Trop J Nat Prod Res, September 2023; 7(9):4026-4031 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Hypoglycemic and Hypolipidemic Effects of Ethanol Extract of Pomegranate Peels, Rinds, and Seeds in Alloxan-Induced Diabetic Rats

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ARTICLE INFO

Article history:
Received 30 July 2023
Revised 14 August 2023
Accepted 02 September 2023
Published online 01 October 2023

ABSTRACT

Pomegranates are one of the fruits that are most frequently consumed in the Middle East and have a medical effect. This study aimed to examine the antidiabetic and antilipidemic effect of pomegranate fruits parts including peels, seeds, and rinds extracted using ethanol on the blood glucose, cholesterol, triglyceride, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very-low-density lipoprotein (VLDL). Control (non-treated) rats and alloxan-diabetic-induced rats were used in this study. The alloxan-diabetic-induced rats were divided into four groups and each group received a subcutaneously injected either peels, seeds, or grind ethanol extract, and a group was treated with the drug glipizide for 28 days. Data showed a significant effect for the pomegranate peels and rind ethanol extract in lowering glucose levels (P = 0.0295) with no significant effect for the juice extract on blood glucose levels. Cholesterol, triglycerides, LDL, and VLDL were all significantly reduced by the pomegranate fruit component extracts employed in this study (P = 0.014). However, no significant effect was observed for any extract on the HDL levels in the blood (P > 0.5). In conclusion, the rind of the pomegranate can be used in optimizing blood lipid profile and glucose, in addition to seeds and peels.

***Keywords*:** Pomegranates, Ethanol extract, Diabetes, Cholesterol, Triglyceride, Low-density lipoprotein, High-density lipoprotein, Very-low-density lipoprotein.

Introduction

The plants' biological activities, progression, and survival are regulated due to the production of several secondary metabolites. These substances are playing a role in plants' defense chemicals against both biotic stresses and abiotic stress.[1,2] Plant extracts have been used for a long time for medical purposes, such as; drugs, nutraceuticals, and cosmetic ingredients. [3] These extracts contain active compounds that are mainly unknown but contribute to the extract's potency.[4] The active compounds mostly consist of secondary metabolites that are divided into four types based on chemical properties and structures; terpenoids, polyphenols, nitrogen, and sulfur-containing compounds.[5]

Fruits can be particularly effective anti-diabetic agents because we consume them every day. Other fruit parts, such as black plum and litchi seeds, lemon peels and leaves, and pomegranate flowers, can also be prioritized in diabetes management. Additionally, reported antidiabetic phytochemicals from these sources (fruits and other plant parts) such as swertisin, quercetin, berberine, hesperidin, kaempferol, 6-gingerol, ellagic acid, pinitol, and other potential phytochemicals should be thoroughly evaluated to reveal novel therapeutics against diabetic disorders.[6]

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Citation: Alqaisi KM, Al-Qaisi TS, Alhmoud JF, Farah HS, Hamdan TA, Ahmed KA, Elbadrawy E. Hypoglycemic and Hypolipidemic Effects of Ethanol Extract of Pomegranate Peels, Rinds, and Seeds in Alloxan-Induced Diabetic Rats. Trop J Nat Prod Res. 2023; 7(9):4026-4031 http://www.doi.org/10.26538/tjnpr/v7i9.26

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Ayurveda, ancient Chinese cultures, and Western medical therapies all address the use of fruits and other plant parts in traditional medicine, demonstrating their enormous potential to be a major source of notable anti-diabetic drugs.[7,8]

