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RESEARCH ARTICLE

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Ameliorative effects of pomegranate peel extract and some of its bioactive components against hyperlipidaemia-induced atherosclerosis in male rats

ABSTRACT:

Pomegranate (*Punica granatum*) peel extracts (PPE) have been shown to possess a significant antioxidant activity, which are attributed to its polyphenols including ellagic acid (EA) and punicalagin (PC). Hence, the present study was designed to evaluate the anti-atherosclerotic effect of PPE, EA and PC in high fat diet (HFD) fed male rats. PPE (50 or 100 mg/kg bw), or EA (1mg/kg bw) or PC (7mg/kg bw) was orally administered for six weeks either with standard diet or after induction of hyperlipidaemia. At the end of experimental duration, serum lipid profile, atherosclerotic ratios, liver antioxidant/oxidative markers, cardiac enzyme (Lactate dehydrogenase LDH), inflammatory marker (TNF-α) and mRNA expression of CD36 gene were analysed. Administration of the HFD caused hypercholesterolemia, hypertriglyceridemia and an increase of LDL-C concentrations, whereas it decreased serum HDL-C in comparison to control rats. Also, HFD resulted in a significant elevation in malondialdehyde level and LDH activity in addition to a significant reduction in catalase activity and CD36 expression level. PPE and its purified polyphenols were able to attenuate all the previous injurious effects of HFD through bolstering the antilipidemic and antioxidant effects. In brief, PPE or EA or PC can be beneficial for the suppression of HFD-induced hyperlipidaemia, oxidative stress as well as regulation of CD36 expression in rats.

KEY WORDS:

pomegranate peel, Ellagic acid, Punicalagin, antilipidemic, antioxidant activity, anti-inflammatory, CD36.

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INTRODUCTION:

Atherosclerosis is the underlying pathophysiologic factor for the majority of cardiovascular diseases, where the majority of cardiovascular diseases follow from complications of atherosclerosis (Grassi *et al.,* 2011; Sahebkar *et al.,* 2016). It considered one of the most leading causes of death, particularly in developed countries (Hasan *et al.,* 2014; Abu-Mweis *et al.,* 2018). There are many metabolic and behavioural risk factors that affect the development and progression of atherosclerotic lesions, the most important are age, cigarette smoking, diabetes mellitus/glucose intolerance, dyslipidaemia, obesity, hypertension, family history and chronic renal insufficiency (Li *et al.,* 2014; Ruscica *et al.,* 2018).

Because oxidative stress plays an important role in atherogenesis, its inhibition by nutritional antioxidants should retard the progression of the disease (Aviram *et al.,* 2008). Therefore, the treatment and prevention of CVDs especially atherosclerotic disease are significant public health focus since many years (Grassi *et al.,* 2010; Sahebkar *et al.,* 2016). There is increasing evidence that a diet rich in fruit and vegetables may be associated with a reduced risk of cardiovascular diseases (CVD), with CVD representing the leading cause of death around the world (Egert and Rimbach, 2011; Yang *et al.,* 2018).

Punica granatum L. is a shrub or small tree and considered to be a native of Iran and Afghanistan. It is also found growing wild in

the warm valleys and outer hills of the Himalayas. The pomegranate fruit consists of the peel, seeds, and the arils. The peel makes up about 50% of the fruit, whereas the arils and seeds make up 40% and 10%, respectively. The peel is rich in many compounds such as phenolics, flavonoids, ellagitannins and proanthocyanin compounds, complex polysaccharides, and many minerals including potassium, nitrogen, calcium, magnesium, phosphorus, and sodium (Aviram and Rosenblat, 2013; Ali *et al.,* 2018).

Previously, it was reported that pomegranate peels extract had the highest free radical scavenging capacity among the tested medicinal plants. It was suggested that hydrolysable polyphenols in pomegranate peel, specifically ellagitannins, are the most active antioxidants among the tannins contained therein. These compounds (ellagic acid, punicalagin, punicalin, and galagid acid) have been shown to hold heightened antioxidant and pleiotropic biological activities and notably, to act synergistically together (Seeram and Heber, 2009; Gullon *et al.,* 2016; Amri *et al.,* 2017).

Ellagic acid and punicalagin are the main bioactive constituents in pomegranate peel (Wu *et al.,* 2013). Higher ellagic acid and punicalagin concentrations are directly associated with the antioxidant activity of pomegranate peel extract (Ismail *et al.,* 2012; Akhtar *et al.,* 2015).

So, the aim is to evaluate the effects of pomegranate peel (PP) in comparison to the pure form of its polyphenols (ellagic acid and punicalagin) as a natural medicine has antioxidant activities on HFD-induced dyslipidaemia in male albino rats.

MATERIAL AND METHODS:

Animals:

Adult male Wistar albino rats, weighing (150 ± 20) g. Rats were obtained from the Veterinary Serum and Vaccine Research Institute (Abbassiya, Cairo, Egypt). The animals were kept under normal condition throughout the experiment. The chosen animals were housed in plastic cages with good aerated covers at 25°C ± 0.5°C as well as 12 h light/dark cycles. Animals were allowed free access to water and were supplied daily with a standard diet. Throughout the experiment, all the procedures and experimental protocols were approved by the Ain Shams University Research Ethics Committee.

Experimental design/groups:

After one week of acclimatization, animals were randomly, divided into ten groups (8 animals each) as follow:

1. Group I (Control): the animals fed the standard diet for 6 weeks.
2. Group II: the animals received an oral daily administration of 50 mg /kg bw PPE for 6 weeks.
3. Group III: animals received an oral daily administration of 100 mg /kg bw PPE for 6 weeks.
4. Group IV: animals received an oral daily administration of 1 mg /kg bw EA for 6 weeks.
5. Group V: animals received an oral daily administration of 7 mg /kg bw PC for 6 weeks.
6. Group VI: the animals fed the high fat diet (HFD), containing (2% Cholesterol, 0.5% Cholic acid, and 20% Cocoa butter), for 6 weeks.
7. Group VII: the animals fed HFD for 6 weeks then fed standard diet with receiving an oral daily administration of 50 mg /kg bw PPE for 6 weeks.
8. Group VIII: the animals fed HFD for 6 weeks then fed the standard diet with receiving an oral daily administration 100 mg /kg bw PPE for 6 weeks.
9. Group IX: the animals fed HFD for 6 weeks then fed the standard diet with receiving an oral daily administration 1 mg /kg bw EA for 6 weeks.
10. Group X: the animals fed HFD for 6 weeks then fed the standard diet with receiving an oral daily administration of 7 mg/kg bw PC for 6 weeks.

