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THE PROTECTIVE ROLE OF PUNICA GRANATUM EXTRACTED ON SOME ANTIOXIDANTS AND LIPID PROFILE IN FEMALE RATS INDUCED BY HYPOTHYROIDISM

Rand Najem Abed[1], Assist. Prof. Dr. Zainab Ibrahim Mohammed[2]
Department of Physiology and Pharmacology/ College of Veterinary Medicine/ AL-Qadisiyah University, Email: rndnjm119@gmail.com, zainab.mohammed@qu.edu.iq

Abstract

The present study has been designed to investigate wheather *punica granatum* has its own antioxidant activity and/or it act through induction of the hypothyroidism. The study has been conducted on adult male rats at the department of physiology, College of Veterinary Medicine, Al-Qadisiya University during the period extended from June, 2022 to December, 2022. Fourty mature female Wistar rats (aged 90 days and weighted 150±10 g) were used in the five experimental periods of the present study. Have been randomly divided randomly into four equal groups, Control group: will give 1ml of distal water daily for 30 days. The first treated group (T1): will give propylthiouracil(PTU) (Singh etal., 2020) daily at dose 8 mg/kg of body weight orally for 30 days. The second treated group (T2): will give polyphenols of punica granatum peel extract (Parmar&Kar,2008) orally at dose 200 mg/kg daily for 30 days. The third treated group (T3): will give (PTU 8mg/kg) and polyphenols of Punica granatum peel extract (200mg/kg) orally for 30 days. The end of each treated and control period, males were anaesthesized (by injection of 0.3ml ketamine + 0.1 ml of xylazine/ kg b.w. *ip*), dissected and blood samples were obtained from abdominal vein in non-heparinized tubes. Blood serum samples were separated for assessment of GSH, CAT, MDA, cholesterol, Triglyceride, HDL and LDL concentrations. The results of the current study showed that there is an effect of PTU and pomegranate extract on Oxidative-Antioxidants and lipid profile.

Introduction

Thyroid disorders were found to be the most common endocrine problems seen in the world. Among various thyroid disorders, the prevalence of hypothyroidism is more with about 4%–5% worldwide. Females are at more risk of developing hypothyroidism than males (Koyyada & Orsu, 2020). Hypothyroidism is a common condition of thyroid hormone deficiency, which is readily diagnosed and managed but potentially fatal in severe cases if untreated. The definition of hypothyroidism is based on statistical reference ranges of the relevant biochemical parameters and is increasingly a matter of debate. Clinical manifestations of hypothyroidism range from life threatening to no signs or symptoms. The most common symptoms in adults are fatigue, lethargy, cold intolerance, weight gain, constipation, change in voice, dry skin, infertility, and menstrual abnormalities, but clinical presentation can differ with age and sex, among other factors (Chaker et al., 2017). Pomegranate (*Punica granatum L.*) is a polyphenols source, which are very important herbal metabolites that can have various effects on hypothyroidism (Arrak,2010). It has been shown that different pomegranate fruit components such as juice and peel have a significant

amount of polyphenols including ellagic acid (EA), punicalagin, anthocyanins, punicalagin, ellagitannins and tannins (Cheshomi et al., 2020). Study the protective role of polyphenols of punica granatum extract on hypothyroidism in female rats induced by propylthiouracil is largely unknown, so the present study was the first in Iraq to investigate the protective role of polyphenols of punica granatum extract to prevention or suppression effects of hypothyroidism on oxidant status and lipedema in adult female rats by studying the following parameters: Oxidant and Anti-oxidant status : catalase, glutathione, and MDA ; lipid profile: total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL).

Materials and Methods

Plant extraction

Polyphenols of *punica granatum* extract was obtained from in package contain (1Kgm) of polyphenols powder, prepare at a dose (200 mg/ kg of B.W). Propylthiouracil was obtained from (Germany) in package contain (250mg) and prepare at a dose (8mg/ kg of B.W).

