fed on standard diet and group II fatty liver group were fed on MCD diet. After a month of the experiment, Group I was subdivided into 4 groups as follows: G1 healthy control, G 2 pomegranate juice (healthy PJ), G 3 pomegranate peels extract (healthy PPE), and group 4 pomegranate seeds extract (healthy PSE). Also Group II was subdivided into 4 groups included: G5 (fatty liver control group), G6(fatty liver + PJ), G7(fatty liver + PPE) and G8(fatty liver + PSE). Results: The results indicated that MCD diet induced significantly hyperlipidemia, significantly elevated nitric oxide concentration and malondialdehyde level, and also significantly depletes reduced glutathione content, catalase, paraoxonase and arylesterase enzymes activities in liver. Moreover MCD diet significantly raised plasma pentaxin-3 and cytokeratin-18 concentrations and significantly elevated liver enzymes. However pomegranate extracts (PJ, PPE and PSE) significantly reduced hyperlipidemia, significantly depleted the elevation of oxidative stress markers and also significantly raised the antioxidants. Moreover, pomegranate extracts improved the liver function and significantly depleted markers for cell death. The results were confirmed by liver histopathology In conclusion: Pomegranate extracts administration attenuated the severity of nonalcoholic fatty liver disease in rats.
KEYWORDS: Pomegranate, nonalcoholic fatty liver disease, oxidative stress, antioxidant, hepatoprotective.

INTRODUCTION
Nonalcoholic fatty liver disease (NAFLD) is a condition in which excess fat accumulates in the liver of a patient without a history of alcohol abuse. The prevalence of NAFLD in the general population ranges from 13 to 15% increasing in subjects with diabetes and with severe obesity and has been reported to range from 25 to 75% or even higher. Nonalcoholic fatty liver disease (NAFLD) is a common disorder, and it is considered as an asymptomatic disease that is typically identified when the liver enzymes alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT) are elevated.[1,2]

In assessing disease severity and risk of progression to cirrhosis, it is useful to divide NAFLD into two categories: nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). The difference between the two entities is histologic. In NASH, there is the presence of hepatic inflammation in contrast to NAFL, which involves only steatosis. NAFL and NASH occur as part of a continuum in which the histology is not exclusively steatosis or steatohepatitis. As one approach to defining the extent and severity of disease, an NAFLD activity score (NAS) has been developed, which assigns numerical values to various histologic measures of steatosis, inflammation, cell injury, and fibrosis.[3] The resulting cumulative score can be used to classify patients as having NAFL, borderline NASH, or fully developed NASH. The distinction between NAFL and NASH is important, since patients with NASH are much more likely to progress to clinically significant cirrhosis, portal hypertension, and liver failure. When cirrhosis develops in the context of NAFLD, there is also a several-fold increased risk of hepatocellular carcinoma.[1,2]

Much of the increase in prevalence of NAFLD is driven by its epidemiologic and pathophysiologic links to oxidative stress-mediated non-communicable diseases including type 2 diabetes mellitus (T2DM) and obesity. Oxidative stress develops when there is an imbalance between reactive oxygen species (ROS) and antioxidants that scavenge oxidative insults. ROS are involved in the etiology of NAFLD by stimulating glutathione depletion, accumulation of lipid peroxides, and oxidative damage of different organelles in liver tissue. Glutathione is the major intracellular antioxidant in hepatocytes and plays an important role in maintaining the reduced cellular homeostasis in hepatocytes, and this is essential for optimum activities of several antioxidant enzymes including catalase.[4] Paraoxonase (PON1)
and arylesterase are antioxidant enzymes closely associated with high-density lipoproteins. They are detoxify lipid peroxides, and are widely distributed in many tissues, including the liver.\[^5\]

Limited evidence exists, which suggests that antioxidant supplements may have a role in preventing or treating NAFLD patients.\[^2\]

The human health benefits of pomegranate (*Punicagra natum L.*) fruit are numerous, and it is used worldwide as a medicinal functional food. Different extracts prepared from pomegranate fruit, such as juice, peels extract, and seeds extract are reported to exhibit strong antioxidant activity.\[^6\]

Pomegranate juice (PJ) is rich in antioxidants of the polyphenolic class which includes tannins and anthocyanins. The antioxidant level in PJ was found to be higher than that in other natural juices such as blueberry, cranberry and orange. Pomegranate peels extract (PPE) is rich in bioactive compounds such as polyphenols, anthocyanidins, tannic acid, gallic acid, and ellagic acid.\[^7,8\]

In turn, seeds contain naringenin and catatheine as major flavonoid compounds, gallic acid as a major phenolic acid, protochatic acid as a second major phenolic acid, and trace amounts of vanillic acid, coumaric acid, ferulic acid, caffeic acid, ellagic acid, and cyaniding.\[^9\]

Recent in vivo, in vitro, and epidemiological studies have shown few medicinal properties of different extracts prepared from pomegranate fruit as an antioxidant\[^6\], against colon cancer\[^10\], type 2 diabetes mellitus (T2DM)\[^11\], and inflammatory mediated diseases.\[^12\]

Also, pomegranate fruit extracts possesses radical scavenging properties in diethylnitrosamine-induced liver injuries, reversed methotrexate toxicity in the liver by decreasing oxidative stress and liver apoptosis, and enhanced the activity of liver enzymes against ROS after CCl4 toxicity.\[^13,14\]

This study aims to explore the potential role of pomegranate extracts (arils, peels, and seeds) on nonalcoholic fatty liver disease in MCD-fed rats. This objective is supported by the rationale that in previous publications, pomegranate extracts ameliorated hepatic oxidative stress and decreased serum lipids in experimental animal models. Therefore, it is important to elucidate the role of pomegranate extracts (arils, peels, and seeds), as a strong antioxidant, in preventing dietary-induced NAFLD.
MATERIALS AND METHODS

1. MATERIALS

1.1. Plant Materials

The pomegranate (Punica granatum L.) fruits were obtained from Ministry of agriculture, Giza, Egypt. The pomegranate extracts were prepared as follows:

1.1.1 Preparation of Pomegranate Juice:

The fresh pomegranate fruits, free of blemishes, or obvious defects were washed and stored at 4°C until use. The fruits were manually peeled, without separating the seeds. Pomegranate juice was obtained by squeezing using a commercial blender (Moulinex, France) and was filtered to remove the residue. The juice was used within 1 h after squeezing and filtration. PJ was administered orally to each rat at a dose calculated as 5 mL/kg body weight per day.\(^{15}\)

