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Hypoglycemic and Antidiabetic Activity of Pomegranate (*Punica granatum*) Fruit Peel Extract in Alloxan-Induced Diabetic Rat Model

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Abstract

Diabetes mellitus is a global health concern which needs safe, effective and economical solutions. This research aimed at the phytochemical composition and antidiabetic activity of ethanol extract and fractions of pomegranate (Punica granatum) fruit peel biowaste against Alloxan induced diabetic rats. The extractable obtained with ethanol extract was 55.82% while the bioactive phytochemicals identified were alkaloids, phytosterols, glycosides, tannins, phenolic compounds, flavonoids, carbohydrates and saponins.

Male Wistar albinos were used to assess the antidiabetic activity *in vivo*. Alloxan (150 mg/kg body weight) was injected once into the peritoneum to induce diabetes. The PG II fraction showed a significant, dose-dependent decrease in fasting blood glucose after 28 days treatment, when administered orally (25 and 50 mg/kg body weight/day). The effects were similar to, or slightly better than, the standard drug, glibenclamide (5 mg/kg) which lowered blood glucose to 95.83 ± 1.85 mg/dl (67.70% reduction). Furthermore, the treatment with PG II fraction in a dose dependent manner significantly inhibited body weight loss and induced weight gain in diabetic control group.

Acute toxicity studies revealed that the PG II fraction was safe up to 1000 mg/kg body weight without any toxicity or mortality. The findings are scientifically significant to validate the traditional use of pomegranate peel in Ayurveda and also reveals that, the PG II fraction is a promising biowaste material that could be used to develop novel phytotherapeutic agents and functional food for diabetes management with safety in future.

Keywords: Diabetes mellitus, Pomegranate peel, PG II fraction, Phototherapy.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by impaired carbohydrate and lipid metabolism due to insufficient insulin production by pancreatic β-cells or insulin resistance. This leads to high blood sug-

ar levels and, over time, serious problems with the eyes, kidneys, nerves, and heart. Diabetes is becoming more common around the world. In 2024, about 589 million adults (ages 20 to 79) will have diabetes, which is about 11.1% of the adult population. This number could reach 853 million by 2050, which shows how important it is to find safe, effective, and cheap therapeutic alternatives, especially in low- and middle-income countries.

Even though traditional antidiabetic drugs work, they often have side effects and are expensive. This is why natural products and traditional medicines are becoming more popular as complementary therapies. *Punica granatum* (pomegranate) is one of the many plant-based alternatives that has gotten a lot of attention because it has been used in traditional medicine for a long time all over the world and because more and more scientific evidence is showing that it works as a medicine (1–3) *Punica granatum*, also known as pomegranate, is an important part of the traditional medicine of many cultures. The Ayurvedic fruit peel has long been regarded as an adjunctive treatment in Ayurveda for managing chronic diseases, including diabetes (4-5). Fruits peels are rich in bioactive compounds that may have medicinal effects, in addition to being used in cooking (6-8). The current study focuses on the biowaste fruit peel of *P. granatum*, which is typically discarded during juice production but represents a sustainable and underutilized resource.

The present study will investigate the beneficial effects of *Punica granatum* fruit peel extract and its fractions on alloxan-induced diabetic rats. We used thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and liquid chromatography-mass spectrometry (LC-MS) to find and measure the bioactive compounds (9-11). Phenolic compounds in the pomegranate peels have been previously isolated by UV-spectrophotometry, TLC, HPLC, and LC-MS, and found to have potential in restricting the risk factors of diabetes.