Chemicals:

Ellagic acid (purity 90%) was purchased from Pure Bulk (Austin Rd. Roseburg OR. 97471, USA). Punicalagin (purity ≥ 80%) was purchased from Xi'an Rongsheng Biotechnology Co., Ltd. (82 Keji Road, Xi'an Hi-tech Zone, China). Cholesterol (purity 95%) and Cholic acid (purity 97%) were purchased from (Acros Organics, Geel, Belgium). Cocoa butter (Al-Alamia Company, Cairo, Egypt).

Preparation of pomegranate peels aqueous extract (PPE):

The preparation of PPE was carried out according to Qnais *et al.* (2007) with some modification as follows; 900 g of ground, air-dried peel was boiled in 9 litres of distilled water for 15 min with continuous stirring. Then the suspension solution left at room temperature until cool. The large particles settled down, the upper layer was filtered through a filter paper. Filtrate was completely evaporated under reduced vacuum at 60°C (Rotatory Vacuum Evaporator).

Blood sampling:

At the end of the experiment, the animals were sacrificed, and the blood was collected in clean centrifuge tubes; without anticoagulant EDTA. The blood was left to coagulate, then was centrifuged in a cooling centrifuge (IEC centra-4R, International Equipment Co., Needham Heights, MA, USA) at 3000 rpm for 15 min. at 4°C to obtain

serum. The serum was separated, aliquoted and stored at – 80ºC until use.

Tissue sampling:

Immediately after sacrificing the animals, liver and aorta were excess perfused with phosphate buffer solution (PBS). Then the liver and dorsal aorta were separated out of the body, cleaned, weighted, sliced into various pieces and stored at – 80°C until used for the biochemical and molecular analysis.

HPLC analysis of pomegranate peel aqueous extract:

Ellagic acid (EA) and punicalagin (PC) content in pomegranate peel aqueous extract was determined by high-performance liquid chromatography (HPLC) analysis according to Masci *et al.* (2016) by using an Agilent Technologies 1100 series HPLC system (Agilent Technologies, Newtown, PA, USA)

Assessment of the lipid profile in serum:

Lipid profile including of total cholesterol (TC), triacylglycerol (TAG) and high-density lipoprotein cholesterol (HDL-C) were carried using spectrum kit (spectrum diagnostics Egyptian company of biotechnology, Cairo, Egypt). Low density lipoproteins cholesterol (LDL-C), very low-density lipoproteins cholesterol (VLDL-C) concentrations were calculated according to Friedewald *et al. (*1972). Atherosclerotic ratios were calculated according to Aboulgasem and Azab (2015) as follow:

- Atherogenic index of plasma (AIP) = TAG/HDL-C.
- Atherogenic coefficient (AC) = (TC– HDL-C) /HDL-C).
- Castelli's risk index I (CRI-I) = (TC/HDL-C).
- Castelli's risk index II (CRI-II) = (LDL-C/HDL-C).

Determination of changes in antioxidant /oxidant agents:

Antioxidant agents included determination of liver glutathione reduced (GSH) level, glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) activities as well as oxidant agents included malondialdehyde (MDA) and nitric oxide (NOx) levels were carried out by using Biodiagnostic kit (Biodiagnostic, Egyptian company of biotechnology, Cairo, Egypt), following the manufacturer's instructions. Serum paraoxonase1 (PON1) activity was determined according to the kinetic spectrophotometric method described by Eckerson *et al*. (1983).

Determination of lactate dehydrogenase (LDH) in serum:

Serum lactate dehydrogenase (LDH) activities were carried out by using spectrum kit (spectrum diagnostics Egyptian company of biotechnology, Cairo, Egypt), following the manufacturer's instructions.

Determination of serum tumour necrosis factor-alpha (TNF-α) level:

Serum TNF-α level was quantitatively determined by sandwich enzyme-linked immune-sorbent assay using CUSABIO TNF-α rat ELISA kit, following the manufacturer's instructions.

Assessment of the CD36 gene expression in dorsal aorta using qRT-PCR:

RNA Extraction and RT-PCR:

Total RNA was isolated from liver samples using BIOZOL reagent purchased from Bioer Technology Co., Ltd. (Hangzhou, China) following manufacturer's instructions. Reverse transcription (RT) of total RNA to cDNA was performed by mixing 1µg of total RNA, 1.5 µl from 10 µM oligo (dT) (Thermo Fisher Scientific Inc., Massachusetts, USA), 2 µl of 10 mM dNTP mix (Promega Corporation, Wisconsin, USA), 4 µl of 25 mM MgCl2 (Promega Corporation), 2 µl of 10 x RT buffer (SibEnzyme Ltd, Novosibirsk, Russia), 2 µl RNase-inhibitor (Promega Corporation), 7.5 µl RT-enzyme (M-Mul V) (SibEnzyme Ltd.). The volume of this reaction mixture was completed to 25 µl of DEPC-treated water and incubated at 70°C for 10 min, then at 37°C for 10 min, and 42°C for 1 hour, followed by final extension stage at 72˚C for 10 min (Zhang *et al.,* 2005). cDNA product was kept at - 20˚C. RT was carried out in Biometra thermocycler (Analytik Jena Company, Göttingen, Germany).

Quantitative Real-time PCR analysis:

PCR was performed with Specific primers for CD36 and GAPDH (glyceraldehydes-3-phosphate dehydrogenase) as a house-keeping gene (Table I). The primers were obtained from Sigma Co (Sigma Aldrich, Egyptian International Centre for Import Cairo, Egypt). All PCR reactions were performed using Maxima SYBR Green qPCR Master Mix (Bioline, London, UK) and were carried out using Agilent Mx3005P QPCR System (Agilent Technologies Co, CA, USA). Mixtures were prepared in a total volume of 20 µl containing 1 µg of cDNA sample, 0.8 µl of forward primer, 0.8 µl of reverse primer, 10 µl of Sybr Green mastermix and the volume was completed to 20 µl with RNase/DNase free sterile water. The PCR reaction consisted of one cycle involved initial denaturation at 95ºC for 10 min, followed by 40 cycles of 15 s at 95ºC, 30 s at 60ºC and 30 s at 72ºC. Each sample was analysed in triplicate. Differences in gene expression between groups were calculated using the $^{\Delta\Delta}$Ct (cycle threshold, Ct) method (Winer *et al.,* 1992) which were normalized against GAPDH and expressed as relative mRNA levels compared with controls. Ct indicates the fractional cycle number at which the amount of amplified target reaches a fixed threshold.

