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Efficacy of Pomergranate (Punica granatum) Extract on Experimentally Metabolic Syndrome Rat with High Fructose Diet

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ABSTRACT

Metabolic Syndrome (MS) denotes a growing threat to population health. Its prevalence has increased dramatically during recent years. Pomegranate Extract (PE) possesses strong hypoglycemic and antioxidant properties. The present work aims to determine whether PE has a therapeutic effect against MS in rats. Male rats (n=40) were divided into two main groups. The first group; control negative rats (n=10) and the second group (n=30) were fed a high-fructose diet (HF; 50%) for six weeks to induce MS. Then this group was divided equally into three sub-groups, control positive (MS), and PE treated groups; rats received orally PE at dose levels of 500 and 750 mg/kg body weight (BW)/ day. After 8 weeks of treatment and at the end of the experiment period (14 weeks). Pro-oxidants malondialdehyde (MDA) were determined. Glucose, insulin, leptin hormone, lipid profile parameters, and uric acid were also measured. Liver tissue samples were examined as well. The results showed that ingestion of a HF diet led rats to exhibit significant ($p<0.001$) hyperinsulinemia, hyperglycemia, dyslipidemia, and hyperuricemia. Additionally, the diet induced a significant ($p<0.001$) increase in leptin hormone and MDA levels in MS group after 14 weeks when compared with control rats. Examination of liver tissues revealed fatty changes of hepatocytes, collagen fibers deposition, marked dilatations, and congestion of hepatic portal blood vessels. Treatment with PE significantly ameliorates all the tested biochemical parameters and helps overcome the histopathological alteration in liver tissues compared with the MS untreated group. The improvement was more pronounced in MS treated with 750 mg/kg BW. It was concluded that PE has a beneficial role in alleviating MS symptoms and oxidative stress induced by HF diet. Therefore, PE may be used as a beneficial supplement for patients with MS.

Key words: *Pomergranate extract, Metabolic syndrome, Rats, High-fructose diet, Antioxidant.*

INTRODUCTION

Metabolic Syndrome (MS) is a complex metabolic disorder defined by a cluster of interconnected factors and leads to substantially increased morbidity of cardiovascular disease and diabetes mellitus type 2. Its prevalence has been increasing dramatically in recent years. Furthermore, MS is considered one of the major burdens in health care systems in many industrialized countries [1-3]. Obesity, reduced physical activity, sedentary lifestyle, rapid transitions toward excessive energy intake, and improper diet are factors that may lead to the development of MS symptoms [4-5]. The primary diagnostic criteria of MS are hyperglycemia, insulin resistance, abdominal obesity, hypertension, and dyslipidemia. Evidence suggests a significant association between fructose consumption and the development of MS [6].

In the past years, a noticeable increase in fructose, sucrose (a disaccharide consisting of 50% fructose) and high fructose corn syrup (HFCS; 55% fructose content) intake, which are common sweeteners used in the food industry, was observed [7-8]. For example, these common sweeteners are used in soft drinks and excess fruit juice (high in HFCS) [9]. Previous studies indicated that, the overconsumption of high fructose products has drastically increased and could have deleterious metabolic effects in humans [10-11]. In addition to that, the overconsumption of high fructose products has coincided with development of MS [7].

Traditional plant medicine is an excellent representative in alternative and complementary medicines [12]. Pomegranate (*Punica granatum* L.) is a deciduous shrub [13]. PE is considered a viable method of controlling MS. Extracts from the different parts of this plant have been researched for numerous beneficial pharmacologic effects such as having anti-diabetic, anti-inflammatory, antioxidant, and antitumor effects *in vivo* and *in vitro* [13-14]. As well as, antibacterial, astringent, and antidiarrheal activities [15-16]. PE also contains an array of compounds that have been attributed to anti-obesity effects [13]. Furthermore, antioxidant activity accompanied with radio protective and anti-fibrotic properties of P. granatum peel extract have been [17-18]. Moreover, a protective role of pomegranate on fatty liver in obesity through improvement of abnormal lipid metabolism was mentioned by Xu et al. [19] and El-Rashedy et al. [20]. On the other hand, the inhibition of carbohydrate digestive enzymes and their phenolic content may contribute to the anti-hyperglycemic effects of pomegranate flower and peel [21]. Abdel Moneim et al. [22] indicated that pomegranate extract could be able to inhibit all-induced oxidative stress and histopathological alternations in the liver and kidney of female rats. These effects may be related to anti-apoptotic and antioxidant activities. Consequently, this study aimed to evaluate the therapeutic role of PE in healing MS in male rats.