Pomegranate (*Punica granatum* L.) fruits, broadly cultivated by Mediterranean nations, including Tunisia, Turkey, Egypt, Spain, Morocco, and Italy, are rich in polyphenols, for example; ellagitannins (ETs), basically including α and β isomers of PC, gallic corrosive (GA), ellagic corrosive (EA), and its glycosylated subordinates, and anthocyanins.[9] Polyphenols are the biggest and most abundant group of bioactive compounds in the plant realm. It has a special structural skeleton comprising at least one fragrant phenyl ring associated with hydroxyl gatherings. Polyphenols display a wide range of wellbeing-related properties including antioxidant protection, anti-inflammatory, anti-allergic, and anti-atherogenic.[10] In addition, it showed numerous anticarcinogenic properties such as; inhibitory impacts for malignancy cell expansion, cancer development, angiogenesis, metastasis, inflammation, and stimulating apoptosis. Moreover, they can modify the response of the immune system and reduce the damage that causes by free radicals and affect normal cells.[11,12]

Pomegranates are significantly handled by food manufacturers to acquire squeezes or jams from the arils, while the peels comprise around half of the organic product weight. It has been accounted that the higher substance produced from the peels are mainly dietary fiber and absolute polyphenols, besides potent antioxidant capacity (AC) than the pulp fraction of the fruit itself. Accordingly, they could be an important wellspring of concentrates for cosmetic and nutraceutical applications.[13] The finding recommends that these mixtures might have defensive action against degenerative chronic diseases, for example, certain kinds of malignancies, type 2 diabetes, atherosclerosis, and cardiovascular infections.[14] Besides, previously published studies focused on the impact of antibacterial and antiviral activity including the inhibition of influenza and herpesvirus replication from the pomegranate peel extracts (PPEs).[15,16,17]

Dyslipidemia is characterized by an imbalance in the levels of total cholesterol, triglycerides, low-density lipoprotein cholesterol, and low levels of high-density lipoprotein cholesterol.[18] One of the risky components of coronary heart disease that raises the possibility of cardiovascular mortality is dyslipidemia.[19] Pomegranate extracts have been shown in multiple published articles to help people lose weight because they reduce dyslipidemia that causes obesity and cardiovascular risk factors.[20,21] Research findings indicated that administering a hydroethanolic extract of *P. granatum* peel has significant anti-hyperlipidemic benefits in rats fed a high-lipid diet. In contrast to saline-treated rats, pomegranate extract lowered blood cholesterol, triglycerides, low-density lipoprotein (LDL), alanine transaminase (ALT), and aspartate aminotransferase (AST), while boosting serum high-density lipoprotein (HDL) levels in high-fat diet-fed rats. In addition, as compared to a saline group, the extract reduced liver damage such as fatty changes in hepatocytes, sinusoid dilatation, and congestion in rats fed a high lipid diet.[22] According to reports, the various sections of pomegranate (*P. granatum*) have been identified as a reservoir of bioactive chemicals with potential biological activity. Pomegranate, particularly the leaves, reduced obesity-related dyslipidemia and coronary heart disease.[20] Pomegranate leaf extracts' potential to reduce obesity.[21]

Pomegranate blossom, on the other hand, has been shown to improve hyperlipidemia and diminish excess cardiac lipid buildup in Zucker diabetic fatty rats, and reduce atherosclerosis in apolipoprotein E defective mice.[23,24] Furthermore, oleanolic acid and ursolic acid, two active components found in pomegranate blossom, have long been known to have anti-hyperlipidemic activities.[25] Gallic acid, another key component of pomegranate blossom, has been shown in animal studies to ameliorate high-fat diet-induced hyperlipidemia and fatty liver.[26] In terms of treatment, a study on the liver of Zucker diabetic fatty rats showed that using the pomegranate flower extract for almost 6 weeks helps to make improvements to fatty liver, by reducing related and total hepatic triglyceride contents and fatty droplet remnants.[27] In conventional Chinese medication, several pomegranate concentrates including the bark, root, and squeeze of the organic product, particularly the dried strips, have been utilized to treat many cases.[20]

