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Antioxidant and Antidiabetic Potentials of *Punica granatum* Peel Extracts in Alloxan-Induced Diabetic Albino Rats

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ABSTRACT

The peel/rind of *Punica granatum* is approximately 60% of the total fruit weight which is generally wasted every year, although, it is known to have a variety of medicinal benefits. The present study was conducted to find the antioxidant activity and antidiabetic potentials of *P. granatum* peels extracts in alloxan-induced diabetic albino rats. The diabetes was induced in albino rats using alloxan at the concentration of 150 mg kg^{-1} body weight. After 15 days, the treated groups with glabinclemide and different varieties of methanolic and aqueous extracts showed a significant ($P<0.05$) decrease in the blood glucose level as compared to diabetic control while slight differences were seen in body weight. The total cholesterol, triglycerides, and low-density lipoprotein, total bilirubin, alkaline phosphatase, alanine aminotransferase, urea, serum creatinine and total proteins were significantly ($P<0.05$) decreased in response to oral dosing of three varieties of *P. granatum* peel methanolic and aqueous extracts which were comparable to healthy control. Moreover, the aqueous extracts and wild *P. grantaum* were more effective than methanolic extracts and other species against hyperglycemia and biochemical profiles of treated rats. It was concluded that the extracts of peels of *P. granatum* possess significant antihyperglycemic and antihyperlipidemic potentials due to presence of phytochemical and have no side effects. Based on the outcome of the study it can be recommended that the peel of *P. granatum* can be further used for the development of antidiabetic drugs or in folk medicines traditionally to treat the diabetes.

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AKA, NS and RAK conceived and designed the experiments. RS, NAK, NUK UK, and FAS performed the experiments. SHZ, MSS, RUK and AAS analyzed the data computationally. AKA, NS and AAS wrote the manuscript.

Key words
Antioxidant activity, Alkaline phosphatase, Alanine aminotransferase, Diabetes mellitus, *Punica granatum*

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder, affecting millions of people all over the world each year with an increasing rate of mortality which results in imposing a major socio-economic challenge (Middha *et al*., 2013; Akhtar *et al*., 2017; Cho, 2018). According to the International Diabetes Federation (IDF) 451 million (M) people were affected all over the world with diabetes in 2017

which could be reckoned to be 693 M by 2045 if effective stratigies are not developed to control it (Abeeleh *et al*., 2009). Among the other non-insulin dependent diabetes mellitus (NIDDM) is the most common type which is due to insulin resistance and dysfunctioning of insulin receptor and ultimately β-cell in results lead to hyperglycemia (Zeb *et al*., 2015). The commercially available antidiabetic drugs including insulin, thiazolidinediones biguanides, and sulfonylureas are less stable and have many other side effects on the body. Albeit, the rising rate of diabetes is consistently increasing with time but no effective treatment with minimum side effects are available so far (Singh and Gupta, 2015). Previously, the antidiabetic potentials of raw extracts of many plants and herbs were determined in albino mice or rats due to their close resemblance with human DM (Thanh *et al*., 2019).

Plant materials have been reported as a major source of natural antidiabetic drugs for the last many years. According to WHO, around 80 % of the population from all over the world rely on traditional medicine (Uttra *et al*., 2018). In the subcontinent, the plants oriented medicine used extensively for the last many decades. Pakistan is also one of the countries which extensively use medicinal plants as traditional medicines (Ismail and Yahyeh, 2009; Uttra *et al*., 2018). Plants posseses the medicinal properties due to the presence of wide range of biochemical constitute (natural drugs) which contributes in many biological activities including antidiuretic, antimicrobial and antioxidant properties (Goncalves and Romeiro, 2019; Shah *et al*., 2019, 2020). Due to significant biological properties of plants they can be used as safest and richest source of natural drugs. Currently, natural products have been reported as a valuable source of drugs, especially in the era of cancer and infectious diseases (Huang *et al*., 2017). Recent decades have witnessed a renewed enhancement in the demand for herb-based medicinal and nutraceutical products because of their cost-effectiveness and fewer side effects (Sagbo *et al*., 2018). These herb-based medicinal and nutraceutical products cannot be prepared through synthetic procedures due to their complex structures. So, therefore the idea of extraction and purification of these biomolecules has been developed and new advancement with time was seen.

The *Punica granatum* (family of Punicaceae) commonly known as pomegranate is the oldest fruit of tropical and subtropical regions and has been used as food for the last many decades (Anwar *et al*., 2007). With diverse varietal distribution between wild and cultivated genotypes the worldwide production of pomegranate is around 1.5 tons (Ay *et al*., 2012). Red pomegranate (locally called Q and harianar) is considered the best variety in Pakistan due to its size and taste, though white and wild pomegranates are also grown in their different parts. Wild pomegranate is sour in taste and small in size, contrary to the cultivated ones, which are sweet and delicious, indicating different biochemical properties. Medicinal properties of *P. granatum* against diarrhea, ulcers, acidosis, hemorrhage, anemia, cardiac and respiratory diseases were reported previously (Ay *et al*., 2012; Middha *et al*., 2012; Shaikh and Bhandary, 2021). Moreover, it also showed immune-boosting effects against bacterial, viral, and fungal infections (Middha *et al*., 2013). The health benefits of *P. granatum* could be attributed to its chemical constituents including phenolics, flavonoids, terpenoids, phytosterols, glycosides, and tannins present in its different parts.