Table I. Sequences of primers used for the RT- PCR analysis

Gene	Primer Sequence
CD36	F: 5`GAGGTCCTTACACATACAGAGTTCGTT 3`
	R: 5`ACAGACAGTGAAGGCTCAAAGATG3`
GAPDH	F: 5-TCAAGAAGGTGGTGAAGCAG-3
	R: 5-AGGTGGAAGAATGGGAGTTG-3

Statistical analysis:

Statistical evaluation was conducted with Instat Program GraphPad. Software, Inc, San Digeo, USA, version 3.6, Copyright©1992-2003 Results were expressed as mean ± SEM. The results were analysed for statistical significance by one-way ANOVA (De Lorio *et al.,* 2004) followed by Tukey-Kramer multiple comparison post-test. Values of $p < 0.05$ were regarded as significant.

RESULTS:

Effect of treatment on body and liver weights:

As shown in figure 1, the % of body weight change and liver weight (g) of rats fed a high-fat diet (HFD) was significantly ($P < 0.001$) higher than that in rats fed a normal diet. While, treatment of hyperlipidaemic groups with PPE at doses (50 and 100 mg/kg bw), EA at dose (1 mg/kg bw) and PC at dose (7 mg/kg bw) significantly reduced ($P < 0.001$) the body weight change and liver weight as compared to HFD group.

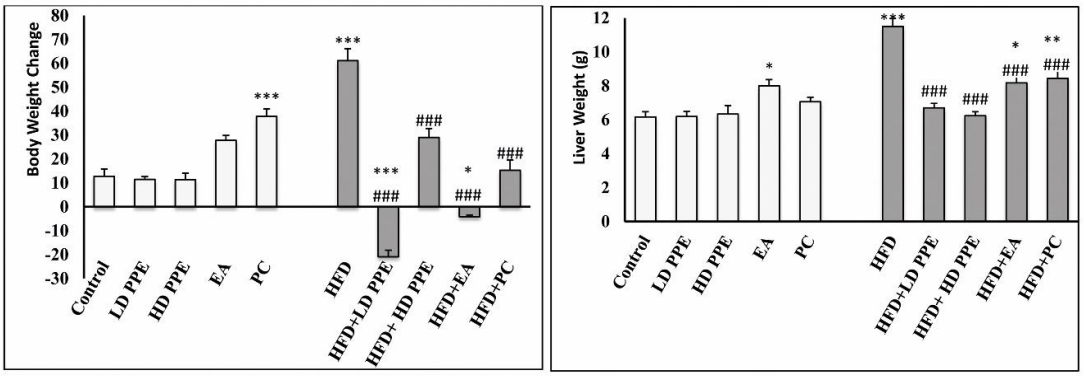
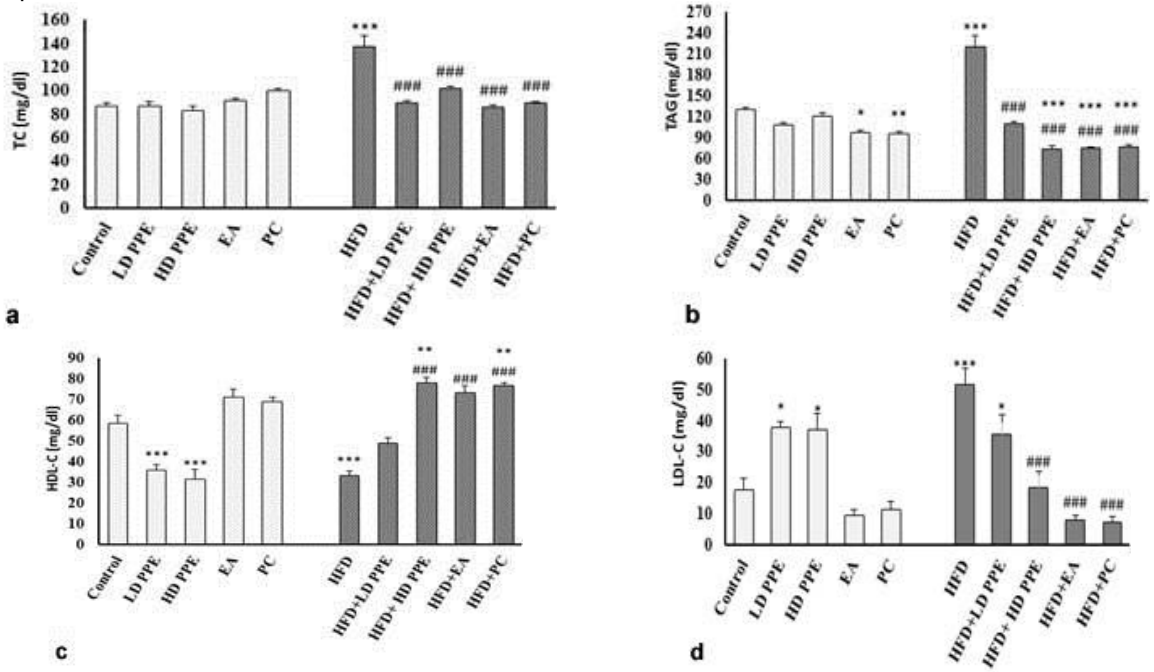


Fig. 1. Body weight change % and Liver weight (g) in non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low or high doses) or ellagic acid or punicalagin. The data represented as Mean ± SEM. P < 0.05 is regarded as significant. *P < 0.05: statistically significant vs. control group, #P < 0.05: statistically significant vs. HFD group. LD: Low Dose, HD: High Dose, PPE: Pomegranate Peel Extract, EA: Ellagic Acid, PC: Punicalagin, HFD: High fat diet.

Effect of treatment on lipid profile:

The levels of lipids (Fig. 2) including TC, TAG, LDL-C and VLDL-C have shown a significant increase ($P < 0.001$) in the serum of HFD group when compared to the control group. On the other hand, oral treatment of hyperlipidaemic groups with PPE at doses (50 and 100 mg/kg bw), EA at dose (1 mg/kg bw) and PC at dose (7 mg/kg bw) significantly decreased ($P < 0.001$) the concentration of TC, TAG, LDL-C and VLDL-C as compared to HFD group.