Pomegranate juice (PJ) has grown in popularity as a result of its phenolic components and therapeutic potential. Its possible hypoglycemic impact has been linked to enzymatic inhibition, insulin release, and pancreatic tissue protection. These effects are determined by a variety of factors, including the amount and composition of phenols in pomegranate juice and the features of the organism that consumes the juice.[28] Hyperglycemia is linked with diabetes mellitus and leads to cause several problems and early death as a result of continual oxidative stress, it is the primary dysfunction that must be addressed. Many pomegranate components, such as the peel, seeds, blossoms, and juice have exhibited hypoglycemic effects linked to their phenolic compounds through the inhibition of carbohydrate metabolism enzymes activation of insulin released by -cells, and preservation of pancreatic tissue.[29,30,31]

Hyperglycemia induces oxidative stress and inflammation by increasing the production of reactive oxygen species (ROS), which is associated with an increase in the production of adhesion molecules and proinflammatory cytokines. Diabetes is therefore a chronic pro-inflammatory syndrome that reduces the antioxidative capability of cells, rendering them more susceptible to harm.[32] Pomegranates are high in phytochemicals such as polyphenols, fatty acids, amino acids, tocopherols, sterols, terpenoids, and alkaloids, which have therapeutic benefits and are used for anti-inflammatory properties, antioxidants, or antineoplastic, hypoglycemic, lipid-lowering, or antimicrobial effects. Pomegranate components have the potential to treat a wide range of diseases and conditions, including cardiovascular disease and diabetes mellitus.[33]

In a recent study, 60 patients received twice-daily administration of 5 g of pomegranate seed powder or a placebo for 8 weeks and found that supplementing with PSP may have a positive effect on fasting blood sugar (FBG) and glycated hemoglobin (HbA1c) in type 2 diabetes mellitus patients. However, its effect on triglycerides (TG) and cholesterol was inconclusive.[34] Another study found that taking pomegranate peel extract for 8 weeks reduced systolic and diastolic blood pressure in patients with type 2 diabetes. This treatment has also been shown to improve blood levels of TG, HDL-C, TBARS, HbA1c, and fatty acid profile in these patients' total plasma lipids, indicating the hypolipidemic, hypoglycemic, and antioxidative potential of pomegranate peel extract. Additional research is required to gain a deeper understanding of the molecular mechanisms underlying these intricate effects of pomegranate peel polyphenols on lipid and glucose metabolism in metabolic disorders.[33]

Although the biological activities of pomegranate have been studied extensively, the effect of all parts of pomegranate fruit on blood glucose and lipid profile have not been the focus of research. Therefore, this study aimed to investigate the hypoglycemic, antihyperlipidemic effect of ethanol extract of pomegranate different fruit parts including grind in groups of rats with alloxan-induced diabetes.

Materials and Methods

Pomegranate peel, rind, and juice ethanol extract

Fresh Turkish pomegranate fruits were purchased from the local market during the autumn season (September / 2017) from Taif City, Kingdom of Saudi Arabia. The plant's scientific name and classification were identified based on International Plant Name Index (IPNI) and Kew Botanic Garden. The fruits were washed well. Then, the fruits were peeled, and the peel, rind, and seeds were separated manually. All the separated parts were washed well followed by washing with distilled water. The extraction methods were used as previously mentioned[35,36] with some modifications. In brief, the peel and rind each were cut into small pieces of approximately 2 cm X 2 cm. These cut pieces and the seeds were then dried for 3 days using the oven at 45^0C. The dried parts were grinded using an electronic grinder, the obtained fine powder was dissolved using 70% Ethanol by stirring at 1000 rpm at room temperature for 3 days. Then, the ethanol extract was filtered twice using filter paper followed by a concentration step which was performed using a rotary evaporator at 90ºC until dried and stored in the fridge until further use.

Animals and treatment

A total of 36 adult Wistar male rats (250 – 300 g) were used in this experiment which was performed at the Animal facility of the College of Medicine at Taif University, Taif, Saudi Arabia. All the procedures that were performed in this experiment have been approved by the institutional review board (2016/23/4332-6) at Taif University by the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH Publication No. 80-23; 1996).

The animals were kept in controlled conditions throughout the experimental procedure (12-h dark/light cycle, temperature of 25^0C, and a relative humidity of approximately 50%) and housed in standard cages where food and water were provided ad libitum. Animals were divided into two main groups as follows:

Group I: Consisted of 6 rats and the animals were injected with only normal saline.