MATERIAL AND METHODS

Chemicals and kits

Casein, cellulose, choline chloride, fructose, cornstarch, vitamin mixture, minerals mixture and all other chemicals and reagents were obtained from Sigma-Aldrich Company (St. Louis, Mo., USA). Corn oil and sucrose obtained from a local market.

Plant material

Pomegranate (*Punica granatum* L.) extract in tablet form was purchased from Holland and Barrett (UK). Each tablet was standardized to 40% ellagic acid and 40% polyphenols. Pomegranate extract was dissolved in carboxymethyl cellulose (CMC), the dose level (500 or 750 mg/kg BW) was orally administered in (1 ml of 1 % w/v, CMC). The dose 500 mg/kg BW was selected based on previous experiments by researchers [23].

Experimental animals

Male Wister Albino rats (n=40 rats) weighing about (200 - 220 g) were allowed one week to acclimate in animal housing conditions before being used for the study. Rats were housed under standard laboratory conditions. All animals were fed a standard nutritionally balanced diet according to Reeves [24], and drinking water *ad libitum*.

Induction of metabolic syndrome

Metabolic syndrome was induced by feeding male rats a high fructose (HF; 55%) diet for 6 weeks according to Nakagawa et al. [25]. After 6 weeks of feeding a HF diet, blood samples from the retro-orbital plexus were collected from each rat by a fine capillary glass tube under anesthesia with diethyl ether, centrifuged at 3000 rpm for 15 min to separate serum. Rats with blood glucose (>200 mg/dl), total cholesterol (>110 mg/dl), triglyceride (>140 mg/dl), high density lipoprotein cholesterol (HDL-C) (< 35 mg/dl), change in body weight (> 8% of initial weight), systolic blood pressure (>130 mm/Hg) confirmed presence of MS. The rats were either fed a normal diet or a HF diet as per the protocol for 8 more weeks.

Experimental groups

Group one: Control negative (n=10) rat are fed standard diet and after 6 weeks orally administered a single dose of (1 ml of 1 % w/v, CMC) daily. Group two: MS group (n=30) rats are fed a diet containing 55% fructose, after 6 weeks were classified equal to three sub-groups, control positive (MS) received orally (1 ml of 1 % w/v, CMC), and PE treated groups; rats received PE orally at a dose level of 500 or 750 mg/kg BW/ day dissolved in (1 ml of 1 % w/v, CMC). After 8 weeks and at the end of the experimental period (14 weeks), rats fasted over night before scarification. Blood samples with drawn by heparinized capillary tubes from the retro-orbital plexus under anesthesia, for serum separation. The liver was dissected out, and then fixed in 10% formalin for histopathological examination.

Biochemical assays

Serum glucose, total cholesterol (TC), triacylglcerol (TAG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C) and uric acid (UA) were measured by enzymatic colorimetric kits. Serum insulin and leptin were measured using ELISA kits (R&D, Inc., USA) according to the manufacturer's instructions.

Determination of lipid peroxides

Lipid peroxides measures as malondialdehyde (MDA) in serum [26]. The principle of the assay depends on the reaction of MDA with thiobarbituric acid in an acidic medium under light temperature. The resultant pink color was extracted by n-butanol and the absorbance was measured to be 540 nm by a spectrophotometer.