1.1.2. Preparation of Pomegranate Peels' Extract

Pomegranate peels were separated from the fruit manually and were cut into small pieces (2 cm x 2 cm). The cut pieces were air-dried. The -dried samples were ground into fine powder by a grinder (Moulinex, Grinder, France) and stored at -20°C until used. The PPE water extract was prepared weekly by mixing dry powder with distilled water (12.5 g dry solids/100 mL) and kept at 4°C until used.\(^{16}\) PPE was administered orally to each rat at a dose calculated as 5 mL/kg body weight per day.\(^{15}\)

1.1.3. Preparation of Pomegranate Seeds' Extract

The seeds were separated from juice and washed carefully to remove sugars and other adhering materials. The separated seeds were air-dried. The dried samples were ground into fine powder by a commercial grinder (Moulinex, Grinder, France). Thereafter, 500 g of pomegranate seed powder were extracted in ethanol (from Science and Technology Center, Egypt) by Soxhlet apparatus.\(^{17}\) The ethanol was completely evaporated (rotary vacuum evaporator, Heidolph, Germany) at 40°C. The PSE was stored in dark place at -20°C until use. PSE was administered orally to each rat at a dose calculated as 5 mL/kg body weight per day.\(^{15}\)

1.2. Animals

The experimental animals used through this study were one hundred healthy adult male albino rats weighing between 180 – 200g and were obtained from National Research Center (NRC), Giza, Egypt. The animals were maintained on standard diet\(^{18}\) and tape water \textit{ad libitum} and kept individually in stainless steel cages in constant environmental conditions.
1.3. Diet
- Balanced diet prepared according to American Institute of Nutrition AIN-93.[18]
- Methionine choline deficient (MCD) diet prepared according to Nagai[19] for the induction of NAFLD in animal.

1.4. Chemicals
- Methionine and choline were obtained from the Al-Gomhouria Company, Cairo, Egypt.
- Kits used for the determination of biochemical measurements were obtained from Biodiagnostic company, Egypt.
- Paraoxonase, arylesterase, plasma pentraxin-3, and cytokeratin-18 kits were obtained from Sigma Chemical Company, Cairo, Egypt.

2. METHODS
2.4. Determination of the bioactive components derived from different pomegranate extracts (arils, peels, and seeds)
High Performance Liquid Chromatography (HPLC) was performed to identify the phenolic compounds and flavonoids content in pomegranate extracts (arils, peels, and seeds).

2.5. Experimental design
One hundred adult male albino rats were used in this study. After adaptation, animals were divided into 2 groups as follows:

**Group I (Healthy Group):** Rats in this group were fed on standard diet for a 1 month.

**Group II (Fatty Liver Group):** Rats in this group were fed on MCD diet (for the initiation of NAFLD) for a 1 month.

After a month of the experiment, 10 rats from each group were sacrificed for biochemical and histopathological analysis. Liver were separated, cleaned and stored frozen at -20°C until used for issue biochemical analysis. Another portion of the liver was kept in 10% formalin for histopathological examination.
In the second month, 40 rats from each groups I and II were subdivided into 8 groups (10 rats/group) as follows:

- **Group (1) (Healthy Control):** rats in this group were continued fed on balanced diet and orally administered distilled water to the end of the experiment at dose 5ml/kg b.wt/day.
- **Group (2) (Healthy PJ):** rats in this group were continued fed on balanced diet and orally administered PJ to the end of the experiment at dose 5ml/kg b.wt/day.
- **Group (3) (Healthy PPE):** rats in this group were continued fed on balanced diet and orally administered PPE to the end of the experiment at dose 5ml/kg b.wt/day.
- **Group (4) (Healthy PSE):** rats in this group were continued fed on balanced diet and orally administered PSE to the end of the experiment at dose 5ml/kg b.wt/day.
- **Group (5) (Fatty liver control):** rats in this group were continued fed on MCD diet and orally administered distilled water to the end of the experiment at dose 5ml/kg b.wt/day.
- **Group (6) (Fatty liver + PJ):** rats in this group were continued fed on MCD diet and orally administered PJ to the end of the experiment at dose 5ml/kg b.wt/day.
- **Group (7) (Fatty liver + PPE):** rats in this group were continued fed on MCD diet and orally administered PPE to the end of the experiment at dose 5ml/kg b.wt/day.
- **Group (8) (Fatty liver + PSE):** rats in this group were continued fed on MCD diet and orally administered PSE to the end of the experiment at dose 5ml/kg b.wt/day.

2.6. **Blood sample collection**

At the end of experimental period, all rats were scarified after for 12 hours fasting with water *ad libitum*. Blood samples were collected in heparinized tubes. Then centrifuged at 4000 rpm for 10 minutes to separate plasma and kept in plastic vials at -20°C until used for biochemical analyses.

2.7. **Tissue sampling**

Liver was separated and cleaned, rinsed and washed by saline solution then plotted on filter paper to remove water residue. Part of the liver was stored frozen at -20°C until used for biochemical analyses. Another portion of the liver was kept in 10% formalin for the purpose of histopathological examination.

2.8. **Biochemical measurements**

2.8.1. **Assessment of Lipid Profile**

Lipid profile measured in liver tissue included cholesterol level[^21], HDL level[^22], LDL[^23] level, and triacylglycerol level[^24].
2.8.2. Assessment of Oxidative Stress Markers
Oxidative stress markers measured in liver tissues included nitric oxide (NO) concentration[25], and malondialdehyde (MDA) level.[26]

2.8.3. Assessment of Paraoxonase (PON-1) and Arylesterase (ARE) activities
The activities of paraoxonase (PON-1), and arylesterase(ARE) were measured by ELISA in liver tissues according to Hang[27] and Nus[28] respectively.

2.8.4. Assessment of Antioxidant Markers
Antioxidant markers measured in liver tissues included reduced glutathione (GSH) content[29] and catalase activity (CAT).[30]

2.8.5. Assessment of Liver Function
The liver function tests included the determination of alanine aminotransferase (ALT)[31] and γ-glutamyltransferase (γ-GT)[32] activities.

2.8.6. Assessment of Liver Apoptosis Markers
Markers of cell death measured in plasma included plasma pentraxin-3 (PTX-3)[33], and cytokeratin-18 (CK-18)[34] concentrations.