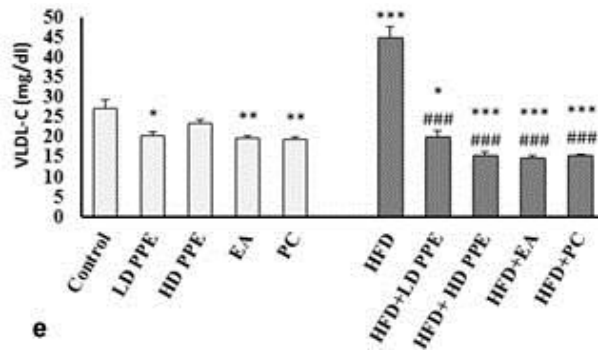


Fig. 2. Total Cholesterol concentration (a), Triacylglycerol concentration (b), High density lipoprotein cholesterol concentration (c), Low density lipoprotein cholesterol concentration (d) and very low-density lipoprotein cholesterol concentration (e) in serum of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low or high doses) or ellagic acid or punicalagin. The data represented as Mean ± SEM.P < 0.05 is regarded as significant. *P < 0.05: statistically significant vs. control group, #P< 0.05: statistically significant vs. HFD group. LD: Low dose, HD: High dose, PPE: Pomegranate Peel extract, EA: Ellagic acid, PC: Punicalagin, HFD: High fat diet.

In contrast, the data presented in figure 2 showed that oral HFD caused a significant decrease (P < 0.001) in HDL-C concentration when compared to control group. Treatment of hyperlipidaemic groups with PPE at dose (100 mg/kg bw), EA at dose (1 mg/kg bw) and PC at dose (7 mg/kg bw) significantly decreased (P < 0.001) the increased the concentration of HDL-C as compared to HFD group.

Effect of treatment on atherogenic index:

As shown in figure 3, oral administration of HFD caused a significant increase (P < 0.001) in AIP and AC when compared to control group. On the other hand, oral treatment of hyperlipidaemic groups with PPE at doses (50 and 100 mg/kg bw), EA at dose (1 mg/kg bw) and PC at dose (7 mg/kg bw) significantly decreased (P < 0.001) the AIP, AC as compared to HFD group.

The data presented in figure 3 showed that oral administration of High fat diet caused a significant increase (P < 0.001) in CRI-I and CRI-II when compared to control group. On the other hand, oral treatment of hyperlipidaemic groups with PPE at dose (100 mg/kg bw), EA at dose (1 mg/kg bw) and PC at dose (7 mg/kg bw) significantly decreased (P < 0.001) the CRI-I, CRI-II as compared to HFD group.

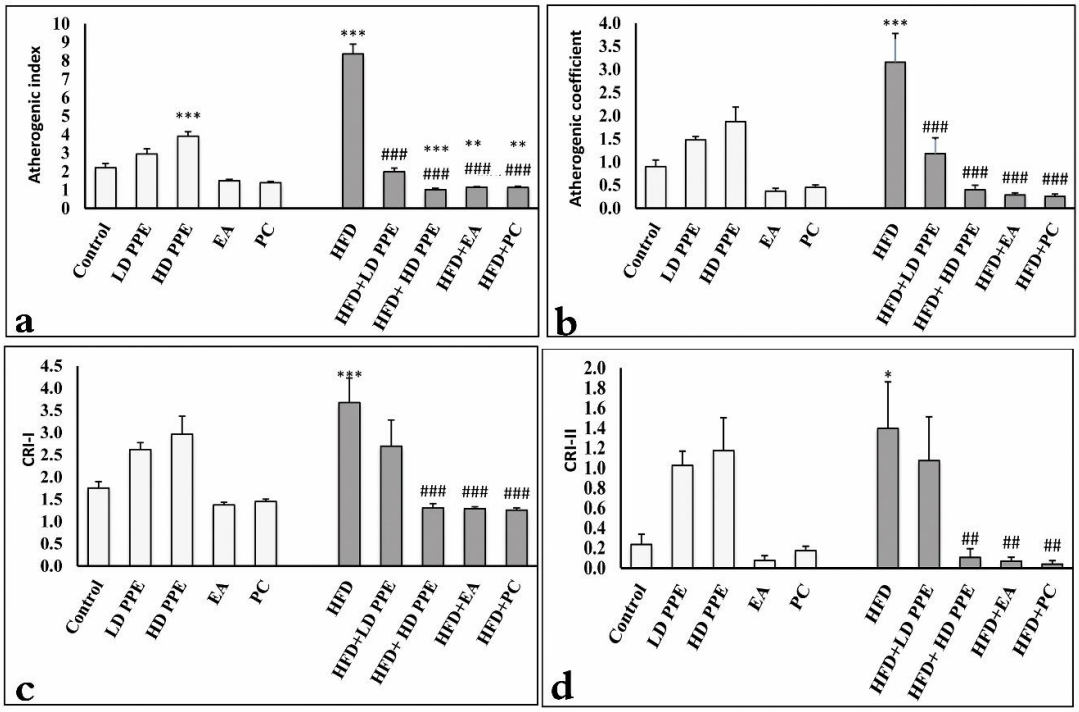


Fig. 3. atherogenic index of plasma(a), atherogenic coefficient (b), Castelli's risk index I & II (c & d) in non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low or high doses) or ellagic acid or punicalagin. The data represented as mean ± SEM. P < 0.05 is regarded as significant. $^{*}P$ < 0.05: statistically significant vs. control group, $^{\#}P$ < 0.05: statistically significant vs. HFD group. LD: Low dose, HD: High dose, PPE: Pomegranate peel extract, EA: Ellagic acid, PC: Punicalagin, HFD: High fat diet.