Experimental design

Forty adult female Wister rats will have divided randomly into four equal groups:

1. Control group: will give 1ml of distal water daily for 30 days.
2. The first treated group (T1): will give propylthiouracil (PTU) daily at dose 8 mg/kg of body weight orally for 30 days.
3. The second treated group (T2): will give polyphenols of punica granatum peel extract orally at dose 200 mg/kg daily for 30 days.
4. The third treated group (T3): will give (PTU 8mg/kg) and polyphenols of Punica granatum peel extract (200mg/kg) orally for 30 days.

Results

Serum Oxidative stress- Antioxidants

GSH Concentration

The results illustrated in table (1) showed a significant differences ($p<0.05$) between experimental groups at 15^{th} and 30^{th} periods of treatment. Where the results of the current study showed that there was a significant decrease in the level of the GSH in the group that was given PUT compared with the control group on the 15^{th} days of the experiment and on the 30^{th} days as well. While the results of the current experiment showed that there was a significant increase in the second group that was treated with *Punica granatum* compared with the control group in both the 15^{th} and 30^{th} time periods. As for the group that was given PUT and *Punica granatum*, there were significant differences between it and the first and second groups compared with the group the control.

Table (1): effect of PUT and *Punica granatum* treatment on serum GSH concentration (µMole/ml) in mature male rats

Groups	15 Days Mean ± SEM	30 Days Mean ± SEM
C	2.41 ± 0.037[B]	2.41 ± 0.03[C]
T1	1.49 ± 0.014[D]	1.08 ± 0.010[D]
T2	3.84 ± 0.056[A]	4.79 ± 0.069[A]
T3	2.12 ± 0.011[C]	3.02 ± 0.023[B]
LSD	0.332	1.009

The results represented as mean ± SE.
different capital letters denotes a significant differences (P>0.05) between groups.
Simillar capital letters denotes the abscence of significant differences (P>0.05) between periods.
C: male rats drenched with drinking water (0.5 ml).
T1: male rats drenched with PUT (200 mg/kg suspended in 0.5 ml of drinking water) to 30 days.
T2: male rats drenched with *Punica granatum* (200 mg/kg suspended in 0.5 ml of drinking water) to 30 days.
T3: male rats drenched with PUT and *Punica granatum* (200 mg/kg suspended in 0.5 ml of drinking water) to 30 days.

CAT Concentration

The results illustrated in table (2) showed a significant differences ($p<0.05$) between experimental groups at 15th and 30th periods of treatment. Where the results of the current study showed that there was a significant decrease in the level of the CAT in the group that was given PUT compared with the control group on the 15th days of the experiment and on the 30th days as well. While the results of the current experiment showed that there was a significant increase in the second group that was treated with *Punica granatum* compared with the control group in both the 15th and 30th time periods. As for the group that was given PUT and *Punica granatum*, there were significant differences between it and the first and second groups compared with the group the control.

Table (2): effect of PUT and *Punica granatum* treatment on serum CAT concentration (U/ml) in mature male rats

Groups	15 Days Mean ± SEM	30 Days Mean ± SEM
C	0.627 ± 0.007[C]	0.627 ± 0.007[C]
T1	0.45 ± 0.017[D]	0.312 ± 0.007[D]
T2	1.10 ± 0.033[A]	1.51 ± 0.024[A]
T3	0.9 ± 0.025[B]	1.02 ± 0.011[B]
LSD	0.021	0.076

MDA Concentration

The results illustrated in table (3) showed a significant differences ($p<0.05$) between experimental groups at 15th and 30th periods of treatment. Where the results of the current study showed that there was a significant increas in the level of the MDA in the group that was given PUT compared with the control group on the 15th days of the experiment and on the 30th days as well. While the results of the current experiment showed that there was a significant decrease in the second group that was treated with *Punica granatum* compared with the control group in both the 15th and 30th time periods. As for the group that was given PUT and *Punica granatum*, there were significant differences between it and the first and second groups compared with the group the control.