The peel/rind of pomegranate is approximately 60 % of the total fruit weight (Middha *et al*., 2012). While the fruit has a variety of applications, its peel is generally wasted, in spite of having a variety of medicinal benefits. Need arises to explore the chemical constituents and medicinal importance of the *P. granatum* rind. Therefore, the rationale of the proposed study was to decipher the antidiabetic and anti-hyperlipedemic potentials of peels of three varieties of *P. granatum* cultivated in Pakistan. Moreover, the antioxidant potential and the effects of peels extracts on body weight liver functions test (LFTs) and renal functions test (RFTs) were also measured.

MATERIALS AND METHODS

Plant collection and extracts preparation

All the three varieties of *Punica granatum* including red, white, and wild fresh and healthy fruits were collected from Mardan, the local market of Khyber Pakhtunkhwa (PK), Pakistan as shown in Figure 1. The plant specimens were identified by Botanist at the Department of Botany, Hazara University, Mansehra, KP, Pakistan, and then deposited in the departmental herbarium. The peels of all three varieties were separated carefully, washed with dH_2O, and dried at room temp (25-30°C) under shadow for two weeks, and ground into fine powdered. Then, 250 ml of commercial methanol (95%) was added in 50 g peel's powder and mixed properly. Then, the mixture was incubated at 25-30°C for 2 weeks with a regular shaking. After the incubation the homogenate was filtered with double-layer cheesecloth followed by Whatman filter paper (Circles: 10 mm to 150 cm (available prepleated) Sheets: 26 x 31 mm to 600 x 600 mm). The methanol from the filtrate was then evaporated to dry mass using a rotary evaporator under reduced pressure at 45°C. Then, 3 g of dried mass was re-suspended in each 15 mL of methanol and dH_2O separately to prepare aqeous and methanolic extracts and stored at 4 °C for further analysis (Das and Barman, 2012).

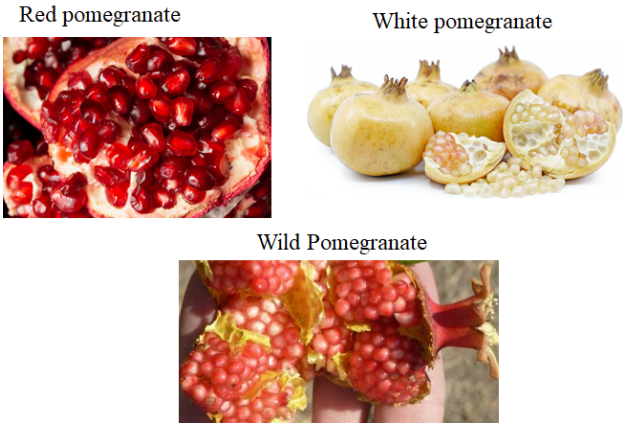

Fig. 1. Three different varieties of pomegranate.

DPPH radical scavinging activity

Antioxidant activity of methanolic and aqueous fractions of all varieties *P. granatum* peel was determined based on the scavenging potentials of 2, 2- diphenyl-2-picrylhydrazyl (DPPH) free radical as reported previously (Brand *et al*., 1995). The three dilutions of each extract 1, 2, and 3mg mL-1 were prepared. 1 mM DPPH was prepared and mixed with each extract (n =3) in 4:1 ratio and incubated for 30 min at room temperature and then the absorbance was measured at 517 nm. Ascorbic acid was used as a positive standard. The inhibition (%) of DPPH was calculated as; Inhibition of DPPH (%) = Abs of sample- Abs of control/ Abs of sample ×100.

In vitro antidiabetic activity

For the determination of activity against α-amylase, each extract was prepared separately. Then, 1.4 % starch solution (Sigma, Aldrich) and 0.05 % α- amylase were prepared in dH_2O. The commercially available antidiabetic drug, Glucophage (metformin-500 mg) (Martin dow) was used as a reference to determine the inhibition of α-amylase (Merck pharma, Karachi Pakistan). The assay mixture consists of 200 µl of 0.02M sodium phosphate buffer, 20 µl of 0.05 % α- amylase enzyme, and 300 µl of three different concentrations (1mg/ml to 3 mg/ml) of each extract were incubated for 10 min at room temperature. Then, 200 µl of the starch solution was added to initiate the reaction by following the addition of 400 µl DNS and placed in a boiling water bath for 5 min to terminate the reaction. The mixture was then cooled at room temp and the absorbance was measured at 540 nm. The mixture without extract was used as a control. The % inhibition was calculated according to the following formula (Lewis and Liu, 2012).

$$\text{Glucose Inhibition (\%)} = \frac{\text{Abs of sample} - \text{Abs of control}}{\text{Abs of sample}} \times 100$$

Experimental animals and induction of diabetes

A total of 54, approximately three months old female albino rats in a weight range of 185–200 g^{-1} were used to evaluate the antidiabetic and hypolipedemic potentials of peels extracts of *P. granatum*. The rats were purchased from the Department of Pharmacy, the University of Sargodha and were maintained in steel cages at room temp (24-26°C) with 12 h light/darkness for 7-21 days in the laboratory. After one week of adaptation, diabetes was induced in albino rats through a single intraperitoneal injection of alloxan, 150 mg kg^{-1} b.w, in the peripheral abdomen. After 8 h of fasting all the rats were given 5% glucose for 6 h and then 20% glucose and foods for 10 h. The blood glucose level through glucometer strips using SD glucometer (G110 Germany) was measured to find the hyperglycemic condition after 48 h of alloxan administration. Rats having fasting blood glucose levels > 200 mg/dl were considered as diabetic and selected for further study. All the rats were grouped into nine different groups (n=6) and given different treatments, provided free food and water, and maintained in specific laboratory conditions. The rats were randomly divided into 9 groups (G1 to G9) each of six. G1, Normal control; G2, Diabetic control; G3, Glibinclamide (10 mg kg^{-1}) (Alliance pharma); G4, wild (daroona) pomegranate peel aqueous extract (DPPw), (150 mg kg^{-1}); G5, red pomegranate peel methanolic extract (RPPm) (150 mg kg^{-1}); G6, white pomegranate peel aqueous extract (WPPw) (150 mg kg^{-1}); G7, wild pomegranate peel methanolic extract (DPPm) (150 mg kg^{-1}); G8, white pomegranate peel methanolic extract (WPPm) (150 mg kg^{-1}); G9, pomegranate peel aqueous extract (RPPw) (150 mg kg^{-1}).