A lot of people think that pomegranate is good for diabetes because it has a lot of phenolic compounds that are strong antioxidants. These compounds may help control glucose metabolism. This paper examines the phytochemical composition of the biowaste peel extract, specifically the PG II fraction, and it's in vivo antidiabetic efficacy. This study elucidates the significance of *Punica granatum* by validating traditional Ayurvedic applications through modern scientific methodologies, thereby highlighting its prospective role in the development of next-generation phytopharmaceuticals and functional foods. Although the antidiabetic effect of Punica granatum peel extracts has been established, there is still a gap in the systematic fractionation and identification of the bioactive fraction that has proven to demonstrate the therapeutic efficacy, especially from biowaste material created during the juice process. The previous studies have been on crude extracts, without the fractionation of specific phytochemicals or the relationship between the phytochemicals and the in vivo anti-diabetic activities in a dose-dependent manner. This study fills this gap by showing that the PG II fraction, which was separated with successive chromatography of the ethanolic peel extract, contains maximum phenolic and flavonoids content and shows good antidiabetic activity than other fractions. The novelty of this work is the complete characterization of the PG II fraction by TLC, HPLC and LC-MS coupled with its blood glucose lowering activity in alloxan induced diabetic rats with blood glucose lowering activity comparable to the standard drug glibenclamide. This makes the choice of the PG II fraction for in vivo detailed studies promising and shows its potential as a sustainable value added phytotherapeutic agent for diabetes mellitus management, from a biowaste source.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents used in this study were of analytical grade and procured from Merck Company, Germany.

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Collection and identification of plant

The sample of biowaste fresh fruit peel of *Punica granatum* was procured from a juice shop (National Handloom Juice centre, Vaishali nagar), Jaipur. Additional plant material was distinguished and entered (Rrg.). No. RUBL -21111) by the department of Botany, University of Rajasthan, Jaipur. Fruit peel was then cut into smaller pieces and then first washed with tap water followed by washing with distilled water. It was then dried in the hot air oven at 40^0 C air temperature and 4 to 5 days. The samples of the plant materials which were dried were powdered using the mechanical grinder and sieved to achieve the particle size of 50- 150 mm. Prior to extraction, the powder was put in polythene bags and placed in room temperature.

Preparation of Crude Extract by Soxhlet Extraction Method

The altered version of extraction procedure was conducted according to the procedures that were previously reported by researchers (12). The peel powder of *P. granatum* fruit (35 g) was packed into the thimble and extracted under 48 hours successively with 95% ethanol (ethanol: distilled water; 95: 5) solvents in Soxhlet extraction unit. The extracts of the plants were filtered and concentrated by using rotary evaporator at 40^0 C and all the extracts were transferred into glass vials and stored at 4^0 C.

Fractionation of biowaste fruit peel by chromatographic techniques

Therefore, the polyphenol biowaste fruit peels of pomegranate were extracted, separated and identified in the previous study of Saxena et al. [2017] (13). Crude ethanolic extract of *Punica granatum* fruit peel was fractionated by column chromatography for the extraction of bioactive fractions. In brief, concentrated ethanol extract (10 g) was adsorbed onto silica gel (60-120 mesh) and then placed on a glass column (3.5 cm internal diameter × 60 cm length) filled with silica gel (100–200 mesh). The column was eluted with a stepwise gradient of increasing polarity. A total of 250 fractions (20 mL/fraction) were manually collected at a flow rate of about 2 mL/min, and grouped into five major fractions (PG I to PG V) with similar Rf values on thin-layer chromatography (TLC). The highest total phenolic content and flavonoid content was observed in the PG II fraction with a yield of 18.6% (w/w) of the crude extract. All the fractions were concentrated under reduced pressure at 40°C in a rotary evaporator and stored at 4°C for further studies.

HPLC and LC-MS analysis

The major bioactive phenolic compounds were identified and quantified in the PG II fraction by HPLC and LC-MS analyses. HPLC analysis was performed on Shimadzu LC-20AD Prominence HPLC system with SPD-M20A UV-Vis photodiode array detector. Separation was performed on a reverse-phase Phenomenex Luna C18 column (250 mm × 4.6 mm, 5-µm particle size) at 30°C. The mobile phase consisted of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B), with a linear gradient elution program: 0–5 min (10% B), 5–30 min (10–60% B), 30–35 min (60–90% B), and 35–40 min (90% B), at a flow rate of 1.0 mL/min. The amount injected was 20µL and the detection was at 280nm. An electrospray ionization (ESI) source was used in both positive and negative ion modes with an Agilent 6520 Accurate-Mass Q-TOF mass spectrometer for LC-MS analysis. Conditions for the mass spectrometry were a capillary voltage of 3500 V, nebulizer pressure of 40 psi, drying gas (N_2) pressure of 10 L/min, and drying gas (N_2) temperature of 325°C. The data was collected in the range of m/z 100–1000. These analytical conditions allowed the identification of the important compounds gallic acid, caffeic acid, and several derivatives of flavonoids in the PG II fraction.