Table (3): effect of PUT and *Punica granatum* treatment on serum MDA concentration (µMole/ml) in mature male rats

Groups	15 Days Mean ± SEM	30 Days Mean ± SEM
C	0.662 ± 0.007[C]	0.662 ± 0.007[C]
T1	2.58 ± 0.035[A]	3.68 ± 0.034[A]
T2	0.497 ± 0.009[D]	0.397 ± 0.006[D]
T3	1.26 ± 0.041[B]	1.85 ± 0.040[B]
LSD	0.004	0.566

Cholesterol Concentration

The results illustrated in table (4) showed a significant differences ($p<0.05$) between experimental groups at 15th and 30th periods of treatment. Where the results of the current study showed that there was a significant increase in the level of the Cholesterol in the group that was given PUT compared with the control group on the 15th days of the experiment and on the 30th

days as well. While the results of the current experiment showed that there was a significant dcrease in the second group that was treated with *Punica granatum* compared with the control group in both the 15th and 30th time periods. As for the group that was given PUT and *Punica granatum*, there were significant differences between it and the first and second groups compared with the group the control.

Table (4): effect of PUT and *Punica granatum* treatment on serum Cholesterol concentration (mg/dl) in mature male rats

Groups	15 Days Mean ± SEM	30 Days Mean ± SEM
C	55.50 ± 0.29[C]	55.50 ± 0.29[C]
T1	85.56 ± 0.26[A]	100.78 ± 0.36[A]
T2	41.42 ± 0.38[D]	34.14 ± 0.38[D]
T3	61.74 ± 0.45[B]	72.12 ± 0.39[B]
LSD	3.899	4.998

Triglyceride Concentration

The results illustrated in table (5) showed a significant differences ($p<0.05$) between experimental groups at 15th and 30th periods of treatment. Where the results of the current study showed that there was a significant increase in the level of the Triglyceride in the group that was given PUT compared with the control group on the 15th days of the experiment and on the 30th days as well. While the results of the current experiment showed that there was a significant decrease in the second group that was treated with *Punica granatum* compared with the control group in both the 15th and 30th time periods. As for the group that was given PUT and *Punica granatum*, there were significant differences between it and the first and second groups compared with the group the control.

Table (5): effect of PUT and *Punica granatum* treatment on serum Triglyceride concentration (mg/dl) in mature male rats

Groups	15 Days Mean ± SEM	30 Days Mean ± SEM
C	144.47 ± 0.68[B]	144.47 ± 0.68[B]
T1	159.807 ± 0.487[D]	130.80 ± 0.22[D]
T2	85.86 ± 0.280[C]	77.45 ± 0.226[C]
T3	95.622 ± 0.303[A]	112.677 ± 0.29[A]
LSD	5.887	6.908

HDL Concentration

The results illustrated in table (6) showed a significant differences ($p<0.05$) between experimental groups at 15th and 30th periods of treatment. Where the results of the current study showed that there was a significant decrease in the level of the HDL in the group that was given PUT compared with the control group on the 15th days of the experiment and on the 30th days as well. While the results of the current experiment showed that there was a significant increase in the second group that was treated with *Punica granatum* compared with the control group in both the 15th and 30th time periods. As for the group that was given PUT and *Punica granatum*, there were significant differences between it and the first and second groups compared with the group the control.

Table (6): effect of PUT and *Punica granatum* treatment on serum HDL concentration (mg/dl) in mature male rats

Groups	15 Days Mean ± SEM	30 Days Mean ± SEM
C	21.27 ± 0.25[C]	21.27 ± 0.25[C]
T1	18.43 ± 0.23[A]	15.26 ± 0.089[A]
T2	35.28 ± 0.38[D]	40.872 ± 0.151[D]
T3	28.12 ± 0.16[B]	31.07 ± 0.31[B]
LSD	1.114	1.879

LDL Concentration

The results illustrated in table (7) showed a significant differences ($p<0.05$) between experimental groups at 15th and 30th periods of treatment. Where the results of the current study showed that there was a significant increase in the level of the LDL in the group that was given PUT compared with the control group on the 15th days of the experiment and on the 30th days as well. While the results of the current experiment showed that there was a significant dcrease in the second group that was treated with *Punica granatum* compared with the control group in both the 15th and 30th time periods. As for the group that was given PUT and *Punica granatum*, there were significant differences between it and the first and second groups compared with the group the control.