Histological examination

Liver tissues from each group were removed, washed immediately with saline, then fixed in 10% buffered formalin, embedded in paraffin, sections cut at 3-5 μm, and stained with Hematoxylin and Eosin [27]. These sections were then examined under a light microscope for histological changes.

Statistical analysis

Statistical analyses of data were carried out using SPSS version 22. Data were expressed as the mean ± SE. Comparisons between groups were done by one-way analysis of variance (ANOVA), followed by L.S.D [28].

RESULTS

Effect of PE on oxidant status in MS rats

The level of serum lipid peroxide as malondialdehyde (MDA) in different groups is shown in Figure (1). Feeding a HF diet for 14 weeks to the MS untreated group induced an increase in oxidative stress, this was clear from drastic significant ($p<0.001$) increase in the serum level of MDA as compared with the control group. Treatment of MS afflicted rats with PE, with either 500 or 750 (mg/kg BW), resulted in significant decrease of MDA level, there were significant differences ($p<0.001$) when compared with MS untreated group. With respect to the control group, treatment with 750 (mg/kg BW) of PE resulted in a decreased level of MDA, almost reaching an equal level with the control group. There was no significant difference between the MS group treated with 750 (mg/kg BW) and the control group.

Metabolic parameters and lipid profile

Fasting serum leptin, insulin, and glucose levels almost doubled in the MS group after 14 weeks, they were significantly ($p<0.001$) increased in MS untreated group when compared with control rats. However, a significant decrease in leptin, insulin, and glucose levels ($p<0.001$) was observed in MS rats treated with either 500 or 750 (mg/kg BW) in comparison to MS untreated rats. In addition, serum leptin, insulin, and glucose levels of MS rats treated with PE 750 (mg/kg BW) returned to control levels of rat serum concentrations. On the other hand, there was no significant different between PE 500 (mg/kg BW) and PE 750 (mg/kg BW) in regard to the serum concentration of leptin, insulin, and glucose Table (1).

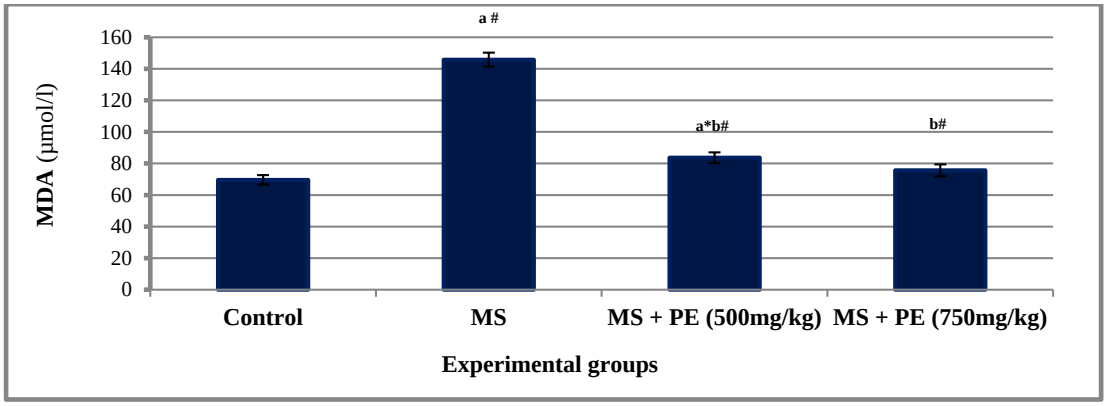

Figure 1: Effect of PE on MDA (µmol/l) in MS rats

MS: Metabolic syndrome, **PE:** Pomegranate extract, **MDA:** Malondialdehyde
Results represent mean of 10 rats ±SE. a Significant difference vs control. b Significant difference vs MS group. c Significant difference between MS treated with 500 and MS treated with 750 mg/kg BW. (* p<0.05, @ p<0.01, and #p<0.001).