Collection of blood samples and biochemical analysis

For the determination of glucose, blood sample was collected from the tail of each rat at different intervals (1^{st} -15^{th} day) during the treatment. The glucose level was measured with glucometer strips using SD glucometer (G110, Germany) at 1^{st}, 5^{th}, $10^{th,}$ and 15^{th} days of treatment. After 15 days all rats were anesthetized by chloroform and blood samples were taken through cardiac puncture for biochemical analysis including Liver functioning tests (LFTs) such as serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), total bilirubin, renal function tests (RFTs) such as urea, serum creatinine, and total proteins and lipid profile including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoproteins (LDL) (Khan *et al*., 2019; Shah *et al*., 2019).

Statistical analysis

The statistical analyses were done in a completely randomized design. The analysis of variance (One way ANOVA) was performed using SPSS (version 18.0). Means of the significantly different parameters were separated by Tukey's test. A P value < 0.05 was considered statistically significant.

RESULTS

DPPH scavenging activity of P. granatum *extracts*

The *in-vitro* antioxidant activity of three different concentrations (1 mg mL^{-1}, 2 mg mL^{-1}, and 3 mg mL^{-1}) of each peels extracts showed DPPH scavenging potentials. The peel extract of wild variety showed the highest activity in both methanolic and aqueous extract which was comparable to standard ascorbic acid as shown in Figure 2.

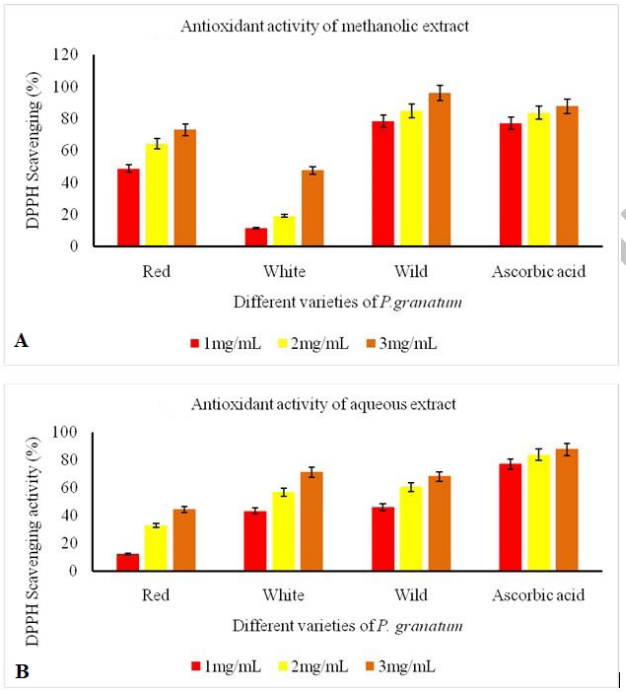

Fig. 2. DPPH scavenging activity of three varieties of *P. granatum* extracts in (A) Methanolic extract (B) Aqueous extract. The red, white and wild are three different varieties of pomegranate. Ascorbic acid (Vitamin C) was used as a standard (antioxidant).

In-vitro antidiabetic activity (α-amylase inhibition) of P. granatum *extract*

The *in-vitro* antidiabetic potentials of peel extracts of all three varieties of *P. granatum* showed significant inhibition against α-amylase in both methanolic and aqueous extract which was comparable with commercially available drug Glucophage (Martin Dow) as shown in Figure 3. The acute toxicity study revealed no mortality or any physical or behavioral changes in rats after oral dosing of peels extract at a dose of 150 mg kg^{-1} b.w. Hence, the same dose was selected further to evaluate the antidiabetic potentials and biochemical profiling of all rats.

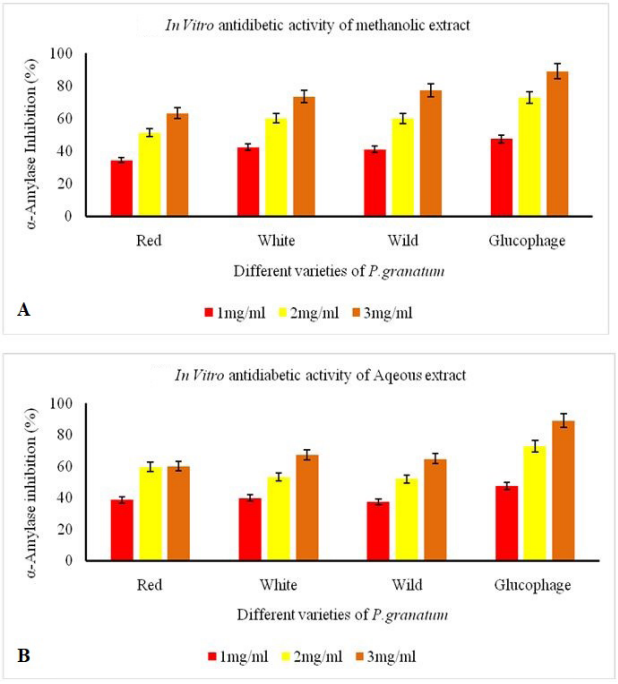

Fig. 3. *In-vitro* antidiabetic activity (α-amylase inhibition) of three varieties of *P. granatum*in (A) Methanolic extract (B) Aqueous extract. The red, white and wild are three different varieties of pomegranate. Glucophase was used as antidiabetic standard.