Group II: This group consisted of 30 rats that were subcutaneously injected with a single dose of alloxan (120 mg/kg) after 12 h fasting with free access to drinking water to induce diabetes. The rats were then kept for one week for the stabilization of diabetes. All rats had their diabetes measured using a blood glucose meter. The rats were then divided into five more subgroups, each with six rats. as follows:

Subgroup I: diabetic group without treatment and injected with only normal saline (diabetic non-treated).

Subgroup II: a diabetic group that received a daily subcutaneous injection of glipizide (5 mg/kg) for 28 days (diabetic treated).

Subgroup III: diabetic group received a daily subcutaneous injection of pomegranate peel ethanol extract (625 µg/kg diluted in saline solution) for 28 days.

Subgroup IV: diabetic group received a daily subcutaneous injection of pomegranate rind ethanol extract (625 µg/kg diluted in saline solution) for 28 days.

Subgroup V: diabetic group received a daily subcutaneous injection of pomegranate juice ethanol extract (625 µg/kg diluted in saline solution) for 28 days.

Blood Sample Collection and Biochemical Analysis

Blood samples were collected in plain tubes every week starting from the beginning of the first week (before the first treatment). Approximately 1 ml of blood was collected from each rat every week through a retro-orbital bleeding procedure and the serum was separated by centrifugation. The collected serum was stored at – 20^{0}C until further analysis.

Glucose levels, cholesterol levels, triglycerides levels, HDL levels, LDL levels, and VLDLc levels were all measured using Hitachi 7020 automatic biochemical analyzer (Japan) by using enzymatic reaction assay, and the absorbances were measured at wavelength range from 510 to 570 nm according to each parameter measured.

Statistical analysis

The statistical analyses were performed using GraphPad Prism 7 Software. Data were checked for normal distribution. The significance of differences between groups was assessed using a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons tests, and an unpaired t-test was used to compare the means of the two groups. All the results were expressed as the mean and standard error of the mean (SEM), and a *P*-value less than 0.05 was considered statistically significant.

Results and Discussion

Effect of pomegranate peel, rind, and seed extracts on glucose levels

The glucose levels were measured in all the studied groups and the results (Figure 1) show a significant difference (P = 0.0295) in the blood glucose levels between the diabetic non-treated group and the diabetic-treated group with glipizide. After approximately 28 days of daily glipizide treatment, the glucose levels in this treated diabetic group reach the levels of control non-diabetic rats (Figure 1). Alloxan is used to induce experimental diabetes in laboratory animals through reactive oxygen species that cause rapid destruction of pancreatic beta cells.[37,38] To compare the effect of pomegranate different fruit parts ethanol extract on glucose levels with widely used diabetic drugs, Glipizide was used in this study as the drug for treating diabetic rats. Glipizide is a "second generation" sulfonylurea compound that is widely used for treating diabetes mellitus Type 2.[39] It promotes insulin release from the pancreatic beta cells, reduces glucose output from the liver, and improves insulin sensitivity at peripheral target sites.[40]

In this study, the glucose levels of the diabetic groups that were treated with pomegranate peel ethanol extract and rind ethanol extract reduced significantly (P = 0.0295) compared to the diabetic non-treated group (Figure 1). The blood glucose levels in these two treated groups reach a level that is similar to the control group (non-diabetic) after 21 days of treatment. Pomegranate fruit and plant have gained the interest of researchers to identify their effect on different blood parameters. In a previous study, the peel alcoholic extract of pomegranate was found to significantly reduce glucose[41] Our data further confirm these results and provide the novel effect of the rind, in addition to the peel, on lowering glucose concentration.