Table 1: Effect of PE on serum leptin hormone, glucose, and insulin levels in MS rats

Parameters	Experimental groups			
	Control	**MS**	**MS + PE** (500 mg/kg BW)	**MS + PE** (750 mg/kg BW)
Leptin (ng/dl)	19.09 ± 1.35	71.66 ± 4.59 $^{a\#}$	30.01 ± 2.23 $^{a*b\#}$	22.85 ± 1.97 $^{b\#}$
Insulin (uU/ml)	22.92 ± 1.30	41.79 ± 2.14 $^{a\#}$	29.09 ± 1.67 $^{a*b\#}$	24.50 ± 1.95 $^{b\#}$
Glucose (mg/dl)	94.79 ± 4.65	202.23 ± 10.78 $^{a\#}$	122.05 ± 6.49 $^{b\#}$	105.23 ± 4.27 $^{b\#}$

MS: Metabolic syndrome, **PE:** Pomegranate extract
Results represent mean of 10 rats ±SE. ^{a} Significant difference *vs.* control. ^{b} Significant difference *vs.* MS group. ^{c} Significant difference between MS treated with 500 and MS treated with 750 mg/kg BW. (* p<0.05, $^{@}$ p<0.01, and $^{\#}$p<0.001).

Results in Table (2) revealed that ingestion of a HF diet to rats induced a marked hyperlipidemic effect, there was significant (p<0.001) increase in TC, TAG, LDL-C and VLDL-C with a concomitant significant (p<0.001) decrease in HDL-C compared with control rats. The alteration in lipid profile was significantly (p<0.001) modulated by administrating rats with PE 500 or 750 (mg/kg BW) for 8 weeks of treatment as compared with MS group. However, treatment of rats with PE 750 (mg/kg BW) returned lipid profile to its normal levels. There was a significant hyperuricemia (p< 0.001) in MS untreated rats compared to the control group. Oral administration of PE 500 or 750 (mg/kg BW) for 8 weeks resulted in significantly decreased (p< 0.001) uric acid levels as compared with MS untreated group. On the other hand, there is no significant difference between MS treated group with PE 500 and 750 (mg/kg BW) in regards to uric acid levels Figure (2).

Table 2: Effect of PE on lipid profile parameters in MS rats

Parameters	Experimental groups			
	Control	**MS**	**MS + PE** (500 mg/kg BW)	**MS + PE** (750 mg/kg BW)
TC (mg/dl)	93.39 ± 2.93	182.35 ± 5.46 $^{a\#}$	108.31 ± 3.25 $^{a*b\#}$	103.10 ± 3.27 $^{b\#}$
TAG (mg/dl)	84.83 ± 2.99	145.13 ± 4.69 $^{a\#}$	101.16 ± 5.24 $^{a*b\#}$	91.96 ± 3.67 $^{b\#}$
HDL-C (mg/dl)	48.87 ± 2.72	27.67 ± 1.71 $^{a\#}$	40.15 ± 1.89 $^{a*b\#}$	43.95 ± 3.07 $^{b\#}$
LDL-C (mg/dl)	27.56 ± 3.06	125.65 ± 8.37 $^{a\#}$	47.93 ±3.78 $^{a*b\#}$	40.76 ±4.45 $^{b\#}$
VLDL-C (mg/dl)	16.96 ± 0.62	29.03 ± 0.35 $^{a\#}$	20.23 ± 1.41 $^{a*b\#}$	18.39 ± 0.75 $^{b\#}$

MS: Metabolic syndrome, **PE:** Pomegranate extract, **TC:** Total cholesterol, **TAG:** Triacylglcerol, **HDL-C:** High-density lipoprotein cholesterol, **LDL-C:** Low-density lipoprotein-cholesterol, **VLDL-C:** Very low-density lipoprotein-cholesterol
Results represent mean of 10 rats ±SE. ^{a} Significant difference *vs.* control. ^{b} Significant difference *vs.* MS group. ^{c} Significant difference between MS treated with 500 and MS treated with 750 mg/kg BW. (* p<0.05, $^{@}$ p<0.01, and $^{\#}$p<0.001).