Effect of peel extract of P. granatum *on blood glucose level*

The effects of orally administered methanolic and aqueous peels fractions of all varieties of *P. granatum* on blood glucose levels in alloxan-induced diabetic albino rats are shown in Table I. The fasting blood glucose level in all untreated diabetic rats (G2-G9) was significantly high compared to control (healthy rats). After oral administration of the methanolic and aqueous extracts of three varieties of *P. grantum* peels at 150mg/kg b.w concentration showed significant (P<0.05) reduction in the blood glucose level at 1st, 5th, 10^{th,} and 15th days of treatment.

Effect of peel extracts of P. granatum *on body weight*

A slight variation in the body weight was seen in treated diabetic rats compared to normal control after the treatment but no significant differences were observed as shown in Table II.

Table I. Effect of peel extract of *P. granatum* on blood glucose level in alloxan induced diabetic albino rats

Samples	Groups	Dose (mg/kg body weight)	Glucose level (mg/dl) at different days interval			
			1st day	5th day	10th day	15th day
Healthy (Untreated)	Normal control	--	86.2±0.4	88.6±0.5	88.4±0.5	86.8±0.2
Diabetic (untreated)	Diabetic control	--	227.1±6.6**	236.2±6.4**	248.2±6.4**	267.2±6.0**
Standard	Glibenclamide	10	211.3±4.5	207.4±5.6+	202.3±5.2+	193.5±4.4+
Aqueous extract	RPPw	150	284.7±5.5	264.7±6.6	197.5±6.6+	148.7±5.4++
	WPPw	150	215.3±4.3	142.3±4.7++	119.4±5.8++	92.6±6.6++
	DPPw	150	288.2±6.2	239.5±4.4	197.2±3.8+	143.5±5.5++
Methanolic extracts	RPPm	150	291.6±4.4	273.6±4.4	239.6±4.6	113.3±5.2++
	WPPm	150	265.2±5.2	223.7±4.3	213.3±5.7	128.4±5.2++
	DPPm	150	262.5±3.7	233.3±4.4	196.4±5.2+	157.3±4.8++

Mean ± SEM (n=6)** indicate significance from the diabetic control group at $P<0.05$ and $P<0.01$ probability level. +,++ indicates significance from the *Punica granatum* peels group at $P<0.05$and $P<0.01$probability level, respectively. RPP, red pomegranate peel; WPP, white pomegrants peel; DPP, wild pomegranate peel.

Table II. Effect of peel extracts of *P. granatum* on body weight in alloxan induced diabetic albino rats.

Samples	Groups	Dose mg (kg body weight)	Body weight (mg) at different time interval			
			1st day	5th day	10th day	15th day
Healthy (Untreated)	Normal control	-----	189.3±5.4	191.2±4.2	191.5±5.3	192.6±5.3
Diabetic (untreated)	Diabetic control	-----	189.5±3.7	187.5±5.3	184.2±6.3	182.5±5.7
Standard	Glibenclamide	10	180.6±4.9	180.2±4.5	182.5±6.5	185.5±7.3
Aqueous extracts	RPP	150	180.3±6.6	180.3±6.6	184.2±6.4	188.3±6.4
	WPP	150	186.2±5.5	186.6±5.5	191.6±6.6	193.3±4.7
	DPP	150	181.6±6.6	185.5±6.5	189.5±4.2	196.2±3.4
Methanolic extracts	RPP	150	190.2±4.6	193.1±4.6	193.5±3.3	196.7±5.5
	WPP	150	186.3±6.4	186.6±6.2	188.3±6.2	193.3±6.3
	DPP	150	182.5±5.6	189.3±5.4	191.6±6.6	198.2±6.6

Mean ± SEM (n=6). Each value indicated the body weight (mg). No significant differences (P>0.05) has been found. For abbreviation, see Table I.

Table III. Effect of peel extracts of *P. granatum* on liver function test (LFTs) in alloxan induced diabetic albino rats.

Samples	Groups	Dose (mg/kg body weight)	Total bilirubin (mg/dl)	ALP (mg/dl)	ALT (mg/dl)
Healthy (Untreated)	Normal control	--	0.78±0.3	53.2±2.2	99.3±2.8
Diabetic (untreated)	Diabetic control	--	1.24±0.2**	121.5±2.2**	187.5±0.1**
Standard	Glebinclamide	10	0.81±0.3++	110.5±3.1++	147.1±2.2++
Aqueous extract	RPP	150	0.87±0.3++	76.2±2.6++	137.5±3.5++
	WPP	150	1.00±0.1++	69.2±0.1++	109.7±4.6++
	DPP	150	0.87±0.2++	73.3±2.7++	115.5±3.5++
Methanolic extracts	RPP	150	0.83±0.3++	88.3±3.3++	141.5±3.2++
	WPP	150	0.80±0.2++	63.4±4.3++	131.5±3.2++
	DPP	150	0.99±0.1++	68.5±0.2++	148.3±3.3++

Mean ± SEM (n=6)** indicate significance from the diabetic control group at $P<0.05$ and $P<0.01$ probability level. +,++ indicates significance from the *Punica granatum* peels group at $P<0.05$and $P<0.01$probability level respectively. ALP, Alkaline phosphatase; ALT, Alanine amino transferase. For abbreviation, see Table I.