2.9. Liver Histopathological Examination
Liver morphology was assessed by light microscopy. Part of the liver was sliced and tissues were fixed in 10% buffered-neutral formalin for 6 hours. Fixed liver tissues were processed and embedded in paraffin. Sections of 4 mm in thickness were subjected to Haemtoxylin and Eosin (H&E) staining before examination.

2.10. Statistical analysis
The data were statistically analyzed by Statistical Package for Social Science (SPSS) version 17.0 statistical packages. Values were presented as mean ± standard deviation (S.D.). Statistical differences between groups were performed using one way ANOVA, the mean difference was significant at the (p≤0.05) level.

3. RESULTS
3.4. Bioactive components derived from pomegranate extracts (arils, peels, and seeds)
Figures (1), (2), (3), and (4) show the percentage of the main phenolic compounds and flavonoids in different pomegranate extracts (arils, peels, and seeds). We found that total
phenolic compound in PJ, PPE, and PSE were 5.57, 206.09, 691.23µg GAE/100g extract, respectively while total flavonoid were 851.47µg CE/100g extract in seed extract only (table 1) illustrates the total phenols and flavonoids found in different pomegranate extracts (arils, peels, and seeds).

3.5. Effect of Pomegranate Extracts (PJ, PPE, and PSE) on Lipid Profile

When we looking for the tables (2) and (3) it could be observed the fatty liver control groups (group II and group 5) showed significant (P<0.05) elevation in total cholesterol by 12.1% and 61.1%, significant (P<0.05) increased in triacylglycerol by 63.34% and 104.96%, as well as a marked increment in low density lipoprotein (LDL) by 11.96% and 94.23% associated with significant reduction in high density lipoprotein (HDL) by 13.1% and 40.94% in liver tissue respectively comparing with healthy control group.

Whereas, all treated groups with pomegranate extracts (G6, G7, G8) resulted significant (P<0.05) improvement in this parameters compared with fatty liver control group (G5).

3.6. Effect of Pomegranate Extracts (PJ, PPE, and PSE) on Oxidative Stress Status

Tables (4) and (5) reveal the effect of pomegranate extracts on the oxidative stress status of fatty liver control rats. After a month on MCD diet liver tissue of rats were observed and evidenced significant elevation of free radicals in fatty liver group (GII) by increased lipid peroxidation. The elevation in lipid peroxidation exemplified by MDA content as a result of NAFLD reached 83.31% in liver tissue, likewise, NO content in group II was significantly increased by 7.79% as compared to healthy control group (G1). Also, there was significant elevation in MDA and NO levels by double fault in fatty liver animals (G5) by 150.45% and 52.16% respectively. When compared with healthy control group (G1).

Oral administration of the pomegranate extracts as treatment showed significant (P<0.05) reduction in the levels of all oxidative stress markers when compared with G5.

3.7. Effect of Pomegranate Extracts (PJ, PPE, and PSE) on Antioxidant Status

Tables (6) and (7) showed that MCD diet which caused hyperlipidemic and increase oxidative stress markers also caused dramatically reduction in antioxidants markers in liver tissue. The non-enzymatic antioxidant (GSH) was significantly (P<0.05) decreased in GII and G5 by 6.59% and 79.72% respectively as compared to the healthy groups. However, oral administration of different pomegranate extracts (PJ, PPE, and PSE) where able promptly
correct this demotion in group 6, 7, and 8 by 573.77%, 694.67%, and 2168.24% respectively as compared to group 5.

Likewise the catalase (CAT) activity was also significantly (P<0.05) depleted by 14.37% and 47.72% in GII and G5 respectively in comparison with healthy control groups. On the other hands, groups 6, 7, and 8 exhibited significant (P<0.05) increase in the catalase activity by 62.6%, 91.1%, and 593.95% respectively as compared to group 5 (table (6)).

Table (4) demonstrates that MCD diet elevates lipid peroxidation as well as increased oxidative stress status. The PON-1 activity was significantly (P<0.05) depletes in GII and G5 as compared to healthy groups by 22.79% and 37.65% respectively. Also the ARE activity by 0.6% and 13.1%.

In the second month, all treated groups (G6, 7, and 8) with pomegranate extracts show improvement in the activity of the PON-1 and ARE.

3.8. Effect of Pomegranate Extracts (PJ, PPE, and PSE) On Liver Function

Tables (8) and (9)illustrated that animal fed MCD diet caused very significant (P<0.05) elevation in liver enzymes activity ALT and GGT by 533.88% and 67.54% respectively in G5 as compared to healthy control group (G1). Whereas, oral administration of pomegranate extracts (PJ, PPE, and PSE) was significantly (P<0.05) reduced liver enzymes activities in all treated groups by 63.63%, 63.84%, and 71.44% respectively for ALT and 4.66%, 29.01%, and 38.22% respectively for GGT in comparison with fatty liver control group (G5).

3.9. Effect of Pomegranate Extracts (PJ, PPE, and PSE) on Liver Apoptosis Biomarkers

Tables (10) and (11) demonstrate the effect of MCD diet on the liver apoptosis biomarkers. Our results revealed that animals fed on MCD diet showed a significant elevation (P<0.05) in PTX-3 by 15.38% and 32.22% for GII and G5 respectively. In addition to increase in CK-18 by 18.92% and 28.12% for the same groups as compared to the healthy control groups.

Oral administration of Pomegranate extracts (PJ, PPE, and PSE) demonstrated very significant (P<0.05) improvement in PTX-3 by 16%, 16.67%, and 26% respectively. Similarly our results show a significant (P<0.05) decreased in CK-18 by 19.01%, 22.4%, and 27.96% for groups 6, 7, and 8 respectively as compared to fatty liver control group (G5).
These results were significantly (P<0.05) reduced after pomegranate extracts oral administration in all treated groups.

3.10. Histopathological Examination of The Liver
General observation of the livers from the healthy control groups (figures 5 and 7) and healthy pomegranate extracts (PJ, PPE, and PSE) groups showed normal red, smooth, and shiny appearance. On the other hand, livers obtained from fatty liver control group appeared enlarged and yellowish with yellow spots, while livers obtained from treated groups (G6, G7 and G8) showed red brownish livers with smooth surfaces like that of the healthy control group.