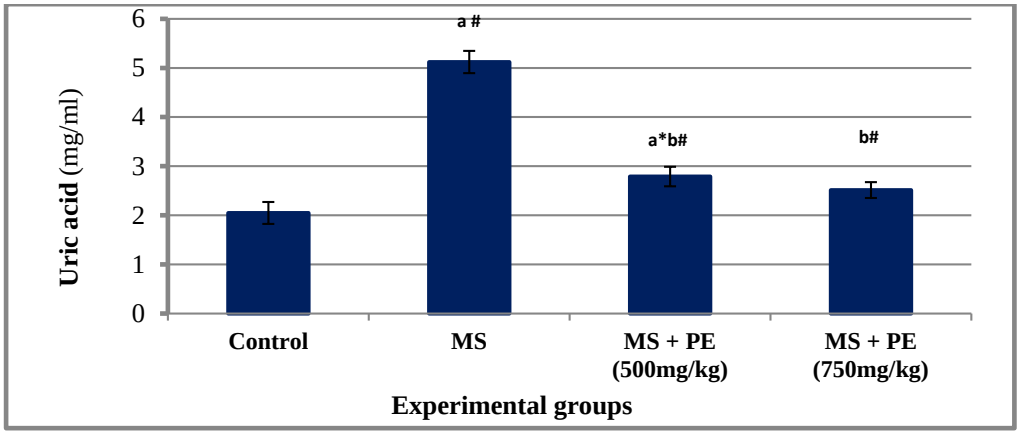

Figure 2: Effect of PE on uric acid (mg/dl) in MS rats

MS: Metabolic syndrome; **PE:** Pomegranate extract.
Results represent mean of 10 rats ±SE. [a] Significant difference *vs*. control. [b] Significant difference *vs.* MS group. [c] Significant difference between MS treated with 500 and MS treated with 750 mg/kg BW. ([*] $p<0.05$, [@] $p<0.01$, and [#] $p<0.001$).

Histopathological examination

Microscopical examination of the liver sections of the control group showing apparent normal structure (Fig. 3(A)). Meanwhile, liver sections of MS rats show fatty changes of hepatocytes, portal infiltration with leucocytic inflammatory cells, vacuoletion of hepatocytes, collagen fiber deposition in the portal triad, congestion of central veins and hepatic sinusoids, marked dilatations and congestion of hepatic portal blood vessels, as well as the appearance of newly formed bile ductules (Fig. 3 (B1-4)). On the other hand, the liver section of MS treated with 500 mg/kg BW PE shows slight congestion and cytoplasmic vacuolization of some hepatocytes (Fig. 3 (C)). While, live sections of MS rats treated with 750 mg/kg BW PE show no histopathological changes, expect some sections showing slight dilatation of hepatic sinusoids with few leucocytes in hepatic sinusoids and binucleation of hepatocytes (Fig. 4 (D1-2)).