Table (7): effect of PUT and *Punica granatum* treatment on serum T4 concentration (ng/ml) in mature male rats

Groups	15 Days Mean ± SEM	30 Days Mean ± SEM
C	6.17 ± 0.061[C]	6.17 ± 0.061[C]
T1	11.22 ± 0.10[A]	13.47 ± 0.192[A]
T2	4.97 ± 0.107[D]	4.06 ± 0.043[D]
T3	7.50 ± 0.051[B]	8.47 ± 0.057[B]
LSD	0.786	1.543

Discussion

Pomegranate (*Punica granatum*) contains several phytochemicals, including punicalagin, ellagitannins, anthocyanins, tannins, hydrolysable tannins, and punicic acid. The punicalagin in *P. granatum* extract is an ellagitannin with unusual and unique antioxidant activity, making it an abundant source of polyphenolic compounds (Arrak, 2010). PUT may disrupt the hypothalamic peptides of the thyroid-releasing hormone, which may contribute to the decrease in thyroid hormones in PUT-exposed animals (Zam et al., 2012). An increase in thyroid hormone in T2 on serum GSH concentration may be due to the antioxidant effect of *P. granatum* or the increase in thyroid hormone or the thyroxine hormone, which stimulates the active form of vitamin D3. However, *P. granatum* peel is a rich source of bioactive components, such as polyphenols, despite not being suitable for human consumption. Depending on the cultivar, environment, and growing region, these compounds are more or less prevalent and serve distinct purposes (Godarzi et al., 2016). The findings are significant because they shed insight into different P's polyphenol and antioxidant content. *granatum* cultivars; this information may be applied to expanding the fruit-processing business and selecting commercially viable *P. granatum* extracts. Arun & Singh (2012) observed that *P. granatum* significantly reduced total cholesterol, LDL, the LDL/HDL ratio, and the total cholesterol/HDL ratio. Patients with hyperlipidemia who consume *P. granatum* juice may have a reduction in cardiovascular risk factors, according to these findings. Arun & Singh (2012) discovered that *P. granatum* polyphenols inhibit breast cancer cell proliferation, invasion, and death. Arun & Singh (2012) found that fermented *P. granatum* juice polyphenols consistently demonstrated twice as much anti-proliferative activity as polyphenols in fresh *P. granatum* juice. Studies on lung cancer have demonstrated that *P. granatum* extract is an effective treatment for the condition. Researchers determined that *P. granatum* has chemopreventive potential against lung cancer. *P. granatum* has been used medicinally for centuries, and various parts of the fruit have been used to cure various diseases. Ancient Egyptians utilised tannin-rich *P. granatum* root treatments to eliminate tapeworms from the human digestive tract. Hippocrates employed *P. granatum* extracts to treat various conditions, including digestive disorders, skin irritation, and eye