Effect of peel extracts of P. granatum *on liver function test*

The effect aqueous and methanolic peels fractions of *Punica granatum* on liver profile such as total bilirubin, ALT and ALP in alloxan-induced diabetic and control rats are shown in Table III. A significant increase was observed in serum total bilirubin, ALT, and ALP in alloxan-induced diabetic groups. The daily oral effect of various fractions of peel extracts of *P. granatum* at the rate of 150 mg/kg b.w in alloxan-induced diabetes rats showed a significant (**P<0.05) decrease in total bilirubin, ALT, and ALP as compared to diabetic control which was comparable to normal control group.

Effect of peel extracts of P. granatum *on renal function test*

The effects of peels fractions at a dose of 150 mg/kg b.w on RFTs including urea, creatinine, and total protein are presented in Table IV. After induction, significant (**P<0.05) increase were seen in RFTs in diabetes-induced rats as compered to healthy rats (control). The daily dose effect of both type of peels extracts (aqueous and methanolic) of *P. granatum* showed a significant (**P<0.05) reduction in treated diabetic groups compared to diabetic control which was compareable to healthy control.

Effect of peel extracts of P. granatum *on lipid profile*

The effect of different varieties of peel extracts of *Punica granatum* on lipid profile in diabetic and control rats are shown in Table V. After induction of diabetes, an increase in TC, TGs, and LDL, and a decrease in high-density lipoprotein (HDL) was observed in diabetic groups as compared to normal control. The daily oral effect of three different varieties of peel fractions of *Punica granatum* at 150 mg kg^{-1} b.w concentration in diabetic rats showed a significant (**P<0.05) decrease in TC, TGs, and LDL and increase in HDL compared to the control groups (diabetic and normal).

Table IV. Effect of peel extracts of *P. granatum* on renal function test (RFTs) in alloxan induced diabetic albino rats.

Samples	Groups	Dose (mg/kg bodyweight)	Urea (mg/dl)	Creatinine (mg/dl)	Total proteins (mg/dl)
Healthy (Untreated)	Normal control	---	30.5±5.5^{++}	0.85±3.5^{++}	6.6±0.1^{++}
Diabetic (untreated)	Diabetic control	150	55.5±2.2**	1.11±2.2**	8.3±0.2**
Standard	Glebinclamide	10	42.5±2.2^{++}	0.61±1.6^{++}	6.3±0.3^{++}
Aqueous extract	RPPw	150	34.3±4.2^{++}	0.95±3.2^{++}	5.3±0.2^{++}
	WPPw	150	38.2±3.2^{++}	1.05±3.3^{++}	5.8±0.2^{++}
	DPPw	150	37.5±2.1^{++}	0.95±0.1^{++}	5.2±0.2^{++}
Methanolic extracts	RPPm	150	39.5±2.5^{++}	0.82±5.1^{++}	5.0±0.2^{++}
	WPPm	150	33.2±3.6^{++}	0.95±0.1^{++}	5.2±0.3^{++}
	DPPm	150	33.5±2.2^{++}	0.93±2.0^{++}	5.1±0.1^{++}

Mean ± SEM (n=6)** indicate significance from the diabetic control group at $P<0.05$ and $P<0.01$ probability level. $^{+}$, $^{++}$ indicates significance from the *Punica granatum* peels group at $P<0.05$and $P<0.01$probability level respectively. For abbreviation, see Table I.

Table V. Effect of peel extracts of *P. granatum* on lipid profile in alloxan induced diabetic albino rats

Samples	Groups	Dose (mg/kg bodyweight)	TCH (mg/dl)	TGs (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Healthy (Untreated)	Normal control	--	72.4±4.2^{++}	108.3±3.3^{++}	65.5±5.2^{++}	35.5±01^{++}
Diabetic (untreated)	Diabetic control	--	92.5±2.2**	197.5±2.0**	46.2±3.6**	83.5±01**
Standard	Glibenclamide	10	68.4±2.2^{++}	154.7±3.7^{++}	49.3±2.1^{++}	71.5±03^{++}
Aqeous extract	RPP	150	73.1±0.1^{++}	186.2±1.2^{++}	48.7±4.6^{++}	47.3±5.0^{++}
	WPP	150	64.5±4.2^{++}	174.5±2.2^{++}	53.7±2.1^{++}	51.5±01^{++}
	DPP	150	73.2±2.1^{++}	124.6±6.3^{++}	59.2±4.1^{++}	42.5±03^{++}
Methanolic extracts	RPP	150	70.3±2.2^{++}	187.7±3.5^{++}	50.5±3.3^{++}	63.5±02^{++}
	WPP	150	72.6±0.1^{++}	114.7±5.5^{++}	60.7±3.3^{++}	56.1±00^{++}
	DPP	150	63.5±3.4^{++}	147.4±6.5^{++}	46.8±4.4^{++}	57.5±02^{++}

Mean ± SEM (n=6)** indicate significance from the diabetic control group at $P<0.05$ and $P<0.01$ probability level. $^{+}$, $^{++}$ indicates significance from the *Punica granatum* peels group at $P<0.05$and $P<0.01$probability level respectively. TCH, Total cholesterol; HDL, High density lipoprotein; TGs, Triglycerides; LDL, Low density lipoprotein. For abbreviation, see Table I.