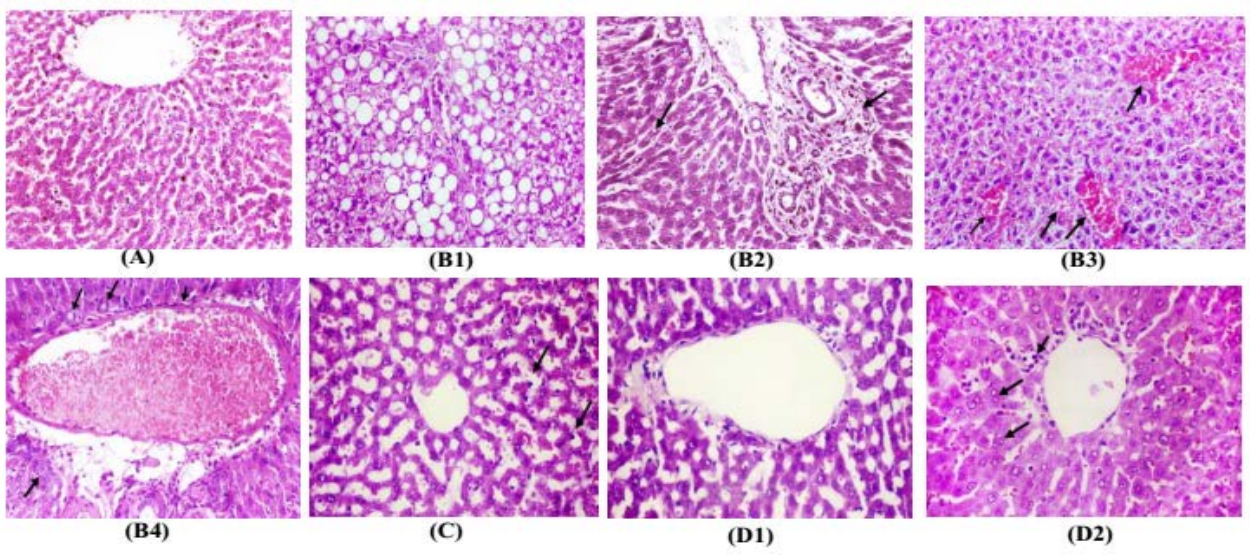

Figure (3): Liver sections of control group (A) showing the normal histological structure of hepatic lobule. Liver sections of MS rats showing fatty changes of hepatocytes (B1), vacuoletion of hepatocytes, collagen fibers deposition in the portal triad (arrows) (B2), congestion of central veins and hepatic sinusoids (arrows) (B3), marked dilatations and congestion of hepatic portal blood vessels (small arrows), as well as appearance of newly formed bile ductules (large arrows) (B4). On the other hand, liver section of MS treated with PE 500 (mg/kg BW) showing slight congestion and cytoplasmic vacuolization of some hepatocytes (arrows) (C). Live sections of MS rats treated with PE 750 (mg/kg BW) showing no histopathological changes (D1), expect some sections showing slight dilatation of hepatic sinusoids (small arrows), with few leucocytes in hepatic sinusoids and binucleation of hepatocytes (large arrows) (D2).

DISCUSSION

Metabolic syndrome, insulin-resistant syndrome, and associated diseases are due to an accumulation of factors including hypertension, dyslipidemia, insulin resistance, obesity, and glucose intolerance that increase subjects' risk of developing CVD and diabetes [29-30]. In animal models high fructose diets have been shown to contribute to a metabolic disturbance leading to insulin resistance. Fructose administration was able to induce systemic hypertension, hyperuricemia, and hypertriglyceridemia [8]. A Diet rich in phytochemicals and antioxidants helps prevent certain diseases.

Pomegranate (*Punica granatum* L.) extract has been used since ancient times to treat many diseases. A number of biological activities such as antioxidant, antitumor, antibacterial and anti-diabetic, anti-inflammatory have been reported for extracts from different parts of *P. granatum* [15, 31-32]. It contains high levels of antioxidant constituents as flavonoids, polyphenols and anthocyanins [33-34]. The levels of antioxidants have been found to be higher than in other natural juices and green tea [35-36]. Therefore, this study aims to assess the efficacy of PE in treating MS in male rats.

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. In the present study, the rats fed a HF diet showed significant increase in serum MDA as compared to the control group. This may be explained by MS 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) [37]. ROS produce a sort of cellular arousal with a subsequent inflammatory effect that has been known as the pathological agent of metabolic disorders [38]. On the other hand, treatment with PE significantly modulated MDA level. Extracts from pomegranate are known to possess enormous antioxidant, anti-inflammatory and anti-tumor effects *in vivo* and *in vitro* [38], it is a rich source of antioxidants from polyphenols, tannins, and anthocyanins, in addition to vitamin C, vitamin E, coenzyme Q10, and lipoic acid [15].