inflammation (Jauhar et al., 2018). According to Jauhar et al. (2018), the *P. granatum* peel contains 63 high molecular weight phenolic, ellagitannins, proanthocyanidins, complex polysaccharides, flavonoids, and appreciable quantities of microelements that, when combined, exhibit potent anti-mutagenic, antioxidant, antimicrobial, and apoptotic properties. Zaglool et al. (2020) state that *P. granatum* extracts have been used to cure diabetes in medicine for quite some time. Some researchers examined the effect of *P. granatum* flower extracts on serum lipid profile, pancreatic lipid peroxidation, and enzymatic and non-enzymatic antioxidant status in mice with diabetes induced by hypothyroidism. Blood glucose, total cholesterol, triglycerides, low-density lipoproteins cholesterol, very low-density lipoproteins, lipid peroxidation level, and high-density lipoprotein cholesterol all rose in these rats, but high-density lipoprotein cholesterol dropped. In addition, the decreased glutathione content and antioxidant enzymes glutathione peroxidase, glutathione reductase, and Glutathione-S-transferase indicate oxidative stress. The authors concluded that *P. granatum* could be utilised as a dietary supplement to treat and prevent chronic diseases characterised by an atherogenic lipoprotein profile, an exacerbated antioxidant status, and impaired glucose metabolism (Bouasla et al., 2016). Dietary treatment with polyphenolic antioxidants slows the course of atherosclerosis in animal models by inhibiting the oxidation of low-density lipoprotein and the formation of macrophage foam cells (Sadeghipour et al., 2014). High polyphenol concentrations in commercial *P. granatum* juice confer potent antioxidant and antiatherosclerotic properties. By preventing oxidative stress or lipid peroxidation in arterial macrophage and lipoprotein, phenol-rich foods decrease the progression of atherosclerosis and lower the incidence of heart disease, according to epidemiological research (Pirinccioglu et al., 2012). Compared to the other groups, group T2 exhibited elevated serum aminotransferase levels, decreased glutathione (GSH) levels, and altered hepatic function. The inability to catalyse aminotransferase reactions is indicative of cell failure. The new study's findings are consistent with those of other studies (E. H. Ali & Al-Okaily, 2016; B. Al-Okaily, 2016; Pirinccioglu et al., 2012; Sayed et al., 2022). In addition, fluoride poisoning induced a considerable increase in aminotransferase activity (Al-Mutary & Abu-Taweel, 2020; Al-Okaily, 2019; Loren et al., 2005; Sharifiyan et al., 2016). This may be a secondary effect of PUT-induced, increasing the leaking of these biomarkers from the cell (Ali & Al-Okaily, 2017). Moreover, the ratio of serum CAT was considerably more significant in the T2 group than in the control. In contrast to the T2 group, there was a distinct drop in T3. While the T1 group was not substantially different from the control group, it was significantly higher than the T1 group. Since testosterone levels are essential for spermatogenesis, a significant fall in testosterone may cause a decrease in the number and activity of somatic and germinal testicular cells and reduce testicular weight (Sharifiyan et al., 2016). In contrast, the testes of rats in the T2 group exhibited complete differentiation and proliferation of spermatogenesis in the centre of seminiferous tubules, with compact sperm and protein material in the lumen of seminiferous tubules. This may be due to a rise in testosterone concentration, which stimulates spermatogenesis and speeds spermatocyte maturation, facilitating the shift from round to elongated sperm (Pirinccioglu et al., 2012). According to Loren et al. (2005), *P. granatum* is purported to have safety and prospective nutritional and therapeutic health benefits. Anthocyanins,