In the current study, there was no significant difference in blood glucose levels between the diabetic non-treated group and the group that was injected with pomegranate juice ethanol extract (P > 0.5, Figure 1). This study result is consistent with a previous finding that showed no significant effect of pomegranate seed juice or powder on glucose levels, despite the slight reduction in the glucose levels after using the juice and powder of seeds.[42]

Effect of pomegranate peel, rind, and seeds extracts on cholesterol, triglyceride levels, and circulating lipoproteins:

The cholesterol levels decreased significantly in the glipizide-treated group when compared to the diabetic non-treated group (P = 0.0015, Figure 2-A). Similarly, this study found that triglyceride levels were significantly lower in the glipizide-treated group (<0.0001) when compared to the diabetic non-treated group (Figure 2-B). In addition, the lipoproteins, LDL, and VLDL were significantly decreased in the glipizide-treated group (P = 0.0041 and 0.0002, respectively) when compared to the diabetic non-treated group (Figures 3-B and C). However, there was no significant change in the levels of HDL between all the diabetic-treated groups and the diabetic non-treated group (P > 0.5, Figure 3-C).

The effect of the drug Glipizide that was used in this study on cholesterol, triglyceride, and LDL blood levels is uncertain. For example, in a study of non-insulin-dependent (Type 2) diabetes patients, glipizide was found to not affect cholesterol, triglyceride, or HDL levels.[43] Another study found a non-significant decrease in cholesterol levels and a non-significant increase in HDL levels when glipizide was used to treat diabetes in patients with non-insulin-dependent diabetes.[44]. Treatment of alloxan-induced diabetic rats in this study with glipizide significantly reduced the levels of glucose, cholesterol, LDL, and VLDLc serum levels. However, there was no effect of glipizide on HDL blood level. The effect of glipizide on cholesterol, LDL, and VLDLc found in this study could be explained by its influence on stabilizing blood glucose levels, which causes changes in lipoprotein metabolism and improves their levels.[45]

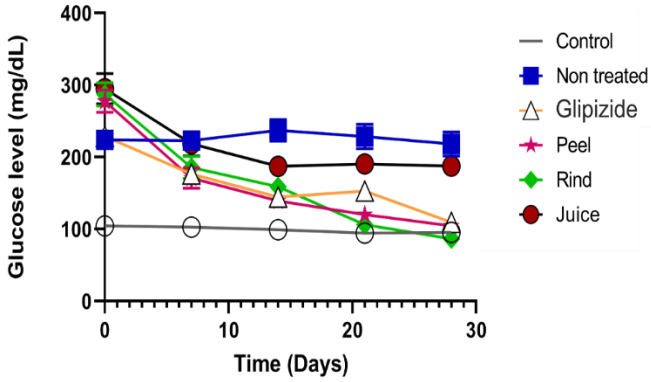

Figure 1: Effect of ethanol extract of pomegranate peel, rind, and seeds on glucose levels. The groups were compared to the non-treated alloxan-diabetic-induced group and the *P* value was as follows: glipizide treated group (0.0295); peel ethanol extract treated group (0.0295); rind ethanol extract treated group (0.0295); juice ethanol extract (>0.5).