The effect of different pomegranate extracts (PJ, PPE, and PSE) on liver of healthy and NAFLD was assessed by histopathological examination (figures 8-10 and 12-14). Healthy control and healthy pomegranate extracts (PJ, PPE, and PSE) groups showed no detectable pathological changes. Yet, in liver of fatty liver control group (GII (figure 6) and G5 (figure 11)), showed fatty changes of hepatocytes (hepatic steatosis), portal infiltration with leucocytic inflammatory cells, vacuolation of hepatocytes, congestion of central veins and hepatic sinusoids, marked dilatations and congestion of hepatic portal blood vessels, whereas, different pomegranate extracts treatment (G6, G7, and G8) resulted in notable improvement with almost normal histology of the hepatic lobule. Table (12), illustrated the degree of congestion, steatosis, inflammation and necrosis found in each group.

4. DISCUSSION
4.1. Bioactive components derived from pomegranate extracts (arils, peels, and seeds)
Pomegranate extracts contain many bioactive components with varying amounts. Gallic acid, ferulic acid, cinnamic acid and sinapic acid are the main phenolic compounds found in PJ\textsuperscript{[35,36]} and PPE\textsuperscript{[37,38]} Whereas, Gallic acid, rosmarinic acid, coumarin, syringic acid, ferulic acid, cinnamic acid, protocatueic acid, vanillic acid, chlorogenic acid, sinapic acid and caffeic acid are the main phenolic compounds found in PSE\textsuperscript{[9]}, while, Catechin is the main flavonoid compound found in the same extract. PSE was found to contain the higher concentration of bioactive components then PPE and finally PJ. The presence of these bioactive components has been associated with decreased oxidative stress and injury in rats groups administered MCD diet\textsuperscript{[6]}. 
4.2. Effect of Pomegranate Extracts (Arils, Peels, and Seeds) on Lipid Profile

An abnormal increase of lipids and lipoproteins such as serum triacylglyceride, total cholesterol and low-density lipoproteins is considered as a prime risk factor for many diseases as NAFLD.\textsuperscript{39}

We used a diet induced model of NAFLD, which is similar to the disease development in humans. The use of MCD diet is considered as the main model used for studying NASH.\textsuperscript{19} The accumulation of fat in the liver resulting from choline deficiency occurs because choline is required to make the phosphatidyl-choline portion in VLDL (very low density lipoprotein) particles.\textsuperscript{40} In the absence of choline, VLDL is not secreted and triacylglycerol builds up in the liver cytosol.

Our results demonstrated that rats fed on MCD diet (G5) showed a significant (P<0.05) increased in total cholesterol, triglycerides and low density lipoprotein LDL associated with decreased in high density lipoprotein HDL comparing with healthy control group.

On the other hand, all tested lipid parameters had highly significant (P<0.05) improvement for all treated fatty liver groups administrated with different pomegranate extracts (PJ, PPE and PSE) comparing with fatty liver control group (G5). Pomegranate extracts are a rich source of polyphenols and other antioxidants.\textsuperscript{7} Polyphenolic compounds was showed to improve the serum lipid profile, this is might be due to it possesses a powerful antioxidant activity and it is able to reduce lipid peroxidation. The polyphenols of pomegranate may accelerate and promote cholesterol metabolism by reversing cholesterol transport via HDL.\textsuperscript{41} Perhaps the greatest mechanism of pomegranate extracts (which include Gallic acid, tannic acid and other polyphenolic compounds that combating cholesterol is associated with an enzyme known as paraoxonase (PON), paraoxonase is an HDL-associated enzyme whose activity is related to cholesterol and atherosclerosis, decreased activity of PON is associated with increase cholesterol and increase risk for atherosclerosis, hypocholesterolemic properties of PON may be related to its ability to protect against lipid peroxidation.\textsuperscript{42} So, the effects of different Pomegranate extracts on the lipid profile may be attributed to its antioxidant activity.

The result came compatible with - Esmailzadeh et al\textsuperscript{43} who reported that consumption of concentrated pomegranate juice for type II diabetic patient with hyperlipidemia caused significant reduction were seen in serum total cholesterol and low density lipoprotein LDL.
and showed a significant increase in HDL-cholesterol. So, pomegranate extracts had an ameliorative effect on NAFLD by reducing cholesterol, triacylglycerols, and LDL and increasing HDL due to the presence of antioxidants.\textsuperscript{[15]}

Also, in previous study evaluating the hypocholesterolemic activities of Gallic acid, in cholesterol- fed rabbits, it was reported that the mechanism of action of Gallic acid is due to decrease of the intestinal absorption (25–75\textper.cent.).\textsuperscript{[15]} This study interprets that the hypolipidemic activity of pomegranate extracts can be due to presence of Gallic acid which reduces absorption of lipids from the intestine to the blood circulation, resulting in low blood lipids level.

As a result, it may be concluded that all the different parts of pomegranate (arils, peels and seeds) extracts possesses antilipidemic activities in NAFLD and pomegranate seed extract may be used as an antidyslipidemic agent. The protective effects are most likely due to their natural constituent of polyphenols and other antioxidants potential.\textsuperscript{[7]}

4.3. Effect of Pomegranate Extracts (Arils, Peels, and Seeds) on Oxidative Stress Status

It has been proposed that over-production of mitochondrial and cytoplasmic superoxide anion, the precursor of reactive oxygen species (ROS), has a pivotal role in NASH development.\textsuperscript{[4]} In addition, lipid peroxidation is often used as an index of oxidative tissue damage, which causes free radical damage to membrane components of the cell and resulting cell necrosis and inflammation.\textsuperscript{[4]}

Malondialdehyde (MDA) is considered the important lipopolysaccharide oxidative stress marker. DNA damage and tissue injury may result in excessive MDA. MDA can react with proteins free amino-groups and form MDA-modified protein adducts. Fifteen Aldehydic products, such as MDA, have relatively longer half-lives as compared with ROS. The products can diffuse to other intra- and extra-cellular places and amplify the effects of oxidative stress. ROS may damage poly-unsaturated fatty acids and cause cell organelle and membrane lipid peroxidation. NAFLD cause significant increase in serum MDA due to raising the grade of mitochondrial beta-oxidation of fatty acids and ketogenesis, which may increase lipid peroxidation and the gathering of reactive oxygen species (ROS).\textsuperscript{[44]}

Similarly, nitric oxide (NO) is determined as a biomarker for reactive nitrogen species (RNS). The increments observed in NO level following fed on MCD diet reflected the
systemic impact in the production of NO caused by the oxidative insult of MCD diet. NO also reacted with O$_2^•−$ to form peroxynitrite anion (ONOO$^•−$) which is a high reactive radical responsible for oxidative damage to macromolecules.$^{[45]}$ Nitric oxide has been implicated in the mechanisms of cell injury and long-term physiological changes in cellular excitability suggested that NO has an important role in modulating oxidant stress and tissue damage.$^{[45]}$

Interestingly, our results indicated that administration of different pomegranate extracts (PJ, PPE and PSE) to fatty liver animals restored these altered biochemical parameter levels to within normal limits and improved liver dysfunction. This could be due to the phytoconstituents detected in the plant materials of pomegranate (arils, peels and seeds) extracts which may be responsible for their antioxidant activity.