ellagic acid, gallic acid, glucose, ascorbic acid, rutin, iron, and amino acids are some of the phenolic compounds in *P. granatum* shown to scavenge free radicals, reduce lipid peroxidation and macrophage oxidative stress, and increase the plasma antioxidant capacity in humans. These help to reduce unhealthy lifestyles or any fatigue, stress or diseases. Hence, this can be related to this present study. When tested for their ability to inhibit platelet responses to collagen and arachidonic acid, *P. granatum* juice and polyphenol-rich extracts from *P. granatum* fruit passed with flying colours; polyphenol-rich extracts from *P. granatum* fruits demonstrated a decisive action in inhibiting platelet activation, active at concentrations comparable to those after ingestion. The researchers concluded that the favourable effects of *P. granatum* on cardiovascular health might partly arise from the polyphenols' ability to inhibit platelet activity (Middha et al., 2013). *P. granatum* polyphenols may improve cholesterol metabolism by inhibiting cholesterol transport in HDL form. Paraoxonase is an enzyme involved in the most crucial process of *P. granatum* extract (which includes ellagic acid, a cholesterol-fighting compound) (Katana1 et al., 2019). Paraoxonase (PON) is an HDL-associated enzyme associated with cholesterol and atherosclerosis; low PON activity is associated with high cholesterol and an increased risk of atherosclerosis; PON's hypocholesterolemic properties may be related to its ability to protect against lipid peroxidation (Middha et al., 2013; Sadeghipour et al., 2014). Researchers have shown that polyphenols can increase HDL blood levels (Al-Okaily, 2016; Naghizadeh-Baghi et al., 2015; Sharifiyan et al., 2016). Because phenolic chemicals activate Lecithin Acyl Transferase (LCAT), a protein that helps integrate cholesterol-free with high-density lipoproteins (HDL), they may be responsible for this rise in HDL. These phenolic substances may enhance performance by activating the lipoprotein lipase enzyme, which plays a role in protein and high-density lipoprotein metabolism. After treatment with *P. granatum* extract, lipid peroxidation and nitric oxide levels in serum and brain cell homogenate are reduced. *P. granatum* extract may neutralise free radicals by removing them from oxidant molecules (Al-Mutary & Abu-Taweel, 2020). Numerous individuals use fresh or juiced *P. granatum* extract. Ancient societies have acknowledged and utilised the medical properties of *P. granatum* extract. After the two-week intervention, those who consumed *P. granatum* juice had significantly greater serum levels of arylesterase, SOD, and GPX than the control group. In addition, compared to the control group, the supplement group with *P. granatum* juice showed reduced MDA levels after the rigorous exercise (Bouasla et al., 2016). The research team led by Fenercioglu et al. discovered that *P. granatum* extract had important antioxidant and anti-lipid peroxidation activities. Schmidt et al. found that high levels of physical effort during training in a cold climate at a moderate altitude were related to increased oxidative stress (Shishavan et al., 2017). Haghighian et al. (2021) previously established that long-term supplementation with *P. granatum* peel extract reduced oxidative stress and improved hepatic structure and function in rats with bile duct ligation. Pre-treatment of rats with *P. granatum* peel extract decreased lipid peroxidation and greatly improved the free-radical scavenging activities of catalase, superoxide dismutase, and peroxidase. This study revealed that treatment with *P. granatum* extract increased GSH, suggesting that GSH may be crucial in protecting cells from oxidative damage. GSH and the activities of glutathione reductase and glutathione peroxidase,