DISCUSSION

DM is a metabolic disorder in which secretion and action of insulin are affected which in result causes the changes not only in carbohydrates but also effects the lipids, and proteins. Insulin hormone is involved in the signal transduction and utilization of carbohydrates by the activation of different signaling molecules, and enzymes involved in the metabolic pathways including glycolysis, glycogen synthesis and lipogenesis (Patterson, 2019). The β cells dysfunctioning or defects in the insulin-stimulated pathway causes impaired carbohydrates metabolism and lead to persistent hyperglycemia and lipedemia (Wang *et al*., 2017). Alloxan is an oxygenated pyrimidine and an analog of glucose is very toxic which is taken by pancreatic β-cells via Glut-2 receptors and led to insulin deficiency (Dwyer-Lindgren *et al*., 2016). After the injection of Alloxan monohydrate elevated level of blood glucose were observed in rats. In this study the methanolic and aqueous extracts of three varieties of *P. granatum* from Mardan, Pakistan were evaluated for *in vitro* and *in vivo* antidiabetic potentials. It was found that both types of extracts from all varieties showed substantial inhibition of α-amylase and in the reduction of blood glucose level in alloxan-induced diabetic albino rats. Furthermore, the effects of peels extracts of all varieties were also measured on lipid profile, and other biochemical parameters related to liver and kidney functioning and found very effective in the reduction of elevated levels of TCs, TGs, LDL, ALT, ALP, bilirubin, and serum creatinine. The methanolic and aqueous peel extracts of all three varieties also showed remarkable antioxidant activity.

It has been previously reported that all parts of *P. granatum* possess varieties of secondary metabolites including polyphenols, flavonoids, and alkaloids (Aragaw *et al*., 2020). The reduction in blood glucose level may be due to the suppression of absorption of glucose due to high concentrations of flavonoids, which could contribute to the control of hyperglycemic conditions (Bloonogarden, 2016). Many studies reported that dietary intake of flavonoids played an effective role in the enhancement of β-cell and revival of insulin, and reduction of glucose which indicated their role in the prevention of T2D (Fang *et al*., 2019; Aragaw *et al*., 2020). The peels extracts reduces the blood glucose may be due to enhanced functions of β-cell by secondary compounds presents in the peels. In line with this study, several studies have been reported the effect of plants extracts containing many secondary metabolites on the regulation of plasma insulin levels (Uttra *et al*., 2018; Pengelly and Bone, 2020; Gurrero-Solano *et al*., 2020). Few studies reported the oral administration of secondary metabolites such as gallic acid, ellagic acid, quercetin other polyphenolic compounds in diabetic mice models and found their pronounced effects in the regeneration of the pancreas, regulation of insulin, and reduction of blood glucose (Singh and Gupta, 2018; Nafees *et al*., 2018; Uhlenbrock *et al*., 2018).

In the current study derangement of ALT, ALP and bilirubin were found in diabetes-induced rats (control) compared to healthy rats (normal control) indicating the abnormal liver function in diabetes. In diabetic rats, the elevation of the serum enzymes is directly associated with changes in related metabolic pathways. The elevated levels of ALT may be due to increased proteolysis and the availability of amino acids in diabetes (Goncalves and Romeiro, 2019). The free availability of amino acids may be responsible for the increased gluconeogenesis and ketogenesis in diabetes. Interestingly, the oral dosing of *P. granatum* peels extracts significantly reduced the level of ALT, ALP, and total bilirubin in the diabetic rats which was comparable with the diabetes-induced rats treated with standard drug glibenclamide (Alliance pharma) and normal control. The restoration of total bilirubin, ALT, and ALP levels in treated rats also indicates a revival of insulin secretion and normal function of the liver. Similarly, the elevated level of creatinine, urea, and decreased level of total protein in diabetic rats indicating renal injuries. It was previously reported that serum creatinine level increases when 40-50% of kidney nephrons were damages (Calin-Sanchez *et al*., 2013; Shah *et al*., 2018, 2019). Our results showed the regeneration in renal function after oral dosing of methanolic and aqueous extracts of peels of *P. granatum* which significantly ($P<0.05$) reduced urea, creatinine, and serum total proteins. The level of serum lipids usually rises in diabetes due to relative or absolute deficiency of insulin and increased lipogenesis and such elevation represents a risk factor for coronary heart disease. In diabetics, the concentration of LDL cholesterol, and TG was significantly increased and HDL cholesterol was decreased from that seen in diabetic control compared to normal controls. The oral administration of aqueous and methanolic peel extract of *P. granatum* at a dose of 150 mg/kg body weight efficiently reduced total cholesterol, TGs, and LDL and increased the HDL diabetes-induced rats. The results were consistent with the previous study that reported significant changes in lipid metabolism in diabetics (Calin-Sanchez *et al*., 2013; Hanani *et al*., 2019; Shah *et al*., 2019). After the treatment, the decrease in TG level may be due to increased insulin release from the β-cells of the pancreas which result favors lipogenesis and may activate a lipoprotein-lipase enzyme that hydrolyse TGs (Hanani *et al*., 2019; Khan *et al*., 2019). Oral administration of various fractions of *P. granatum* peels was found to be safe because no apparent toxicity was observed under the experimental conditions

as well as no organ toxicity (liver, heart, and kidney) was seen in healthy rats. Although, to confirm the adverse effect of various fractions of *P. granatum* peels extract analysis were also performed in healthy rats which indicated no side effects of orally administered extracts at a dose of up to 2500 mg kg^{-1} b.w. The current study confirms that peel extracts of *P. granatum* can be contributory to reduce the complications of diabetics including hyperlipidemia and nephropathy as it lowers the lipids contents and recovers the liver and kidney function parameters.

CONCULSION

It was concluded that the extracts of peels of *Punica granatum* possess significant antihyperglycemic and antihyperlipidemic potentials due to antidiabetic compounds. The methanolic extract of pomegranate peel was found more effective than aqeous extract. The glucose level, lipid and liver profile was signficantlty affected in diabetic compared compared to normal control. It was significantly improved after oral doing of *Punica granatum* peel extract in alloxan induced diabetic rats which was compareable to normal control. It is recommended that peel of *Punica granatum* can be used for antidiabetic drugs development or in folk medicines to treat diabetes.