4.4. Effect of Pomegranate Extracts (Arils, Peels, and Seeds) on Antioxidant Status

Endogenous protection against oxidative stress is achieved by enzymatic and non-enzymatic antioxidant agents that scavenge ROS. Our results highlight the protective role of GSH, catalase (CAT), paraoxonase-1 (PON1), and arylesterase (ARE) as an intracellular antioxidant in the liver.

Both GSH and catalase are considered to be free-radical scavengers in the cells. Thus, the decrease in GSH level and catalase activity leading to an indirect increase in oxidative DNA damage, which suggests that, catalase plays a role in the suppression of oxygen free-radical formation and the decrease of NO generation.

In our study, the depletion of the catalase activity and GSH concentration in the animals of fatty liver group could be either the result of their increased utilization for conjugation and/or their participation in achieving free radical products induced by MCD diet.$^{[15,17]}$

Similarly, Paraoxonase-1(PON1) and arylesterase (ARE) are both esterase enzymes that have lipophilic antioxidant characteristics.$^{[5]}$ These enzymes play a role in decreasing oxidative stress. PON1 in particular is an important endogenous free radical scavenging system in the human body.$^{[5]}$ Serum PON1 acts in conjunction with ARE to function as a single enzyme.$^{[5]}$

Some studies reported that PON1 and ARE deficiencies are associated to hepatic steatosis in mice fed a MCD diet. These changes are accompanied by severe metabolic alterations, and with increased oxidative stress and inflammation. The original function attributed to PON1 was that protects LDL from oxidation seems to be related to its capacity to hydrolyze
oxidized fatty acids derived from phospholipids, cholesterylester and triglycerides hydroperoxides that are potentially atherogenic compounds.[46]

The present study revealed that catalase, PON1 and ARE activities and GSH level significantly increased by different pomegranate extracts (PJ, PPE and PSE). The protective effect of these plant extracts may be explained depending on the fact that these extracts contain polyphenolic compounds which may scavenge free radicals offering protection and their antioxidant potential mechanism suggesting that the extracts of these plants may be useful to prevent the oxidative stress inducing damage and protein oxidation.[47,48]

4.5. Effect of Pomegranate Extracts (Arils, Peels, and Seeds) on Hepatic Function

There are different types of enzymes that could be secreted from different organs there are specific enzymes secreted in specific organs like ALT which is a specific enzyme for liver cells.[3] High levels of serum ALT indicated that there was a liver dysfunction. NAFLD which accompanied with elevated liver ALT enzyme was represented to hepatocellular damage and systemic inflammation.[4] Also, γ-glutamyltransferase(GGT) in the serum is frequently elevated in patients with NAFLD and it has been reported to be associated with advanced fibrosis in NAFLD patients.[4]

Our results revealed that MCD diet significantly caused oxidative stress and elevation of hepatic specific enzyme activities. This elevation in liver enzymes may be due to degeneration and necrosis of hepatocytes which attributes an increased permeability of the cell membrane that results in the release of transaminases into the blood stream as a result of the MCD diet model induced NAFLD. The present study is in a good agreement with the previous study approved that following feeding on MCD diet caused a significant increase in the serum ALT and GGT.[19]

However, treatments with different Pomegranate Extracts (PJ, PPE and PSE) were able to alleviate the liver damage caused by feeding MCD diet as revealed by remarkable decrease in these enzymes. This could be due to the impressive amount of polyphenols present in different pomegranate extracts which decrease the oxidative stress resulting in restoration the functions of the plasma membrane and decreasing release of enzymes into serum.[49,50]
4.6. Effect of Pomegranate Extracts (Arils, Peels, and Seeds) on Liver Apoptosis

Several clinical studies investigated the relationship between plasma pentraxin3 PTX3 levels and liver fibrosis of NAFLD, including NASH. The results demonstrated that plasma PTX3 levels can be used to reliably differentiate NASH patient from non-NASH patients and the elevation of plasma PTX3 levels in NASH patients could be as a result from the severity of liver fibrosis.\[51\]

The long pentraxin PTX3 are acute-phase proteins produced in vivo during inflammatory reactions. PTX3 is produced in tissues under the control of primary proinflammatory signals, such as lipopolysaccharide, IL-1 beta, and tumor necrosis factor-alpha, which induce the release of PTX3 by endothelial cells and mononuclear phagocytes. Cell death commonly occurs during inflammatory reactions. PTX3 is therefore an intriguing candidate for interaction with cells dying at inflammatory sites.\[51,52\]

The alteration in the glycosylation pathways, which occurs during cell death, may contribute to their recognition by pentraxins3. Further studies indicated the role for these molecules as binding sites for PTX3 on the membrane of dying cells.\[51\]

In our study, marked elevation of the plasma PTX3 level was observed in the fatty liver control animals in comparison with the healthy control group (P<0.05). This elevation in liver plasma PTX3 level may be due to degeneration and necrosis of hepatocytes. Treatment with different pomegranate extracts (PJ, PPE and PSE) to fatty liver animals was able to alleviate the liver damage caused by MCD diet as revealed by remarkable decrease in plasma PTX3 level.\[51,52\]

On the other hand, Cytokeratin-18 one of the features of NASH is hepatocytes apoptosis.\[53-55\] During the apoptotic process, various caspases are activated with the subsequent cleavage of different substrates. The main substrate in the liver is cytokeratin 18 (CK-18), which is an intermediate filament protein. Numerous studies have shown when hepatocytes are chronically exposed to oxidative stress, they become ballooned, accumulate fat, show a disruption in the keratin intermediate filament network, and form Mallory bodies. A Mallory body is composed of abnormally phosphorylated and cross-linked keratins, such as cytokeratin (CK18) and stress-induced proteins. Since hepatocytes containing Mallory bodies are susceptible to apoptosis, so those levels of Mallory body-associated proteins released from hepatocytes into peripheral blood may be increased in NASH patients and change in
accordance with disease activity. Further studies show this caspase cleaved CK18 fragment is a sensitive and specific biomarker for NASH that rises with increasing severity of NASH. This suggests that CK18 is a valuable soluble marker for diagnosis of NASH and a biomarker that can demonstrate the efficacy of NASH treatments.