Experimental animals

Male Wistar albino rats (150–200 g) or Wistar albino mice (20–25 g) were purchased from the experimental animal facility of Indian Veterinary Research Institute (IVRI), Bareilly, Utter Pradesh, India and kept them in a clean

rodent room. Animals were allowed to consume regular food diet and tap water at will. The rats were acclimatized to a period of 7 days under normal environmental conditions of temperature, relative humidity, and housed in polypropylene cages lined with husk and maintained in a semi natural light/dark environment (12 hours light/12 hours dark) after randomization into different groups and prior to commencement of experiment. Animals termed as fasting were denied food and water within 16 h ad libitum. Jayoti Vidyapeeth Women University, Jaipur conducted animal study with the permission of Institutional Animal Ethics Committee (R.No. 1402/a/10/CPCSEA).

Acute toxicity study

The acute toxicity test of PG II fraction was performed as per OECD Guideline 423 (Acute Toxic Class Method). Male Wistar albino mice weighing 20-25 g were divided into 6 groups (n=6) each group was given a single dose of PG II fraction for oral administration at the doses of 50, 100, 200, 400, 600, 800, and 1000 mg/kg bwt. Animals were closely monitored for any changes in behavior, clinical signs of toxicity or death for the first 4 hours following dosing and then periodically for up to 14 days. Various toxicological indicators including body weight, food and water consumption, skin/mur condition, respiration, appearance of eyes and mucous membranes, and neurological responses (e.g., tremors, convulsions, sedation) were observed. The mortality, behavior changes, and toxicity were not assessed at any of the doses studied, showing that the PG II fraction is safe and non-toxic up to a dose of 1000 mg/kg.

Safe dose studies

The Wistar albino mice (20-25 g) were randomly split into six groups and were orally administered with extracts of *P. granatum* of 50, 100, 200, 400, 600, 800 and 1000 mg/kg body weight doses in order to examine safe dose. Animals subjected to *P. granatum* extracts had no behavioural alterations, toxic response or death. The fraction was found to be safe at the dose of 1000 mg/kg, p.o. (2).

Table 1.: Experimental design

Group I	Group II	Group III	Group IV	Group V
Normal control (sterilized distilled water)	Alloxan treated control (150 mg /Kg b.w. (i.p.) intraperitoneally)	Alloxan (150 mg / Kg b.w. i.p.) induced diabetic rats treated with standard drug Glibenclamide (5 mg/kg b.w., p.o.)	alloxan (150 mg/ kg b.w. i.p.) induced diabetic rats treated with 25 mg/ kg b.w. of most active fraction of P. granatum (PG II fraction)	alloxan (150 mg/ kg b.w., i.p.) induced diabetic rats treated with 50 mg/ kg b.w. of most active fraction of P. granatum (PG II fraction)

*The animals were randomly divided into the 5 groups with six animals in each group.

Induction of Diabetes

Diabetes was induced in rats who were starved (24 h) before being subjected to it. A single intraperitoneal injection of freshly prepared alloxan monohydrate (Loba Chemie, Bombay) in normal saline induced diabetes (150 mg / kg body weight). Alloxan was initially weighed on each animal on a case-by-case basis based on the weight and then dissolved in double distilled water only before injecting. Diabetes was developed by assessing blood glucose levels in 48 h of alloxan injection.

Determination of Blood Glucose and body weight

Blood samples were obtained through end cutting method and blood glucose content in blood samples was measured through use of kit method (Omron Blood Glucose Meter HEA-230 and HEA- 232). Rats whose blood glucose

level was 200 mg/dl and above were regarded as diabetic and in case of fasting, the level of glucose in blood was greater than 120 mg/dl which was regarded as diabetic. Blood samples were drawn at 1, 7, 14, 21 and 28 days intervals till the end of study (i.e. 3 weeks). Monitoring of fasting blood glucose estimation and body weight were done weekly and compared with the control group.