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Ethical and IRB approval

Approval to conduct the study was obtained from bioethical committee of Hazara University, Mansehra. The research project was approved by IRB of Hazara university, Mansehra.

Satement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abeeleh, M.A., Ismail, Z.B., Alzaben, K.R., Abu-Halaweh, S.A., Al-Essa, M.K., Abuabeeleh, J. and Alsmady M.M., 2009. Induction of diabetes mellitus in rats using intraperitoneal streptozotocin: A comparison between 2 strains of rats. *Eur. J. Sci. Res.,* **32:** 398-402.

Akhtar, K., Shah, S.W.A., Shah, A.A., Shoaib, M., Haleem, S.K. and Sultana, N., 2017. Pharmacological effect of *Rubus ulmifolius* Schott as antihyperglycemic and antihyperlipidemic on streptozotocin (STZ)-induced albino mice. *Appl. biol. Chem.,* **60**: 411-418. https://doi.org/10.1007/s13765-017-0293-9

Anwar, F., Latif, S., Ashraf, M. and Gilani, A.H., 2007. *Moringa oleifera*: A food plant with multiple medicinal uses. *Phytother. Res.,* **21:** 17-25. https://doi.org/10.1002/ptr.2023

Aragaw, T.J., Afework, D.T. and Getahun, K.A. 2020. Assessment of knowledge, attitude, and utilization of traditional medicine among the communities of Debre Tabor Town, Amhara Regional State, North Central Ethiopia: A cross-sectional study. *Evid. Based Complement. Altern. Med.,* **10**: 1-9. https://doi.org/10.1155/2020/6565131

Ay, C.O., Özcan, A.S., Erdoğan, Y. and Özcan, A., 2012. Characterization of *Punica granatum* L. peels and quantitatively determination of its biosorption behavior towards lead (II) ions and acid blue 40. *Colloids Surf. B. Biointerf,* **100**: 197-204. https://doi.org/10.1016/j.colsurfb.2012.05.013

Bloomgarden, Z., 2016. Questioning glucose measurements used in the International Diabetes Federation (IDF). *Atlas J. Diabet.,* **8**: 746–747. https://doi.org/10.1111/1753-0407.12453

Brand-Williams, W., Cuvelier, M.E. and Berset, C.L.W.T.. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT Fd. Sci. Technol.,* **28**: 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

Calín-Sánchez, Á., Figiel, A., Hernández, F., Melgarejo, P., Lech, K. and Carbonell-Barrachina, Á.A., 2013. Chemical composition, antioxidant capacity, and sensory quality of pomegranate (*Punica granatum* L.) arils and rind as affected by drying method. *Fd. Bioprocess. Technol.,* **6:** 1644-1654. https://doi.org/10.1007/s11947-012-0790-0

Cho, N.H., 2018. IDF Diabetes atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res. Clin. Pract.,* **138**: 271–281. https://doi.org/10.1016/j.diabres.2018.02.023

Das, S. and Barman, S., 2012. Antidiabetic and antihyperlipidemic effects of ethanolic extract of leaves of *Punica granatum* in alloxan-induced non–insulin-dependent diabetes mellitus albino rats. *Indian J. Pharmacol.,* **44**: 219. https://doi.org/10.4103/0253-7613.93853

Dwyer-Lindgren, L., Mackenbach, J.P., Van Lenthe, F.J., Flaxman, A.D. and Mokdad, A.H., 2016.

Diagnosed and undiagnosed diabetes prevalence by County in the U.S., 1999–2012. *Diabetes Care,* **39**: 1556–1562. https://doi.org/10.2337/dc16-0678

Fang, J.Y., Lin, C.H., Huang, T.H. and Chuang, S.Y. 2019. *In vivo* rodent models of type 2 diabetes and their usefulness for evaluating flavonoid bioactivity. *Nutrition,* **11**: 530. https://doi.org/10.3390/nu11030530

Gonçalves, P.B. and Romeiro, N.C., 2019. Multi-target natural products as alternatives against oxidative stress in chronic obstructive pulmonary disease (COPD). *Eur. J. med. Chem.,* **163**: 911-931. https://doi.org/10.1016/j.ejmech.2018.12.020

Guerrero-Solano, J.A., Jaramillo-Morales, O.A., Jiménez-Cabrera, T., Urrutia-Hernández, T.A., Chehue-Romero, A., Olvera-Hernández, E.G. and Bautista M., 2020. *Punica protopunica* balf the forgotten sister of the common pomegranate (*Punica granatum* L.): Features and medicinal properties. A review. *Plant,* **9:** 1214. https://doi.org/10.3390/plants9091214

Hanani, Z.N., Yee, F.C. and Nor-Khaizura, M.A. 2019. Effect of pomegranate (*Punica granatum* L.) peel powder on the antioxidant and antimicrobial properties of fish gelatin films as active packaging. *Fd. Hydrocoll.,* **89**: 253-259. https://doi.org/10.1016/j.foodhyd.2018.10.007

Huang, H., Liao, D., Chen, G., Chen, H. and Zhu, Y., 2017. Lack of efficacy of pomegranate supplementation for glucose management, insulin levels and sensitivity: evidence from a systematic review and meta-analysis. *Nutr. J.,* **16:** 67. https://doi.org/10.1186/s12937-017-0290-1

Ismail, M.Y.M. and Yaheya, M., 2009. Clinical evaluation of antidiabetic activity of *Trigonella* seeds and *Aegle marmelos* leaves. *World appl. Sci. J.,* **7**: 1231-1234.