In the current study we found statistically significant differences (P<0.05) between fatty liver control group (G5) and the healthy control group (G1) regarding the plasma CK18 level. Our results are similar to Wieckowska et al., who found that CK18 could serve as an indicator of hepatocyte apoptosis. This could be explained by the hepatocyte apoptosis process which is one of the key components involved in the progression of NAFL to NASH. This process is mediated by the caspase activity. It is reported that Caspase 3 is activated in NASH liver, which induce hepatocyte apoptosis and release the CK18 fragments into the sera of NASH patients.

Interestingly, our results indicated that administration of different pomegranate extracts (PJ, PPE and PSE) to fatty liver animals restored these altered CK-18 levels to within normal limits and improved liver dysfunction. This could be due to the phytoconstituents detected in the plant materials of radish and leek juices which may be responsible for their hepatoprotective activity.

In a study done by Gonsebatt et al., who found that, hepatic mRNA expression of CK18 has been reported to be up-regulated by oxidative stress in mice; which could be another explanation for the elevated levels of serum CK18 in NASH patients. In another study, CK-18 correlated most strongly with steatosis and liver function of NASH patients.

The results of the current study goes with the results of the study of Vuppalanchi et al., who found that changes in serum levels of cytokeratin fragments appear to reflect changes in liver histology in patients with NASH. They found that every 100-U/L decline in serum cytokeratin fragment (CK-18) level was significantly associated with overall histological improvement (P < 0.001); resolution of NASH (P = 0.002); and improvement of at least 1 point in steatosis grade, hepatocellular ballooning, and nonalcoholic fatty liver disease (NAFLD) score (P < .001 for all).
4.7. Histopathological Examination of The Liver

Livers obtained from fatty liver control group (GII and G5) showed yellowish and enlarged livers with yellow spots by general observation. These yellow spots may be due to deposition of fat inside hepatic cells, this can be attributed to sinusoidal dilatation, microvesicular steatosis, or fibrosis resulting from an increase in connective tissue following hepatocellular necrosis.\[4\]

In the present study, the histopathological analysis was done to examine hepatocytes and liver tissues for any abnormalities or fat droplets. Microscopically, livers of rats fed on MCD diet for a month (GII) showed small fat droplets that accumulate in the hepatocytes cytoplasm. Meanwhile, liver tissues of fatty liver group after 2 months (G5) revealed severe hepatic microvesicular and macrovesicular steatosis with few necrosis which indicates severe NAFLD. In addition, disturbed hepatic architecture of the liver which observed in this group (G5) was explained as a result of oxidative damage in hepatocellular proteins or necrotic changes in hepatocytes that lead to irregularity in the orientation of the hepatocyte plates and disturbing hepatic architecture. On the other hand, Histopathological study revealed that the animals administered with different pomegranate extracts (G6, G7, and G8) showed significant decreased in histopathological damage of liver including fatty change in hepatocyte which in turn significantly reduce hepatic steatosis, ballooning, dilation of sinusoid, and congestion. Furthermore, there was no necrosis in hepatocytes, lower lobular inflammation and portal inflammation in all of pomegranate extracts treated groups. These results indicated that the protective effects of PJ, PPE, and PSE on NAFLD were partly due to their antioxidant activities.\[61,62\]

This finding was supported by previous reported that PPE and PSE extract had a decreased lipid accumulation in the liver by activating hepatic gene expression and by decreasing fatty acid oxidation.\[63\] Based on the results of this study, PPE and PSE act as a powerful antioxidant against the rat liver of high fat dietary induced-NAFLD. In another study, antioxidant effect of polyphenols that found in pomegranate juice enhanced the process of regeneration; this might be due to the destruction of free radicals, supplying a competitive substrate for unsaturated lipids in the membrane and/or accelerating the repair mechanism of damaged cell membrane.\[63\] This finding was indicated that the pomegranate extracts (arils, peels and seeds) had a protective effect against fat accumulation in the liver. It is concluded
that may be due to reduced lipid peroxidation and/or enhancement of antioxidant action by different pomegranate extracts.\cite{64}

5. CONCLUSION

From the present study and its results it can be concluded that the administration of pomegranate extracts (arils, peels and seeds) act as a modulator tool for improving health status and alleviating the high risk factors of NAFLD. This is due to its content of phytochemicals and their potential improving anti-oxidant activity which reduces the oxidative stress status and act as protective to preserve the damage in the hepatocyte that occurs due to MCD diet. Moreover, our findings clearly showed that pomegranate extracts have hepatoprotective, hypolipidemic, hypocholesterolimic and anti-inflammatory effects on MCD fed rats. In addition, the results revealed that the pomegranate seeds' extract is most effective followed by pomegranate peels' extract and finally pomegranate juice on the risk factors of NAFLD.

**Figure (1):** Percentage of main phenolic compounds derived from pomegranate juice.

**Figure (2):** Percentage of main phenolic compounds derived from pomegranate peels extract.
Figure (3): Percentage of the main phenolic compounds derived from pomegranate seeds' extract.

Figure (4): Percentage of the main flavonoid compound derived from pomegranate seeds' extract.

Table (1): The total phenols and total flavonoids in different pomegranate extracts (arils, peels, and seeds).