Statistical Analysis

The data from all experiments were presented as mean ± SEM (n=5 animals per group). The one-way analysis of variance (ANOVA) was applied and a multiple comparison among the group test was conducted using Tukey. A significance level of $p < 0.05$ was used. All statistical calculations were performed using graphpad prism software version 8 (Graphpad software inc, San Diego, CA, USA). This statistical method was used to assess the significance of the differences among mean Fasting Blood Glucose and body weight changes in the normal control group, diabetic control group, standard drug treated group and PG II fraction treated group. Each replication conducted on the experimental studies was taken five times, ascertained to be the statistics. The values were presented in the form of mean of total number of replicates + SE and compared with diabetic control.

Administration of plant fractions

Punica granatum plant was force-fed on rats with the plant fractions (PG II Fraction). This was done through tube feeding of the baby orally by inserting a tube in the gastric region of the rat and that tube was linked to a syringe that contained the extract. To maximize bioavailability the animals were fasted 30 min prior to and following the treatment.

A*ntidiabetic Screening*

Experimental Animals: Wistar Albino rats (150-200 g) of male sex were bought at IVRI, Bareilly. Acclimatization of animals was done in 7 days under normal environmental conditions.

Experimental Design

The rats were separated into 5 antidiabetic screening groups. Diabetes was induced by intraperitoneal injection of alloxan monohydrate (150 mg/kg body weight). Plant fractions were given by mouth and the level of glucose in the blood was checked periodically.

Results and Discussion

The findings suggest that the *P. granatum*, with regard to its antidiabetic activity, is mainly comprised of a fraction of PG II, which was observed to have an effect on blood glucose levels and body weight of diabetic rats induced by alloxan as illustrated in Figure 1 & Table.2. Other than these, the influence of Punica granatum PG II fraction on fasting blood glucose levels of Alloxan-induced diabetic rats has also been shown in a graphical format in Figure 2. The identified effects can be explained by the existence of bioactive compounds that have antioxidant properties. The findings are in line with the conventional applications of P. grana*tum* in the treatment of diabetes and this validates their possible use as therapeutic agents in diabetes mellitus.

The paper focuses on the role of natural sources in the antidiabetic treatment development and the discovery of the relationship between antioxidant activity and antidiabetic effects. The phytochemical analysis of bioactive compounds The Qualitative phytochemical screening of bioactive compounds of *P. granatum* fruit peels has been summarized in the Sharma et al., (2015) (14). The total phenolic and flavonoids content and chromatographic identification of extract and fractions of *P. granatum* fruit peel were summarized in the earlier study of Saxena et al. (2017) (13). The study of Saxena et al., (2017) revealed the highest total phenolic and flavonoid content was observed in the PG II fraction of *P. granatum* (617 ± 0.017 and 546.33 ± 0.032 mg/g respectively).

The isolation and characterization of individual bioactive compounds and clinical trials should be further strengthened in future to help

validate and establish the full potential of PG II fraction of *P. granatum* in managing diabetes.

The study of Saxena et al., (2017) reported that HPLC chromatogram of standard gallic and caffeic acid were shown retention time at 2.69 min ± 0.011 and 2.82 min ± 0.002 respectively (13). Retention time (Rt) of standard gallic acid and caffeic acid as phenolic compounds were coincided with the retention time of A and B compounds in PG II fraction of *P. granatum*. According to this study, the two phenolic compounds from PG II fraction of *P. granatum* were identified by the interpretation of their fragmentation patterns obtained from mass spectra and retention times of peaks. The results of U.V. spectrophotometer and HPLC analysis showed that PG II fraction of *P. granatum* plant have the highest content of total phenolic compounds. Therefore, this fraction was selected for further confirmation of phenolic compounds by LCMS techniques.

The findings indicated that *P. granatum* fraction of the PG II had a significant antidiabetic effect among the rats. After four weeks of treatment with this fraction, there was a significant decrease in blood glucose level in alloxan induced diabetic rats. Alloxan monohydrate produced a considerable rise in serum glucose of diabetic control rats (Group II). The standard drug was glibenclamide (5 mg/kg b.w. p.o.) which demonstrated a reduction in the level of blood glucose levels in rats as compared to diabetic control rats (Group II) (Table.3). After 4 weeks, the diabetic control group lost weight. Giving PG II at different doses (25 and 50 mg/kg b.w.) made a big difference in body weight. PG II showed that it could speed up growth, which means that the person was getting better from diabetes.