Effect of treatment on antioxidant capacities in liver tissue:

Antioxidant capacities in the liver tissues are shown in figure 4. Administration of HFD caused a non-significant decrease in GSH level, GR activity, GPx activity and SOD activity when compared to control group. While, a significant decrease ($P < 0.001$) in CAT activity was observed. A significant decrease in hepatic GPx ($P < 0.001$) and SOD ($P < 0.05$) activities following treatment with PEE (50mg/kg bw) was reported as compared to control. Whereas, treatment with PPE at doses (100 mg/kg bw) significantly decrease ($P < 0.05$) SOD and CAT activity. Treatment of hyperlipidaemic rats with PC at dose (7 mg/kg bw) caused a significant increase in the GSH level ($P < 0.001$), and the activities of GR ($P < 0.001$), SOD ($P < 0.001$) and CAT ($P < 0.05$) as compared to hyperlipidaemic animals (Fig. 4). On the other hand, EA at dose (1 mg/kg bw) induced a significant increase in the activities of GR ($P < 0.001$), GPx ($P < 0.05$) and SOD ($P < 0.001$) as compared to hyperlipidaemic animals (Fig. 4).

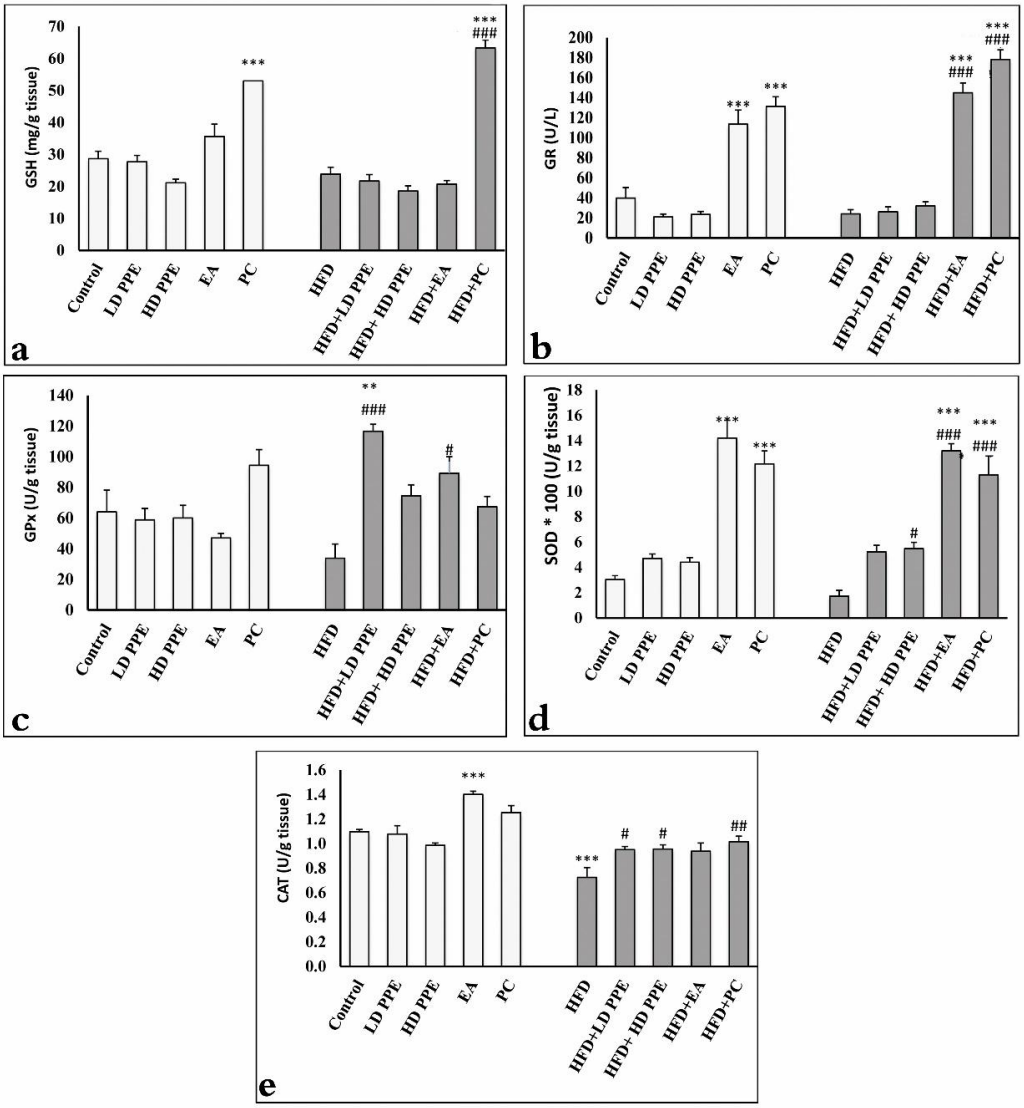


Fig. 4. Reduced glutathione level (a), Glutathione reductase activity (b), Glutathione peroxidase activity (c), Superoxide dismutase activity (d) and Catalase activity (e) in liver tissue of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with Pomegranate peel extract (low or high doses) or Ellagic acid or punicalagin. The data represented as mean ± SEM.$P < 0.05$ is regarded as significant. $^{*}P < 0.05$: statistically significant vs. control group, $^{\#}P < 0.05$: statistically significant vs. HFD group. LD: Low dose, HD: High dose, PPE: Pomegranate Peel Extract, EA: Ellagic Acid, PC: Punicalagin, HFD: High fat diet.

Effect of treatment on hepatic MDA, NOx and serum PON1:

A highly significant increase ($P < 0.001$) in the malondialdehyde (MDA) level was recorded after HFD administration when compared to control group. Whereas, treatment of hyperlipidaemic rats with PPE at doses (50 and 100 mg/kg bw), EA at dose (1 mg/kg bw) and PC at dose (7 mg/kg bw) significantly decreased ($P < 0.001$) the level of MDA as compared to HFD group (Fig. 5). Administration of rats with HFD resulted in a non-significant increase in NOx level in liver tissue and PON1 activity in serum when compared to control group. While treatment of

hyperlipidaemic rat with EA at dose (1 mg/kg bw) and PC at dose (7 mg/kg bw) significantly (P < 0.001) increased the level of NOx as compared to HFD group, however a significant increase (P < 0.01) was observed in the activity of PON1after treatment with PPE at dose (100 mg/kg bw) (Fig. 5).