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total Phenols (µgGAE/100g)</th>
<th>Total flavonoids (µgCE/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pomegranate Juice</td>
<td>5.57</td>
<td>-</td>
</tr>
<tr>
<td>Pomegranate Peels Extract</td>
<td>206.09</td>
<td>-</td>
</tr>
<tr>
<td>Pomegranate Seeds Extract</td>
<td>691.23</td>
<td>851.47</td>
</tr>
</tbody>
</table>

GAE=Gallic Acid Equivalent CE=Cathachine Equivalent
Table (2): Effect of feeding methionine choline deficient (MCD) diet on hepatic lipid profile

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>After The First Month</th>
<th>After The Second Month</th>
<th>LSD (P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy (G1)</td>
<td>Fatty Liver (GII)</td>
<td>Healthy Control (G1)</td>
</tr>
<tr>
<td>Cholesterol (mg/g. tissue)</td>
<td>57.89±3.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>64.88±2.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.91±2.82&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triacylglycerol (mg/g. tissue)</td>
<td>31.12±4.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.80±18.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.03±4.68&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL-C (mg/g. tissue)</td>
<td>12.43±1.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.80±0.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.46±1.68&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDL-C (mg/g. tissue)</td>
<td>39.23±3.59&lt;sup&gt;c&lt;/sup&gt;</td>
<td>43.92±5.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.33±3.06&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.D, n=10.

There is no significant difference between means have the same letter in the same column (P<0.05)
Table (3): Effect of consuming pomegranate extracts (PJ, PPE, and PSE) on hepatic lipid profile in different experimental groups.

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Healthy Groups</th>
<th>Fatty Liver Groups</th>
<th>LSD (P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (G1)</td>
<td>+PJ (G2)</td>
<td>+PPE (G3)</td>
</tr>
<tr>
<td>Cholesterol (mg/g. tissue)</td>
<td>54.91± 2.82c</td>
<td>44.28± 3.32d</td>
<td>42.18 ± 2.15d</td>
</tr>
<tr>
<td>Triacylglycerol (mg/g. tissue)</td>
<td>29.03±4.68b</td>
<td>24.70± 1.31bc</td>
<td>23.65 ± 3.83bc</td>
</tr>
<tr>
<td>HDL-C (mg/g. tissue)</td>
<td>13.46±1.68d</td>
<td>16.32±1.26c</td>
<td>22.53±3.41a</td>
</tr>
<tr>
<td>LDL-C (mg/g. tissue)</td>
<td>35.33±3.06e</td>
<td>25.89±3.97d</td>
<td>14.91±2.62e</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.D, n=10.

There is no significant difference between means have the same letter in the same column (P<0.05)

Table (4): Effect of feeding methionine choline deficient (MCD) diet on some hepatic oxidative stress status.

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>After The First Month</th>
<th>After The Second Month</th>
<th>LSD (P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy (G1)</td>
<td>Fatty Liver (GII)</td>
<td></td>
</tr>
<tr>
<td>NO (µmol/g. tissue)</td>
<td>57.87±6.46c</td>
<td>62.38±0.59b</td>
<td></td>
</tr>
<tr>
<td>MDA (nmol/g. tissue)</td>
<td>68.81±4.54c</td>
<td>125.11±7.09b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Healthy Control (G1)</td>
<td>Fatty Liver Control (G5)</td>
<td></td>
</tr>
<tr>
<td>NO (µmol/g. tissue)</td>
<td>58.01±3.85c</td>
<td>88.25±4.61a</td>
<td>4.139</td>
</tr>
<tr>
<td>MDA (nmol/g. tissue)</td>
<td>64.84±1.65c</td>
<td>162.39±3.09c</td>
<td>4.268</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.D, n=10.

There is no significant difference between means have the same letter in the same column (P<0.05)

Table (5): Effect of consuming pomegranate extracts (PJ, PPE, and PSE) on some hepatic oxidative stress status in different experimental groups

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Healthy Groups</th>
<th>Fatty Liver Groups</th>
<th>LSD (P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (G1)</td>
<td>+PJ (G2)</td>
<td>+PPE (G3)</td>
</tr>
<tr>
<td>NO (µmol/g. tissue)</td>
<td>58.01±3.85a</td>
<td>56.28±2.37dc</td>
<td>54.44±3.75c</td>
</tr>
<tr>
<td>MDA (nmol/g. tissue)</td>
<td>64.84±1.65c</td>
<td>64.59±7.30c</td>
<td>53.75±7.24a</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.D, n=10.

There is no significant difference between means have the same letter in the same column (P<0.05)
Table (6): Effect of feeding methionine choline deficient (MCD) diet on some hepatic antioxidant status.

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>After The First Month</th>
<th>After The Second Month</th>
<th>LSD (P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy (G1)</td>
<td>Fatty Liver (GII)</td>
<td>Healthy Control (G1)</td>
</tr>
<tr>
<td>CAT activity (U/g.tissue)</td>
<td>17.19±1.15b</td>
<td>14.57±3.99c</td>
<td>20.87±0.99a</td>
</tr>
<tr>
<td>GSH (mg/g.tissue)</td>
<td>21.24±2.81b</td>
<td>19.84±2.39b</td>
<td>24.97±2.86a</td>
</tr>
<tr>
<td>PON1 activity (ng/ml)</td>
<td>14.92±0.99b</td>
<td>11.52±0.36c</td>
<td>16.84±1.60a</td>
</tr>
<tr>
<td>ARE activity (ng/ml)</td>
<td>114.23±1.45a</td>
<td>113.54±0.48a</td>
<td>114.49±2.35a</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.D, n=10.

There is no significant difference between means have the same letter in the same column (P<0.05)

Table (7): Effect of consuming pomegranate extracts (PJ, PPE, and PSE) on some hepatic antioxidants status in different experimental groups.