The results of the body weight of wistar rats of diabetic control (Group II), standard (Group III) and four trial drug groups (Group IV and V) of plant fractions were summarized in Table. From the results, it was observed that the body weight of all the rats of diabetic control group was reduced from its initial level (135.66 ± 2.36 gm) after the trial period of 4 weeks (89.66 ± 1.92 gm). Whereas, the alloxan induced diabetic rats were treated with standard drug glibenclamide (5 mg/k.g. b.w.) in group III and it has seen that the body weight of all the rats were reduced from 138.83 ± 0.98 gm to 119.5 ± 0.84 gm in the first week. It was further increased within 28 days (139.33 ± 1.17 gm). Even though the body weight of rats of group IV (a) and group V (a) treated with PG II fraction of *P. granatum* at two different concentrations (25 and 50 mg/kg b.w.) were also showed significant increase in body weights (132.5 ± 1.92 gm to 133.34 ± 1.14 gm and 134.33 ± 1.33 gm to 143.5 ± 0.95 gm respectively) which were statistically significant. From day seven on, PG II had clear hypoglycemic effects, with a big drop on day 28. Diabetic control rats (Group II) showed a rise in their average fasting blood glucose levels. PG II fractions at concentrations of 25 and 50 mg/kg b.w. significantly reduced blood glucose levels in comparison to diabetic control rats. The findings indicated that the hypoglycemic effect of the PG II fraction at a dosage of 50 mg/kg b.w. was more pronounced than that of the standard drug glibenclamide. PG II fractions exhibited a dose-dependent reduction in blood glucose levels. The PG II fraction of *P. granatum* did not induce significant alterations in behavioral or neurological responses at doses up to 1000 mg/kg body weight. The analysis of safe doses corroborated the non-toxic characteristics of P. granatum, consistent with prior research. The antioxidant characteristics of the PG II fraction of *P. granatum* were associated with their antidiabetic efficacy. The bioactive compounds (gallic acid, caffeic acid, quercetin) in these fractions were found to be responsible for their healing effects. The data were expressed as mean ± SEM (n =6). Statistical significance observation of antidiabetic activity of both selected plant against body weight and glucose level were determined by one way ANOVA test. A 95 % confidence terval, P values less than 0.05 were considered significant.

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(A) (B)

Figure 1: Punica granatum (A) Whole plant and (B) Selected part fruit Peels

Table 2: The effect of body weight on normal, alloxan induced and treated rats with PG II Fraction of P. granatum and Standard Glibenclamide

Group (n=5)	Alteration of body weight of rats (gm) (Mean ± SEM)					
	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
Group I Normal Control rats (Distilled water)	140.33 ± 2.34	145.16 ± 2.16	151.83 ± 1.57	159.66 ± 1.6	165.5 ± 1.92	165.8 ± 0.6
Group II Alloxan induced diabetic rats + control	135.66 ± 2.36	122.5 ± 2.1	109 ± 2.08	95.5 ± 1.72	89.66 ± 1.92	85.36 ± 1.6
Group III Alloxan induced diabetic rats + glibenclamide (5 mg/kg b.w., p.o.)	138.83 ± 0.98	119.5 ± 0.84	125.66 ± 1.33	130.66 ± 0.8	138.33 ± 1.17	138.87 ± 0.7
Group IV (a) Alloxan induced diabetic rats + PG II Fraction (25 mg/kg b.w.)	132.5 ± 1.92	117.5 ± 0.88	120.5 ± 1.08	124.5 ± 0.76	133.34 ± 1.14	133.5 ± 0.9
Group V (a) Alloxan induced diabetic rats + PG IIFraction (50 mg/kg b.w.)	134.33 ± 1.33	119.83 ± 1.42	124.16 ± 1.04	132.66 ± 0.71	143.5 ± 0.95	144.15 ± 0.83

*Values are expressed as mean ± S.E.M.