both essential components of the GSH-redox cycle, play a crucial role in limiting the propagation of free radical reactions, which would otherwise lead to extensive lipid peroxidation, and thus provide significant protection in oxidative injury (Sharifiyan et al., 2016). Hussen & Arrack (2014) found a favourable correlation between the phenolic content of *P. granatum* peel extracts made with methanol, water, and acetone and their antioxidant activity using the beta-carotene-linoleate model system. Faddladdeen & Ojaimi (2019) examined the antioxidant activities of *P. granatum* peel extracts and compared them to punicalagin, the primary polyphenol in *P. granatum*. This indicated a synergistic effect between the numerous phenolic components in the peel extract since the extract exhibited more excellent antioxidative activity than punicalagin. Possible reasons for the reported GSH levels include a rise in oxidative stress state supported by lower GSH levels and interference of *P. granatum* extract with GSH formation or metabolism. After consuming *P. granatum* extract, oxidative damage to biomolecules decreased, showing that the first hypothesis is implausible (Mustafa & Ali, 2010). GR is responsible for regenerating GSH from glutathione disulfide (GSSG), formed during oxidation processes. It was discovered that when *P. granatum* was consumed, GR activity was lowered. The activity of this enzyme may have decreased due to a decrease in total GSH (Haghighian et al., 2021). When glutathione levels are low, GR activity cannot avoid being diminished. Tannic acid and coumarins are polyphenols that have been demonstrated to decrease GR activity, and their presence in *P. granatum* may account for the observed GR inhibition. GST, a phase-II enzyme, is an additional GSH-related enzyme that decontaminates several substrates (Hasan et al., 2016). A decrease in GST activity may mirror the decrease in protein damage. Additionally, it has been hypothesised that *P. granatum* polyphenols (such as ellagic and tannic acid) regulate this enzyme in a manner comparable to how GR is regulated (Pirinccioglu et al., 2012). Lastly, *P. granatum*'s polyphenols are responsible for its effect on GSH level; these polyphenols are known to modulate the transcription and expression of proteins involved in endogenous antioxidant defence by interacting with antioxidant response elements in the promoter regions of genes encoding proteins involved in oxidative injury management (Sayed et al., 2022). CAT is the primary mechanism for peroxide detoxification. When iron is a catalyst, the antioxidant enzyme CAT destroys hydrogen peroxide (H2O2), producing less damaging hydroxyl radicals (OH). Through the GSH redox cycle, GSH and GPx turn H2O2 and lipid peroxides into non-toxic molecules. The phenolic components of *P. granatum* have been used as antioxidants to prevent organ damage caused by lipid peroxidation. Rats treated with *P. granatum* had elevated SOD and CAT activity in the cells, while MDA levels (a consequence of lipid peroxidation) were dramatically reduced. These findings indicate that *P. granatum* juice possesses potent antioxidant effects (Pirinccioglu et al., 2012). Some individuals living at sea level have plasma MDA and exhaled pentane levels above the norm. Given that this tripeptide, glutathione, protects against oxidative damage, its fluctuating levels were studied (Al-Okaily, 2016). When the GSH/GSSG ratio falls below a certain level indicative of a particular cell population, the viability of the cells is jeopardised. In hypoxia, GSSG levels are a biomarker of oxidative stress since they are believed to be especially sensitive to this state. Because glutathione reductase activity is lower in muscle and blood, GSSH levels are higher (Middha et al., 2013).

Understanding oxidative stress is crucial as one of the most fundamental causes of PUT's chronic illnesses. It enhances oxidative intermediates' development, increasing oxidative damage. SOD, as one of the most important antioxidant enzymes, prevents the generation of damaging free radicals by converting harmful superoxide radicals into innocuous hydrogen peroxide (Ali et al., 2017). GSH also assists glutathione peroxidase in neutralising free radicals, thereby protecting cells from oxidative damage. Therefore, their concentrations decrease with oxidative stress. Lipid peroxidation is a sign of oxidative stress generated by the interaction of polyunsaturated fatty acids (PUFAs) with free radicals, which generates malondialdehyde (MDA) and has adverse effects such as cell necrosis and inflammation (Mustafa & Ali, 2010). Due to their redox properties, phenolic compounds enhance the antioxidant properties of *P. granatum* extract. They neutralise lipid free radicals and prevent the decomposition of hydroperoxides into free radicals. In contrast to the findings of earlier investigations, we observed that *P. granatum* extract did not significantly raise the levels of SOD or MDA in the body (Pirinccioglu et al., 2012). The combination of *P. granatum* extract and sitagliptin has not been investigated in any previous clinical trials. Given that *P. granatum* extract and sitagliptin have their unique mechanism of action, the observed results indicate their combined effects. GSH and SOD levels increased considerably compared to the group with PUT (Bouasla et al., 2016). Compared to controls, significantly lower levels of SOD were found. The GSH levels in the buffer control group returned to their pre-treatment levels after being considerably reduced. Renal cell histopathology results improved the most when *P. granatum* extract was administered in conjunction with conventional treatment. Routine renal histopathology is the norm (Hasan et al., 2016). Cysteine, glycine, and glutamic acid are the three amino acids that comprise glutathione in its reduced form. It can detoxify reactive oxygen species through the enzyme glutathione peroxidase or function non-enzymatically through the interactions of reactive oxygen species with sulfhydryl (SH) groups (Loren et al., 2005). Rats treated with lead acetate demonstrate a considerable increase in lipid peroxidation and a decrease in glutathione levels. Lead's strong affinity for attaching to the sulfhydryl groups of glutathione may be responsible for the observed oxidative damage. Rats treated with lead acetate and given *P. granatum* extract exhibited a significant increase in glutathione and a decrease in malondialdehyde. This study reveals that *P. granatum* extract reduces oxidative stress in lead-treated rats by increasing glutathione levels and decreasing MDA levels (Sadeghipour et al., 2014). GST, which has a considerable affinity for glutathione, is one such enzyme. GST activity is considerably increased in lead-exposed individuals due to the protein's high affinity for glutathione. After co-treatment, GST activity reduces dramatically, and it decreases much more in the exposed group compared to the control group. Many antioxidant enzymes cannot perform their functions without cofactors (Zaglool et al., 2020). A considerable drop in the activity of superoxide dismutase, catalase, and glutathione peroxidase is indicative of a redox imbalance, which precipitates oxidative stress. As its prosthetic group, catalase is one of the essential antioxidant enzymes (Shishavan et al., 2017). Evidence shows that SOD, CAT, and GST form a coordinated defence against reactive oxygen species. Here, we demonstrate that PUT strongly inhibits the activity of several antioxidant enzymes. Some examples are catalase, superoxide dismutase,