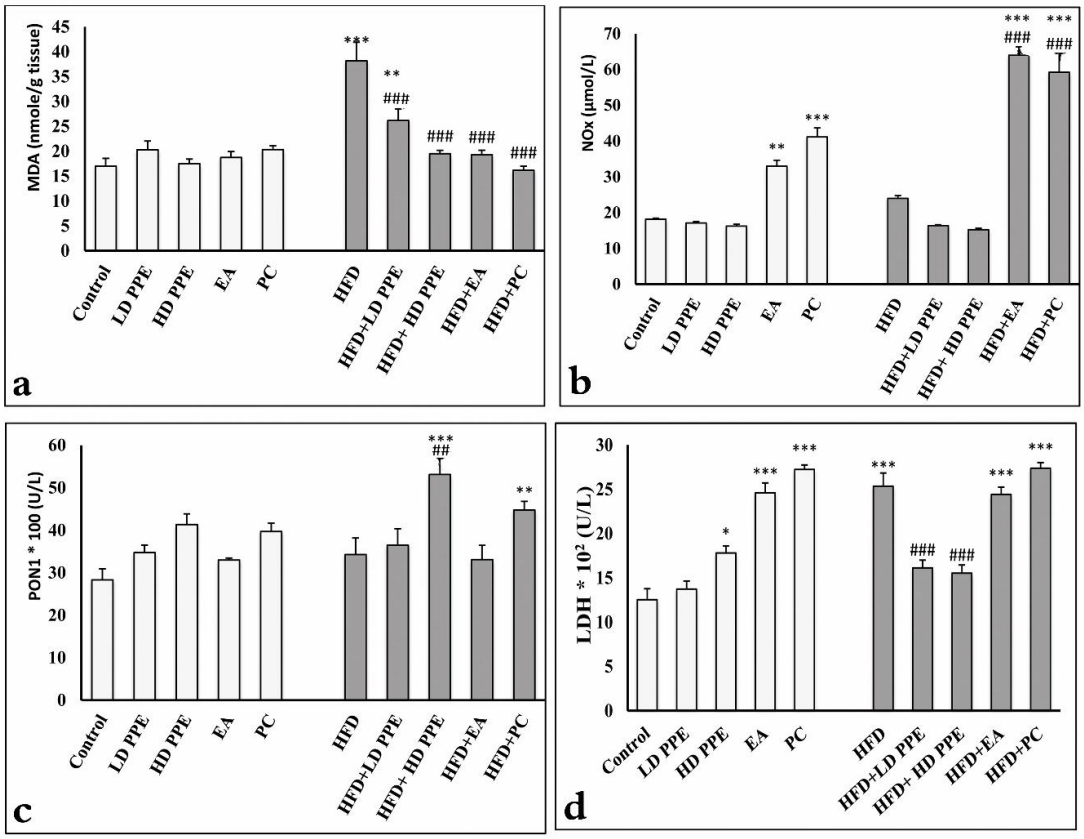


Fig. 5. Malondialdehyde level (a), Nitric oxide level (b), Paraoxonase 1 activity (c) and Lactate dehydrogenase (d) in liver tissue of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with Pomegranate peel extract (low or high doses) or ellagic acid or Punicalagin. The data represented as mean ± SEM. P < 0.05 is regarded as significant. $^{*}P$< 0.05: statistically significant vs. control group, $^{\#}P$ < 0.05: statistically significant vs. HFD group. LD: Low dose, HD: High dose, PPE: Pomegranate peel extract, EA: Ellagic acid, PC: Punicalagin, HFD: High fat diet.

Effect of treatment on serum lactate dehydrogenase activity (U/L):

High fat diet induced a significant increase (P < 0.001) in LDH activity when compared to control group. Oral treatment of hyperlipidaemic groups with PPE at doses (50 and 100 mg/kg bw) significantly (P < 0.001) decreased the activity of LDH (Fig. 5).

Effect of treatment on TNF-α level in serum:

Treatment of hyperlipidaemic group with PPE at doses (50 and 100 mg/kg bw) resulted in a robust inflammatory response as indicated by a marked elevation of serum TNF-α (P < 0.01 and P < 0.001, respectively) as compared to the control group (Fig. 6).

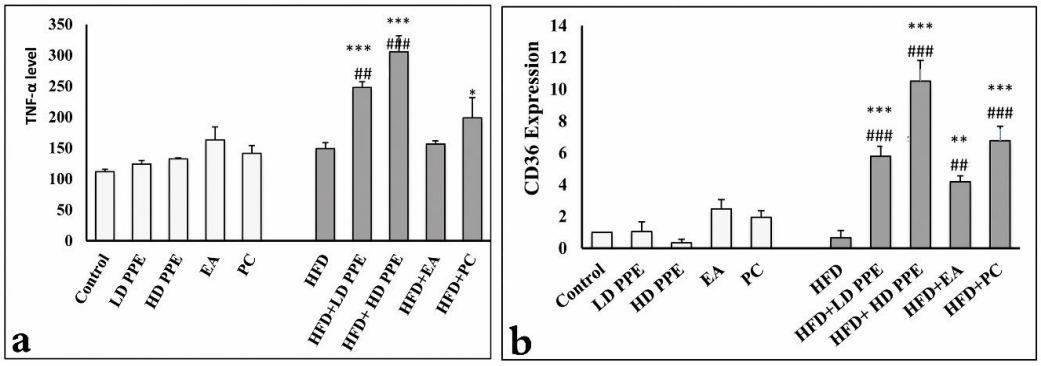


Fig. 6. TNF-α level (a) and CD36 expression (b) in liver tissue of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with Pomegranate Peel extract (low or high doses) or ellagic acid or punicalagin. The data represented as mean ± SEM.P < 0.05 is regarded as significant. $^{*}P$ < 0.05: statistically significant vs. control group, $^{\#}P$ < 0.05: statistically significant vs. HFD group. LD: Low Dose, HD: High Dose, PPE: Pomegranate Peel extract, EA: Ellagic acid, PC: Punicalagin, HFD: High fat diet.

Effect of treatment on the expression of CD36 in dorsal aorta:

RT-PCR analysis demonstrated that oral treatment of hyperlipidaemic groups with PPE at doses (50 and 100 mg/kg bw), EA at dose (1 mg/kg bw) and PC at dose (7 mg/kg bw) significantly increased ($P < 0.001$, $P < 0.01$ and $P < 0.001$, respectively) the expression of CD36 as compared to HFD group (Fig. 6).

DISCUSSION:

Hyperlipidaemia plays a key role in the pathogenesis of atherosclerosis (Guaraldi *et al.,* 2018) that is the vascular lesion responsible for most cardiovascular-related complications including myocardial infarction, stroke, and kidney injury (Abbas and Sakr, 2013). Recently, there is a great interest with the pomegranate as a good medicinal and nutritional source because it is a good source of polyphenolic components such as ellagic acid and ellagitannin which act as natural antioxidants (Sayed-Ahmed, 2014; Gullon *et al.,* 2016; Amri *et al.,* 2017).