Table 3: The effect of fasting blood glucose levels in normal, alloxan induced and treated rats with PG II Fraction of P. granatum and Standard Glibenclamide

Group (n=5)	Blood glucose levels (mg/dl) of rats in fasting condition (Mean ± SEM)					
	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
Group I Normal Control rats (Distilled water)	81.07 ± 0.62	79.65 ± 0.90	82.20 ± 0.60	85.33 ± 0.80	83.54 ± 0.80	84.92 ± 0.95
Group II Alloxan induced diabetic control rats	281 ± 2.21	290. 83 ± 1.70	293.16 ± 1.64	296.66 ± 2.26	308.83 ± 2.82	324.64 ± 1.52
Group III Alloxan induced diabetic rats + glibenclamide (5 mg/kg b.w., p.o.)	296.66 ± 2.67	230.16 ± 2.48 22.42%	159.16 ± 2.24 46.35%	112.83 ± 2.66 61.97%	95.83 ± 1.85 67.70%	95..5 ± 1.12
Group IV (a) Alloxan induced diabetic rats + PG II Fraction (25 mg/kg b.w.)	288.33 ± 2.24	236.83 ± 2.18 17.86%	165.66 ± 4.17 42.55%	138.66 ± 2.29 51.91%	99.35 ± 2.12 65.54%	98.94 ± 1.96
Group V (a) Alloxan induced diabetic rats + PG II Fraction (50 mg/kg b.w.)	289.83 ± 1.92	229.5 ± 2.39 20.82%	161.16 ± 2.62 44.39%	130. 5 ± 2.60 54.97%	92.33 ± 2.44 68.14%	92.11 ± 1.92

*Values are expressed as mean ± S.E.M.

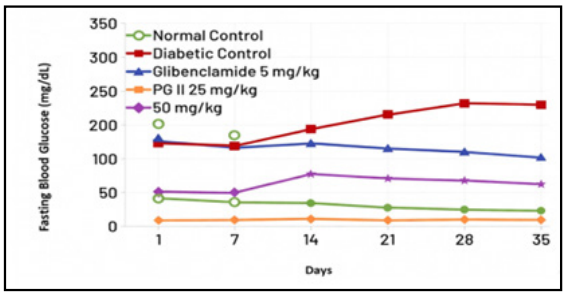

Figure 2.: Effect of Punica granatumn PG II fraction on fasting blood glucose levels in Alloxan-induced diabetic rats

DISCUSSION

The results of this research confirm the strong antidiabetic potential of the biowaste peel of *Punica granatum* (pomegranate), particularly its PG II fraction, in alloxan-induced diabetic rats. Oral administration of the PG II fraction at 25 mg/kg and 50 mg/kg body weight for 28 consecutive days led to a significant reduction in fasting blood glucose levels and a marked improvement in body weight compared to the diabetic control group. Notably, the higher dose (50 mg/kg) exhibited hypoglycemic activity that was comparable to, or in some aspects superior to, the standard drug glibenclamide (5 mg/kg). These findings provide scientific support for the traditional Ayurvedic use of pomegranate peel in managing chronic metabolic conditions similar with the study of Maphetu et al., (2022) and Apaydin Yildirim et al., (2025) (15-16).

The ethanolic extract of pomegranate peel yielded 55.82% extractives and was found to be rich in various bioactive secondary metabolites, including alkaloids, phytosterols, glycosides, tannins, phenolics, flavonoids, carbohydrates, and saponins. Among the fractions, PG II contained the highest levels of total phenolics and flavonoids. HPLC and LC-MS analyses revealed the presence of key compounds such as gallic acid, caffeic acid, and several flavonoid derivatives. These observations align with earlier reports highlighting the abundance of polyphenols in pomegranate peel, especially hydrolysable tannins (ellagitannins), gallic acid, ellagic acid, and caffeic acid, which are well known for their potent antioxidant properties.

In the present study, diabetic control rats exhibited a steady decline in body weight due to muscle wasting and dehydration associated with persistent hyperglycemia. Treatment with the PG II fraction reversed this trend in a dose-dependent manner, indicating improved metabolic status and recovery from the diabetic state. This growth-promoting effect is consistent with earlier findings where pomegranate peel extracts helped restore normal energy utilization and insulin levels in diabetic animals.