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Control (G1)</th>
<th>PJ (G2)</th>
<th>PPE (G3)</th>
<th>PSE (G4)</th>
<th>Control (G5)</th>
<th>PJ (G6)</th>
<th>PPE (G7)</th>
<th>PSE (G8)</th>
<th>LSD (P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT activity (U/g.tissue)</td>
<td>20.86 ± 0.99e</td>
<td>30.35 ± 6.41d</td>
<td>46.36 ± 4.24c</td>
<td>80.81 ± 1.63a</td>
<td>10.91 ± 3.08a</td>
<td>17.74 ± 2.58d</td>
<td>20.85 ± 0.97a</td>
<td>75.74 ± 1.64b</td>
<td>2.95</td>
</tr>
<tr>
<td>GSH (mg/g.tissue)</td>
<td>24.97 ± 2.86f</td>
<td>27.01 ± 4.37d</td>
<td>29.50 ± 7.41c</td>
<td>54.32 ± 4.01c</td>
<td>5.07 ± 1.84c</td>
<td>34.16 ± 2.52d</td>
<td>40.29 ± 5.19b</td>
<td>115.00 ± 6.56a</td>
<td>4.32</td>
</tr>
<tr>
<td>PON1 activity (ng/ml)</td>
<td>16.84 ± 1.60c</td>
<td>17.80 ± 0.91bc</td>
<td>27.02 ± 3.36a</td>
<td>28.04 ± 2.81a</td>
<td>10.50 ± 0.35e</td>
<td>13.55 ± 2.07d</td>
<td>18.00 ± 0.65bc</td>
<td>19.05 ± 0.59b</td>
<td>1.71</td>
</tr>
<tr>
<td>ARE activity (ng/ml)</td>
<td>114.49 ± 2.35d</td>
<td>120.16 ± 0.79b</td>
<td>121.77 ± 1.36b</td>
<td>126.77 ± 3.51a</td>
<td>99.48 ± 1.24c</td>
<td>117.43 ± 1.54c</td>
<td>117.77 ± 2.90c</td>
<td>126.47 ± 1.21a</td>
<td>1.89</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.D, n=10.

There is no significant difference between means have the same letter in the same column (P<0.05)
Table (8): Effect of feeding methionine choline deficient diet on liver function.

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>After The First Month</th>
<th>After The Second Month</th>
<th>LSD (P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy (G1)</td>
<td>Fatty Liver (GII)</td>
<td>Healthy Control (G1)</td>
</tr>
<tr>
<td>GPT activity (U/L)</td>
<td>44.66±3.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>129.9±3.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.33±3.44&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>GGT activity (U/L)</td>
<td>9.44±0.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.98±0.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.40±0.25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.D, n=10.

There is no significant difference between means have the same letter in the same column (P<0.05)

Table (9): Effect of consuming pomegranate extracts (PJ, PPE, and PSE) on liver function in different experimental groups

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Healthy Groups</th>
<th>Fatty Liver Groups</th>
<th>LSD (P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (G1)</td>
<td>+PJ (G2)</td>
<td>+PPE (G3)</td>
</tr>
<tr>
<td>GPT activity(U/L)</td>
<td>44.33±3.44&lt;sup&gt;d&lt;/sup&gt;</td>
<td>41.68±5.73&lt;sup&gt;d&lt;/sup&gt;</td>
<td>26.18±5.54&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>GGT activity(U/L)</td>
<td>11.40±0.25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.64±1.08&lt;sup&gt;e&lt;/sup&gt;</td>
<td>9.25±0.82&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.D, n=10.

There is no significant difference between means have the same letter in the same column (P<0.05)

Table (10): Effect of feeding methionine choline deficient (MCD) diet on liver apoptosis markers plasma pentraxin-3 and cytokeratin-18.

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>After The First Month</th>
<th>After The Second Month</th>
<th>LSD (P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy (G1)</td>
<td>Fatty Liver (GII)</td>
<td>Healthy Control (G1)</td>
</tr>
<tr>
<td>PTX-3(µg/L)</td>
<td>1.84±0.19&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.38±0.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.58±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CK-18(µg/L)</td>
<td>26.96±0.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.25±2.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.07±1.27&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.D, n=10.

There is no significant difference between means have the same letter in the same column (P<0.05)
Table (11): Effect of consuming pomegranate extracts (PJ, PPE, and PSE) on liver apoptosis markers in different experimental groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy Groups</th>
<th>Fatty Liver Groups</th>
<th>LSD (P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (G1)</td>
<td>+PJ (G2)</td>
<td>+PPE (G3)</td>
</tr>
<tr>
<td>(PTX-3)(µg/L)</td>
<td>2.58±0.13b</td>
<td>2.35±0.09da</td>
<td>2.24±0.07da</td>
</tr>
<tr>
<td>(CK-18)(µg/L)</td>
<td>26.07±1.27b</td>
<td>25.20±0.81cd</td>
<td>24.36±1.60d</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.D, n=10.

There is no significant difference between means have the same letter in the same column (P<0.05)

Table (12): The effect of pomegranate extracts (PJ, PPE, and PSE) on the degree of congestion, steatosis, inflammation and necrosis in different experimental groups

<table>
<thead>
<tr>
<th>Histopathological lesions</th>
<th>Group Healthy (G1)</th>
<th>Group Fatty Liver (GII)</th>
<th>Healthy Control (G1)</th>
<th>Healthy Juice (G2)</th>
<th>Healthy Peel (G3)</th>
<th>healthy Seeds (G4)</th>
<th>Fatty liver Control (G5)</th>
<th>Fatty liver Juice (G6)</th>
<th>Fatty liver Peel (G7)</th>
<th>Fatty liver Seeds (G8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congestion of hepatic sinusoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hepatic steatosis</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>inflammatory cells infiltration</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Necrosis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(-) no change (+) mild change (++) moderate change (+++) severe change
Figure (5): Liver section of rat from healthy group (GI) after 1 month showing the normal histological structure of hepatic lobule (H & E X 400).

Figure (6): Liver section of rat from fatty liver group (GII) after 1 month showing fatty change of hepatocytes associated with few inflammatory cells infiltration (H & E X 400).

Figure (7): Liver section of rat from healthy control group (G1) after 2 month showing no histopathological changes (H & E X 400).
Figure (8): Liver section of rat from healthy juice group (G2) showing no histopathological changes (H & E X 400).

Figure (9): Liver section of rat from healthy peels group (G3) showing no histopathological changes (H & E X 400).

Figure (10): Liver section of rat from healthy seeds group (G4) showing no histopathological changes (H & E X 400).
Figure (11): Liver section of rat from fatty liver control group (G5) after 2 month showing marked hepatic steatosis (steatosis of hepatocytes) (H & E X 400).

Figure (12): Liver section of rat from fatty liver juice group (G6) showing slight congestion of hepatic sinusoids and activation of Kupffer cells (H & E X 400).

Figure (13): Liver section of rat from fatty liver peels extract group (G7) showing almost normal liver tissue (H & E X 400).
Figure (14): Liver section of rat from fatty liver seeds extract group (G8) after 2 month showing no histopathological changes (H & E X 400).

6. REFERENCES
1. Estes, C.; Razavi, H.; Loomba, R.; Younossi, Z. and Sanyal A. "Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease" J. Hept., 2017; 00(00): 1-11.