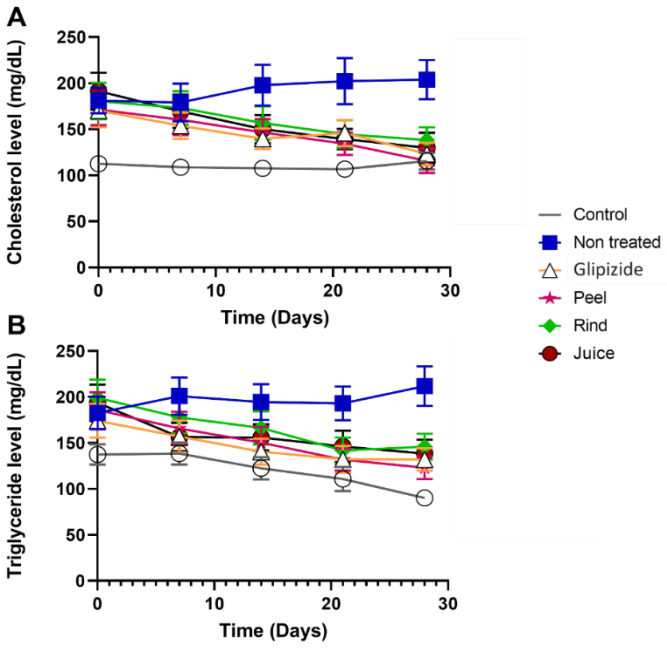

Figure 2: Effect of ethanol extract of pomegranate peel, rind, and seeds on cholesterol and triglyceride levels. The groups were compared to the non-treated alloxan-diabetic-induced group and the *P* value for cholesterol was as follows: glipizide treated group (0.0002); peel ethanol extract treated group

(0.0002); rind ethanol extract treated group (0.0015); juice ethanol extract (0.0015). The *P* value for triglyceride was as follows: glipizide treated group (<0.0001); peel ethanol extract treated group (0.0001); rind ethanol extract treated group (0.0022); juice ethanol extract (0.0005).

Using alloxan-injected rats in this experiment was the choice to examine the anti-glycemic and antilipidemic effect of pomegranate peel, rind, and juice ethanol extract in rats. Furthermore, in addition to using alloxan to induce diabetes in animal models, alloxan also can cause an increase in the levels of cholesterol, triglyceride, and LDL.[41,46-48] The results of the current study are consistent with the previous studies and found that cholesterol, triglyceride, LDL, and VLDLs levels were increased in the diabetic non-treated group when compared to the control group. However, this study showed that alloxan treatment did not significantly affect HDL. This finding is in parallel with Szabadfi and colleagues'[38] findings in that HDL levels were not changed significantly between the alloxan treated and the control groups.

In the current study, the cholesterol levels were reduced significantly in all the treated groups with pomegranate peel ethanol extract, rind ethanol extract, and juice ethanol extract (*P* = 0.0002, 0.0015, and 0.0015, respectively) compared to the diabetic non-treated group (Figure 2-A). After 28 days of treatment, the cholesterol levels in all treated groups reached the cholesterol levels in the control group. Moreover, this study found that triglyceride levels were significantly lower in all the treated groups when compared to the diabetic non-treated group (Figure 2-B). The *P* values were as follows: 0.0001 for the peel ethanol extract-treated group, 0.0022 for the rind ethanol extract-treated group, and 0.0005 for the juice ethanol extract-treated group. For the studied lipoproteins, HDL, LDL, and VLDL (Figure 3), it was found that LDL and VLDL significantly decreased in all the diabetic-treated groups when compared to the diabetic non-treated group (Figures 3-B and C). For instance, LDL and VLDL reduced significantly in the peel ethanol extract-treated group (*P* = 0.0029 and 0.0004, respectively), rind ethanol extract-treated group (*P* = 0.0136 and 0.0015, respectively), and juice ethanol extract-treated group (*P* = 0.0134 and 0.0008, respectively). However, there was no significant change in the levels of HDL between all the diabetic-treated groups and the diabetic non-treated group (*P* > 0.5, Figure 3-C).

This study's findings are directly in line with previous findings that showed the peel alcoholic extract of pomegranate significantly reduces LDL, and VLDL levels.[41] Moreover, it decreased cholesterol levels in hyperlipidemic rats.[49] Our data further confirm these results and provide the novel effect of the rind, in addition to the peel, on lowering cholesterol, triglyceride, LDL, and VLDL concentrations. However, the HDL levels were not changed after treating alloxan-diabetic rats with any treatment used in this study. This study result is not consistent with previous findings. For instance, it has been found that HDL increased after treatment with pomegranate peel hydro-methanol extracts in alloxan-induced diabetic rats,[49] and the ethanol extract in human subjects.[33] For pomegranate seed juice extract and consistent with most of this study results, it was found that the seed juice and powder significantly lowered cholesterol and glyceride levels, with no effect on LDL. However, concentrated pomegranate does not affect the lipid profile in human subjects.[50]