The safety of the PG II fraction further strengthens its therapeutic promise. Acute toxicity studies in mice demonstrated that doses up to 1000 mg/kg body weight produced no behavioural changes, toxic symptoms, or mortality, indicating a wide safety margin. This aligns with the long history of safe traditional use of pomegranate-based preparations (Shetty et al., 2026) (17).

Overall, the study establishes a clear correlation between the high phenolic and flavonoid content of the peel and its antidiabetic activity. The limitation of this study is that, it was conducted only in an alloxan-induced rat model and for better efficacy in other diabatic model or human study need to be explored. Apart from these, multiple clinical trials also recommended to ensure the safety and efficacy.

In conclusion, the biowaste fruit peel of *Punica granatum*, especially the PG II fraction, represents a valuable and sustainable source of bioactive compounds with significant potential for diabetes management. By utilizing an often-discarded industrial byproduct, this work supports the principles of waste valorization and green chemistry, while paving the way for the development of novel plant-based pharmaceuticals and functional foods for diabetes care. Future studies should focus on isolating individual active constituents, elucidating their precise molecular mechanisms using advanced omics approaches, and conducting clinical trials to evaluate their efficacy and safety in humans.

CONCLUSION

The present investigation revealed that ethanol extract of biowaste peel of *Punica granatum* exhibited remarkable blood glucose lowering potential in alloxan induced diabetic rats. Phytochemical analysis reconfirmed the presence of various bioactive secondary metabolites such as flavonoids, tannins, alkaloids, glycosides and saponins. TLC, HPLC and LC-MS established the presence of major compounds like gallic acid, caffeic acid, and flavonoid derivatives. Maximum amount of total phenolics and flavonoids was observed in PG II fraction. Administration of PG II fraction (25 mg/kg and 50 mg/kg b.w.) p.o. daily for 28 days showed a significant dose dependent reduction in fasting blood glucose level as well as increased the body weight significantly when compared with diabetic control rats. The antihyperglycemic effect of 50 mg/kg dose of fraction was found to be comparable or better than the standard drug glibenclamide (5 mg/kg). The fraction was found to be non toxic as no signs of behavioural abnormalities or mortality were observed even at the doses up to 1000 mg/kg b.w.Antioxidant activity and insulin-secretagogue activity of the phytoconstituents may be responsible for the beneficial effects. It can modulate glucose homeostasis, lower hepatic glucose production and diabetic-induced oxidative stress. This study supported the Ayurvedic claim of using pomegranate fruit peel as an herbal remedy for chronic ailments. Pomegranate peels are an inexpensive by-product and potential waste material after extraction of juice. Therefore, using pomegranate peel allows valorization of waste material in an economical and environment-friendly approach to develop novel phytotherapetic agents, nutraceuticals and functional foods for diabetes.

Author Contributions

Conceptualization: Richa Saxena, Richa Sharma; Methodology: Richa Sharma, Richa Saxena.; Writing: – Richa Sharma, Richa Saxena; Writing: – review & editing-Brij Kishore Tiwari, Ratnesh Kumar Singh, Dipti Bharti

Conflict of Interest

The authors declare no conflicts of interest.

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Data Availability Statement:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval:

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References

1. Wu S, Tian L. Diverse phytochemicals and bioactivities in the ancient fruit and modern functional food pomegranate (Punica granatum). Molecules. 2017 Sep 25;22(10):1606. doi: 10.3390/molecules22101606

2. Ghosh S, Bishal A, Ghosh SK, Jana K, Gayen B, Sahu S, Debnath B. Herbal medicines: A potent approach to human diseases, their chief compounds, formulations, present status, and future aspects. Int. J. Membr. Sci. Technol. 2023 Oct 11; 10:442-64. doi: 10.15379/ijmst.v10i1.2608

3. Islam MR, Jony MH, Thufa GK, Akash S, Dhar PS, Rahman MM, Afroz T, Ahmed M, Hemeg HA, Rauf A, Thiruvengadam M. A clinical study and future prospects for bioactive compounds and semi-synthetic molecules in the therapies for Huntington's disease. Molecular Neurobiology. 2024 Mar;61(3):1237-70. doi: 10.1007/s12035-023-03292-2