glutathione peroxidase, glutathione reductase, and glutathione S-transferase. The decrease in antioxidant enzyme activity following intravenous administration of PUT is most likely due to protein inactivation induced by reactive oxygen species (Middha et al., 2013). Regardless of whether the supplement is taken in conjunction with acrylamides, our findings indicate that supplementation with *P. granatum* extract significantly increases SOD, GSH, and CAT levels and decreases MDA levels in the blood. These findings are comparable with those presented in Naghizadeh-Baghi et al. (2015), which demonstrated that *P. granatum* extracts decreased MDA levels and increased GSH-Px activities, supporting the positive effects of the *P. granatum* extract on function and oxidative markers (Ali & Al-Okaily, 2016). Increased GSH levels following therapy with *P. granatum* extract may aid in preventing oxidative damage to cells. In the GSH redox cycle, GSH, glutathione reductase, and glutathione peroxidase govern the propagation of free-radical reactions that lead to lipid peroxidation (Middha et al., 2013). Caruso et al. (2020) reported an increase in the malondialdehyde (MDA) level (a marker of lipid peroxidation) and enhanced activity of antioxidant enzymes (GR and GPx) in *P. granatum*-administered groups, resulting in the enhanced ability to scavenge toxic free radicals such as hydrogen peroxide and lipid peroxy (Sadeghipour et al., 2014). The edible portion of *P. granatum* is abundant in vitamin C, and the overexpression of hormones and enzymes has been related to the aetiology of the disease. To maintain safe levels of reactive oxygen species (ROS), antioxidant mechanisms mix with them to produce less reactive molecules. Several recent studies have reached the same conclusion, demonstrating that acrylamides are detrimental because they promote lipid peroxidation and impair antioxidant enzyme systems, resulting in adverse effects on cells (Katana1 et al., 2019). Zaglool et al. (2020) reported that these protective effects could be attributed to lower levels of TGF-1 and MDA and increased GSH-Px activity, Nrf2 and HO-1 expression; this suggests that antioxidative activity may be a potential mechanism for *P. granatum*'s protection against acrylamide toxicity, which is consistent with our findings. Following the administration of *P. granatum* extract, low TNF- and MDA levels were detected, according to the present study's findings. TNF- and MDA levels remained significantly higher than the control groups, although GSH and SOD levels rose dramatically compared to the diabetes group. While GSH and SOD levels improved modestly, they remained significantly below those of the control group. In a prior study, *P. granatum* extract administration was reported to decrease TNF- and MDA levels while dramatically increasing GSH and SOD levels (Adebisi et al., 2022).

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