Several mechanisms have been postulated to explain the hypoglycemic and hypolipidemic effects of pomegranate extract. The ability to reduce oxidative stress by acting as an anti-oxidant or activating antioxidant enzymes, including β-cells in islets of Langerhans within the pancreas, is the most commonly suggested.[42,51,52] It was suggested that the antioxidant properties of the pomegranate peel extract come from abundant phenolic compounds. Furthermore, a positive correlation between antioxidant activity and total phenolic in the pomegranate peels and seeds.[53] Furthermore, it was concluded that pomegranate extract with abundant phenolic compounds has also antidiabetic and antihyperlipidemic effects.[52,54] In our study, the rind ethanol extract was found to stabilize glucose, cholesterol, and lipoprotein levels which might be explained as a result of phenolic compounds in the extract similar to seeds and peels. However, further studies are needed to identify the different compounds in the rind extract and if it has a toxic effect on the liver.

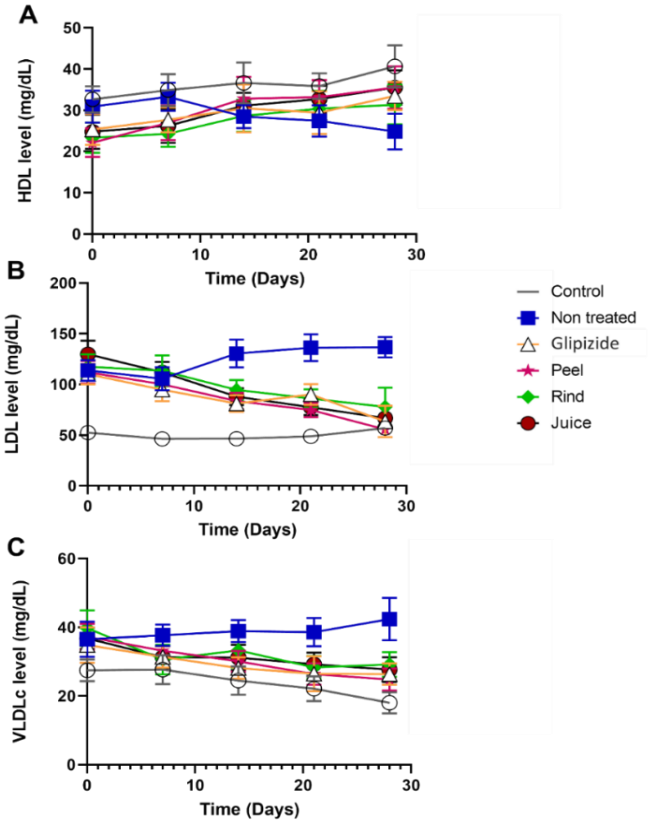

Figure 3: Effect of ethanol extract of pomegranate peel, rind, and seeds on circulating lipoproteins. The groups were compared to the non-treated alloxan-diabetic-induced group and the *P* value for high-density lipoprotein (HDL) was as follows: glipizide treated group (>0.5); peel ethanol extract treated group (>0.5); rind ethanol extract treated group (>0.5); juice ethanol extract (>0.5). The *P* value for low-density lipoprotein (LDL) was as follows: glipizide treated group (0.0041); peel ethanol extract treated group (0.0029); rind ethanol extract treated group (0.0136); juice ethanol extract (0.0134). The *P* value for very-low-density lipoprotein (VLDLc) was as follows: glipizide treated group (0.0002); peel ethanol extract treated group (0.0004); rind ethanol extract treated group (0.0015); juice ethanol extract (0.0008).

Conclusion

The extracts of the pomegranate seeds, peels, and grind extract have a hypoglycemic effect, lower cholesterol, triglyceride, LDL, and VLDL concentrations in the blood, and can be used for stabilizing glucose and lipids. This study reports the unique beneficial effect of the pomegranate grinds similar to widely studied peels and seeds. Further research is needed to determine the toxicity of pomegranate extracts on the liver and their effect on stress markers.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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