4. Ge S, Duo L, Wang J, Yang J, Li Z, Tu Y. A unique understanding of traditional medicine of pomegranate, Punica granatum L. and its current research sta-

tus. Journal of ethnopharmacology. 2021 May 10;271:113877. doi: 10.1016/j.jep.2021.113877

5. Ochora DO, Moodley T, Hayeshi R. Phytochemistry, Pharmacological Activities, and Drug Interactions of Pomegranate, *Punica granatum L.*(Punicaceae). InMedicinal Plants-Harnessing the Healing Power of Plants 2023 Nov 23. IntechOpen. doi: 10.5772/intechopen.1003779

6. Hussain H, Mamadalieva NZ, Hussain A, Hassan U, Rabnawaz A, Ahmed I, Green IR. Fruit peels: Food waste as a valuable source of bioactive natural products for drug discovery. Current issues in molecular biology. 2022 Apr 30;44(5):1960-94. doi: 10.3390/cimb44050134

7. Gandhi Y, Kumar R, Grewal J, Rawat H, Mishra SK, Kumar V, Shakya SK, Jain V, Babu G, Sharma P, Singh A. Advances in anti-inflammatory medicinal plants and phytochemicals in the management of arthritis: A comprehensive review. Food chemistry advances. 2022 Oct 1;1:100085. doi: 10.1016/j.focha.2022.100085

8. Gunupati S, Biradavolu S, Nitin P, Kalyani T. Punica granatum (Pomegranate) A Potential Herbal Medicine in Treating Periodontal Disease in the Modern World of Dentistry. Pharmacological Studies in Natural Oral Care. 2023 Oct 3:435-51. doi: 10.1002/9781394167197.ch22

9. Mondal P, Anweshan A, Purkait MK. Green synthesis and environmental application of iron-based nanomaterials and nanocomposite: A review. Chemosphere. 2020 Nov 1;259:127509. doi: 10.1016/j.chemosphere.2020.127509

10. Carpena M, García-Oliveira P, Pereira AG, Soria-Lopez A, Chamorro F, Collazo N, Jarboui A, Simal-Gandara J, Prieto MA. Plant antioxidants from agricultural waste: synergistic potential with other biological properties and possible applications. In-Plant antioxidants and health 2022 May 27 (pp. 343-380). Cham: Springer International Publishing. doi: 10.1007/978-3-030-78160-6_35

11. Patyra A, Kołtun-Jasion M, Jakubiak O, Kiss AK. Extraction techniques and analytical methods for isolation and characterization of lignans. Plants. 2022 Sep 5;11(17):2323. doi:10.3390/plants11172323

12. Yu M, Gouvinhas I, Rocha J, Barros AI. Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. Scientific reports. 2021 May 11;11(1):10041. doi:10.1038/s41598-021-89479-w

13. Saxena R, Sharma R, Nandy BC. Chromatographic determination of phenolic profile from Punica granatum fruit peels. DOI: 10.7897/2230-8407.080112

14. Sharma R, Saxena R, Nandy BC. Extraction of bioactive chemical compounds and antibacterial activity of biowaste fruit peels extract of punica granatum. Journal of Biomedical and Pharmaceutical Research. 2016;4(5):53-61.

15. Yu M, Gouvinhas I, Rocha J, Barros AI. Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. Scientific reports. 2021 May 11;11(1):10041. doi:10.1038/s41598-021-89479-w

16. Maphetu N, Unuofin JO, Masuku NP, Olisah C, Lebelo SL. Medicinal uses, pharmacological activities, phytochemistry, and the molecular mechanisms of Punica granatum L.(pomegranate) plant extracts: A review. Biomedicine & Pharmacotherapy. 2022 Sep 1;153:113256. doi: 10.1016/j.biopha.2022.113256.

17. Shetty A, Dubey A, Rodrigues VP, Kamath S, Fernandes L, Mahadev M, Jain P, Hebbar S. Nutritional and Pharmacological Significance of Pomegranate (Punica granatum L): A Comprehensive Narrative Review. Nutrition Reviews. 2026 Feb;84(2):409-34. https://doi.org/10.1093/nutrit/nuaf165