Increased glucose, insulin, leptin hormone, TC, TAG, LDL-C, VLDLC, uric acid and low HDL-C are associated with MS, which agree with [39-41]. Amini and Janghorbani [42] found that MS induced impaired glucose regulation, which resulted in an increased risk of developing type II diabetes and dislipdemia. High fructose diet resulting in glycation-induced insulin resistance, thus leads to compensatory hyperinsulinaemia, thus reduce ß-cells function, leading to raised blood glucose and further deterioration of ß-cells function and insulin sensitivity via glucose toxicity [43]. Another mechanism may be attributed to the different metabolic effects of glucose and fructose, where fructose does not stimulate insulin secretion *in vitro*, probably because of the pancreatic ß-cells lack of fructose transporter GLUT5 thus causing more marked insulin resistance. In addition, hyperglycemia in MS is associated with a large ROS generation and critical oxidative damage in various tissues, such as those of the liver [7,44].

Chronic fructose consumption induces leptin resistance and accelerates high fat [45]. This effect may be attributed to central leptin resistance that can impair leptin signal transduction [46]. Another possibility is that the exogenous leptin failed to reach target sites in the brain, the resistance might be associated with impaired leptin transport across the blood-brain barrier (BBB). Moreover, Serum triglycerides are known to impair the ability of the BBB to transport leptin, which may cause leptin resistance [47].

Insulin resistance and hyperinsulinemia play an important role in the cause of liver disorder. Fructose induces insulin resistance status [48], it is converted into glycerol-3-phosphate, and acetyl-coenzyme-A in the liver, which is used for VLDL production in the liver [8]. The increase of blood cholesterol is related to the reduction of endogenous cholesterol absorption or excretion [49]. Insulin resistance increases hydrolysis of stored TAG in adipocytes and their presence in the blood stream, causing a decrease in plasma levels of HDL-C [50].

Mahmoud and Elshazly [51] reported that rats receiving fructose (10%) in drinking water for 12 weeks resulted in a highly significant increase in UC level. These effects are attributed to an increased UA production and decreased uric acid urinary excretion. Fructose raises serum UA, which predicts for the development of obesity, hypertension and may have a pathogenetic role in MS [25]. These may be explained by hyperinsulinemia that can lead to

impairment in urate excretion [52], fructose results in lactate production, which is a competitive inhibitor for urate excretion. As well as, hyperuricemia causes endothelial dysfunction and renal vasoconstriction, which is known to impair urate excretion [53].

Interestingly, administration of PE reversed the effects of feeding a HF diet, where it induced a significant decrease in pro-oxidant MDA, leptin hormone, glucose, insulin, lipid parameters and uric acid compared with MS untreated group. Reduced leptin level can be attributed to improved leptin sensitivity, thus resulting in increased energy expenditure and decreased food intake and consequently reduced body weight, which was observed in the present study. Hypoglycemic effects of PE, which were associated with decreased insulin levels, are similar to results obtained by de Oliveira et al. [54], Kam et al. [21] and Banihani et al. [55]. Thus may have resulted from decreased hepatic gluconeogesis and improved insulin sensitivity [56]. Moreover, PE has strong antioxidant properties and free radical scavenging capability, that might be effective against MS oxidative damage caused by a HF diet [57-59].

Similar to biochemical findings and in agreement with previous studies [60-62], histological examination revealed fatty changes of hepatocytes, collagen fiber deposition marked dilatations and congestion of hepatic portal blood vessels in MS group, while PE treatment overcame the histopathological alteration in the liver tissues. Large ROS generation and critical oxidative damage in various tissues, such as the liver in MS. Hwu et al. [63] reported that increase consumption of fructose has been shown to be strongly linked with a high prevalence of non-alcoholic fatty liver disease. Similar to these results, are previous studies by Jang and Choung [60], Wang et al. [61] and [33]. These findings revealed that PE treatment could effectively inhibit oxidative stress and lipid accumulation in liver tissues. In conclusion, PE treatment ameliorated deleterious effects associated with MS. The hypoglycemic, hypolipidemic, hypouremic and antioxidant properties of PE may contribute to its beneficial effects.

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