The present study was designed to evaluate the effect of PPE in comparison to two its bioactive components ellagic acid and punicalagin on HFD-induced hyperlipidaemia in male albino rats.

It is commonly known that HFD resulted in significant alterations in lipid profile and a depressed antioxidant defence system (Chen *et al*., 2011; Yang *et al*., 2018). In the current study, rats fed HFD showed a significant increase in serum levels of TC, TAG, LDL-C, VLDL-C and atherosclerotic ratios, as well as a significant decrease in HDL-C concentration were observed. These findings are in accordance with those reported in the previous studies (Yokozawa *et al.,* 2006; Hossin, 2009; Suanarunsawat *et al.,* 2010; Kalaivani *et al.,* 2018), which investigated the significant impairment of the lipid profile combined with significant increase in atherosclerotic ratios of HFD-fed rats as compared with normal diet-fed rats.

Elevation of atherosclerotic ratio in current model confirmed that hypercholesterolemia and hypertriglyceridemia are independent risk factors that can accelerate the development of coronary artery disease and the progression of atherosclerotic lesions as reported by McKenney (2001).

Post treatment of HFD-fed rats by PPE at both doses, EA and PC showed marked hypolipidemic effect directly via a significant reduction in serum TC, TAGs, LDL-C, and VLDL-C, combined with significant increase in serum HDL-C levels in comparison to HFD group. These results agree with Neyrinck *et al.* (2013), Sadeghipour *et al.* (2014), and Cao *et al.* (2015).

The observed antilipidemic effect of PPE and its purified polyphenols; EA and PC, suggested that they could have protective effect on the cardiovascular system, because there is an inverse relationship between cholesterol, triglycerides and the incidence of coronary heart disease. This confirmed by a significant reduction in the atherosclerotic ratios as compared to HFD-fed rats in the current study.

In harmony with the current results, Esmaillzadeh *et al*. (2006) reported that pomegranate reduced cholesterol absorption, increased cholesterol excretion in faeces, exerted positive effects on cholesterol metabolizing enzymes, markedly decreased TC and LDL-C and improved the CRI-I and CRI-II. Moreover, Hosomi *et al*. (2011) found that the cholesterol-lowering effect of EA may affect the cholesterol metabolism via decomposing cholesterol into bile acid in the liver by regulating transporter factors.

Oxidative stress is defined as a disturbance in the prooxidant and antioxidant balance within tissues (Küçükgergin *et al.,* 2010; Sies, 2018). Looking to oxidative stress markers, we observed an alteration in the antioxidant enzymes activities of GR, SOD, CAT, GPx, and GSH level during fed HFD. The depletion in antioxidant enzymes activities was associated with elevation of lipid peroxidation in liver of hyperlipidaemic rats.

Hypercholesterolemia leads to the increased production of oxygen free radicals (Khorrami *et al.,* 2018) which exert their cytotoxic effect by causing lipid peroxidation with the formation of MDA as shown in the present study. Elevated levels of lipid peroxidation products may be responsible for some of the pathological effects of hyperlipidaemia.

Baynes (1991) reported that free radicals may also be formed via the auto-oxidation of unsaturated lipids in plasma and membrane lipids. The produced free radicals may react with polyunsaturated fatty acids in cell membranes leading to lipid peroxidation. This increase in liver MDA could be due to increases in the production of reactive oxygen species (ROS) and decreases in the antioxidants. Hypercholesterolemia increases the levels of ROS through various mechanisms. A high-cholesterol diet increases liver superoxide anion generation and NADPH oxidase expression (Csont *et al.,* 2007) that utilizes the antioxidant capacity of the liver (Cui *et al.,* 2011; Prasanna and Purnima, 2011; Olorunnisola *et al.,* 2012)

Oral administration of EA showed a significant increase the activities of GR, SOD, and CAT, while administration of PC significantly increased GSH level, GR, and SOD activities. Additionally, administration of PPE or its pure form polyphenols (EA or PC)

to HFD-fed rats significantly decreased the level of MDA in liver when compared with untreated rats fed with HFD only. This significant improvement in the levels of GSH, MDA, GR, SOD, and CAT activities by administration of PPE or EA or PC suggest their ability to combat oxidative stress.

The observed antioxidant action may be due to their direct ability to capture and scavenge free radicals such as superoxide radical or hydrogen peroxide or may be due to their ability to chelate metal cations like iron involved in free radical formation (Amarowicz *et al.,* 2004). It has been reported that the four hydroxyl and two lactone functional groups of EA structure act, respectively as hydrogen bond acceptors and donors, enabling EA to scavenge $O^{2\bullet-}$, $HO^{\bullet}$, H_2O_2, and $ONOO^{\bullet}$ (Nugroho *et al*., 2014).

In addition, it is reported that EA, besides acting as an antioxidant, enhances the antioxidant status at both enzymatic and non-enzymatic levels in the tissues and also decreases the lipid peroxidation in tissues (Devipriya *et al.,* 2007).

Paraoxonase1 (PON1) is synthesized in the liver and is physically associated with HDL, on which it is almost exclusively located. Several studies have indicated that PON1 has a unique antioxidant property and can prevent lipid peroxide accumulation on LDL both *in vitro* and *in vivo* (Abbott *et al.,* 1995). Pomegranate juice contains punicalagin, which is a potent antioxidant, decreases oxidative stress in serum and increases PON1 association with HDL (Rosenblat *et al*., 2006; Rock *et al.,* 2008). The present results showed a significant increase in serum PON1 activity after treatment with PPE and PC in comparison to HFD-fed rats.

Most studies have reported that HFD induces cardiac dysfunction as a result of increased ROS production (Zeng *et al.,* 2015). In the current study, HFD caused significant increase the activity of serum cardiac enzyme (LDH), which is widely used as a predictor for heart damage.

Ahmed *et al*. (2016) demonstrated that treatment with pomegranate extract reversed this increase of cardiac enzyme LDH activity. In agreement, the present study demonstrated that oral treatment of hyperlipidaemic groups with PPE significantly decreased the activity of serum LDH, indicating strong reversal effect of this extract against HFD-induced damage in myocardium, probably through maintaining membrane integrity and/or permeability thereby preventing leakage of these cardiac biomarkers into the blood (Mollazadeh *et al*., 2016).