Khan, I.U., Shah, A.A., Sahibzada, F.A., Hayyat, A., Nazar, M., Mobashar, M. and Sultana, N., 2019. Carcass characteristics and serum biochemical profile of Japanese quail by the supplementation of pine needles and vitamin E powder. *Biology,* **74**: 993-1000. https://doi.org/10.2478/s11756-019-00225-y

Lewis, D.R. and Liu, D.J., 2012. Direct measurement of lipase inhibition by orlistat using a dissolution linked *in vitro* assay. *Clin. Pharmacol. Biopharmacol.,* **1:** 1-19. https://doi.org/10.4172/2167-065X.1000103

Middha, S.K., Usha, T. and Pande, V., 2013. A review on antihyperglycemic and antihepatoprotective activity of eco-friendly *Punica granatum* peel waste. *Evid. Based Complement. Altern. Med.,* pp. 1-9. https://doi.org/10.1155/2013/656172

Middha, S.K., Usha, T. and Ravi-Kiran, T., 2012. Influence of *Punica granatum* L. on region specific responses in rat brain during alloxan-induced diabetes. *Asian Pac. J. Trop. Biomed.,* **2**: S905-S909. https://doi.org/10.1016/S2221-1691(12)60334-7

Nafees, M., Jaskani, M.J., Naqvi, S.A., Haider, M.S. and Khan, I.A., 2018. Evaluation of elite pomegranate genotypes of Balochistan based on morphological, biochemical and molecular traits. *Int. J. Agric. Biol.,* **20:** 1405–1412.

Patterson, C.C., 2019. Trends and cyclical variation in the incidence of childhood type 1 diabetes in 26 European centres in the 25 year period 1989–2013: A multicentre prospective registration study. *Diabetology,* **62**: 408–417. https://doi.org/10.1007/s00125-018-4763-3

Pengelly, A. and Bone, K., 2020. *The constituents of medicinal plants: An introduction to the chemistry and therapeutics of herbal medicine* (2nd ed.). Routledge, **4**: 1-9. https://doi.org/10.4324/9781003117964

Sagbo, I.J., van de Venter, M., Koekemoer, T. and Bradley, G., 2018. *In vitro* antidiabetic activity and mechanism of action of *Brachylaena elliptica* (Thunb.) DC. *Evid. Based Complement. Altern. Med.,* pp. 1-13. https://doi.org/10.1155/2018/4170372

Shah, A.A., Khan, I.U., Sahibzada, F.A., Tauseef, I., Kalsoom, U.E. and Sultana, N., 2019. Biological and biochemical characteristics of male reproductive system, serum metabolites and carcass quality of Japanese quails by the supplementation of *Pinus ponderosa* leaves and α-tocopherol acetate. *Reprod. Domeist. Anim.* **54**: 1348-1356. https://doi.org/10.1111/rda.13521

Shah, A.A., Liu, Z., Qian, C., Wu, J., Sultana, N. and Zhong, X., 2020. Potential effect of the microbial fermented feed utilization on physicochemical traits, antioxidant enzyme and trace mineral analysis in rabbit meat. *J. Anim. Physiol. Anim. Nutr.,* **104:** 767-775. https://doi.org/10.1111/jpn.13252

Shah, A.A., Yuan, X., Khan, R.U. and Shao, T., 2018. Effect of lactic acid bacteria-treated King grass silage on the performance traits and serum metabolites in New Zealand white rabbits (*Oryctolagus cuniculus*). *Anim. Physiol. Anim. Nutri.,* **102**: e902-e908. https://doi.org/10.1111/jpn.12854

Shaikh, S.B. and Bhandary, Y.P., 2021. Therapeutic properties of *Punica granatum* L (pomegranate) and its applications in lung-based diseases: A

detailed review. *J. Fd. Biochem*., **45**: e13684. https://doi.org/10.1111/jfbc.13684

Singh, T.J. and Gupta, T., 2018. Morphological and quality traits performance of the fruits of wild pomegranate (*Punica granatum* L.) in Himachal Pradesh. *Int. J. Bio-Res. Str. Manage.,* **9**: 341-344. https://doi.org/10.23910/IJBSM/2018.9.3.3C0997

Thanh, H.N., Huyen, N.T., Van Khanh, N., Thu, D.K. and Tung, B.T., 2019. Phytochemicals and antidiabetic activity of the aqueous extract of the *Punica granatum* fruit in streptozotocin-induced diabetic mice. *J. Basic Clin. Physiol. Pharmacol.*, **30**: 2019006. https://doi.org/10.1515/jbcpp-2019-0061

Uhlenbrock, L., Sixt, M., Tegtmeier, M., Schulz, H., Hagels, H., Ditz, R. and Strube, F., 2018. Natural products extraction of the future sustainable manufacturing solutions for societal needs. *Processes,* **6:** 177. https://doi.org/10.3390/pr6100177

Uttra, A.M., Ahsan, H., Hasan, U.H. and Chaudhary, M.A., 2018. Traditional medicines of plant origin used for the treatment of inflammatory disorders in Pakistan: A review. *J. Tradit. Chin. Med.,* **38**: 636-656. https://doi.org/10.1016/S0254-6272(18)30897-5

Wang, L., Gao, P., Zhang, M., Huang. Z., Zhang, D., Deng, Q., Li, Y., Zhao, Z., Qin, X., Jin, D. and Zhou, M., 2017. Prevaence and ethnic pattern of diabetes and prediabetes in China in 2013. *J. Am. med. Assoc.,* **317**: 2515–2523. https://doi.org/10.1001/jama.2017.7596

Zeb, M.A., Sajid, M., Rahman, T.U., Khattak, K.F. and Halim, M., 2015. Phytochemical screening and antibacterial activity of *Opuntia dillenii* and *Onosma bracteata*. *J. Microbiol. Exp.*, **3**: 74. https://doi.org/10.15406/jmen.2015.02.00074