Inflammation plays a pivotal role in the development of metabolic syndrome features, including dyslipidaemia and altered glucose tolerance. TNF-α is a potent inflammatory cytokine, that is secreted by activated mononuclear leukocytes, and a wide variety of other immune and non-immune cell types, including fibroblasts, smooth muscle cells, astrocytes, and neurons. (Elkind *et al*., 2002; Pober and Sessa*,* 2015; Mitoma *et al.,* 2018).

In the present study, hyperlipidaemic rat model was accompanied by insignificant increase of TNF-α level. This result is agreement with Cani *et al.* (2008) and Clements *et al.* (2018) who found that increase in TNF-α mRNA concentration after high-fat feeding. This may be due to either oxidative stress that is frequently associated with inflammation and metabolic dysfunction in adipose depots (Houstis *et al.*, 2006) or due to high-fat feeding that is associated with adipose tissue macrophage infiltration (Kanda *et al.,* 2006; Cani *et al.*, 2007).

On the other hand, treatment of hyperlipidaemic groups with PPE at both doses resulted in significantly increase in TNF-α level. These results are agreement with Mueller *et al.* (2010) who reported that pomegranate extract increased the TNF-α secretion to 250–340%.

CD36, a scavenger receptor for oxidized LDL, played an important role in the pathogenesis of atherosclerosis (Glatz and Luiken, 2018). In the current study, HFD-hyperlipidaemic rat model showed decrease in aortic CD36 expression although it did not reach the significance level. On the other hand, oral treatment of hyperlipidaemic groups with PPE doses, EA and PC significantly increased the expression of CD36.

The present data confirmed the previously reported studies that revealed the association of CD36 deficiency with hypercholesterolemia. The aforementioned data suggest that CD36 mRNA downregulation is associated with the dyslipidemia induced atherosclerosis. Thus, CD36 may turn out to be a good expression marker for the atherosclerosis in hyperlipidaemic rats.

In this respect, also Rać *et al*. (2007) and Sun *et al.* (2018) reported that CD36 deficiency underlies defective fatty acid metabolism and hypertriglyceridemia in spontaneously hypertensive rats. In agreement with observations by Goudriaan *et al*. (2005) and Glatz and Luiken (2018) have shown that the absence of the fatty acid translocase CD36 in mice leads to increased plasma free fatty acids levels concomitant with increased TAG and VLDL-C levels.

Other observations in humans and rodents have suggested the possible pathological involvement CD36 deficiency in cardiomyopathy (Hirooka e*t al*., 2001; Kintaka *et al*., 2002; Ojima *et al.,* 2017) and this is agreement with the present results of cardiac

marker elevation combined with CD36 downregulation in HFD group.

Taking all the above results together, it can be considered that CD36 deficiency play an important role in cardiovascular disease and may be predisposed to cardiovascular disease in the presence of risk factors, such as dyslipidaemia and oxidative stress. While upregulation effect of PPE and its pure form of EA and PC on CD36 expression is considered as beneficial anti-atherogenic properties in hyperlipidaemic rats. This is harmony with the previous studies that reported the upregulated effect of anti-atherogenic drugs such as aspirin and atorvastatin on CD36 expression *in vitro* (Ruiz-Velasco *et al*., 2004; Viñals *et al*., 2005).

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التأثيرات المُحسنة لمستخلص قشر الرُمان وبعض مكوناته النشطة حيويا ضد تصلب الشرايين المحدث بارتفاع دهون الدم في ذكور الجرذان

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ثبت أن مستخلص قشر الرمان (*Punica granatum*) (PPE) له نشاط مضاد للأكسدة ويعزى ذلك النشاط إلى محتواه من عديد الفينولات polyphenols بما في ذلك حمض الإيلاجيك (EA) وبيونكالاجين (PC). ومن هنا، تم تصميم الدراسة الحالية لتقييم التأثير المضاد لتصلب الشرايين لكل من مستخلص قشر الرمان وحمض الإيلاجيك وبيونكالاجين عند تغذية ذكور الجرذان على نظام غذائي عالي الدهون (HFD). ولذلك تم إعطاء مستخلص قشر الرمان (50 أو 100 مجم / كجم من وزن الجسم) أو حمض الإيلاجيك (1 مجم / كجم من وزن الجسم) أو بيونكالاجين (7 مجم / كجم من وزن الجسم) عن طريق الفم لمدة ستة أسابيع للجرذان إما مع اتباع نظام غذائي معياري أو بعد الاصابة بارتفاع الدهون في الدم. في نهاية مدة المعاملة، تم تحليل مستوى الدهون في الدم، ونسب تصلب الشرايين، ومضادات الأكسدة / علامات التأكسد في الكبد، انزيم القلب لاكتيت ديهيدروجينيز (LDH)، ومعامل الالتهاب (TNF-α) ومستوى التعبير الجيني ل CD36.

وقد تسبب النظام الغذائي عالي الدهون بزيادة كوليسترول ودهون الدم وتركيزات الكوليسترول في الليبيدات البروتينية المنخفضة الكثافة LDL-C، بينما حدث انخفاض الكوليسترول في الليبيدات البروتينية العالية الكثافة HDL-C بالمقارنة بالمجموعة الضابطة. وتسبب أيضا في ارتفاع كبير في مستوى المالون داى الديهيد (MDA) ونشاط انزيم اللاكتيت ديهيدروجينيز بالإضافة إلى انخفاض كبير في نشاط الكتاليز (CAT) ومستوى التعبير الجينى CD36. وكان لمستخلص قشر الرمان وحمض الإيلاجيك وبيونكالاجين قدرة على تخفيف جميع الآثار الضارة السابقة للنظام الغذائي عالي الدهون من خلال تعزيز مضادات الاكسدة والتأثيرات المضادة لخلل الدهون. وهذه النتائج تدل على أن مستخلص قشر الرمان أو حمض الإيلاجيك أو بيونكالاجين يمكن أن يكون مفيد كخافض لدهون الدم المُحدثة بواسطة النظام الغذائي عالي الدهون وكذلك الإجهاد التأكسدي وتنظيم تعبير CD36 في الجرذان.

نشر البحث = 1600 جنيه

صفحات زيادة = 21 × 20 = 420 جنيه

أشكال زيادة = 2 × 20 = 40 جنيه

المجموع = 2060 جنيه