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The potential for interaction of tolbutamide with pomegranate juice against diabetic induced complications in rats

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ABSTRACT

Background: Pomegranate can inhibit cytochrome P450 (CYP) 2C9 activity, which is largely responsible for the metabolism of sulfonylureas. The present study was undertaken to evaluate the pharmacokinetic and pharmacodynamic interaction of pomegranate and tolbutamide (TOL) against diabetic-induced complications.

Methods: Diabetes was induced via administration of alloxan (150 mg/kg, intraperitoneally). Rats ($n = 8$) were treated with pomegranate juice (PJ) [3 mL/animal, per os (p.o.)], TOL (20 mg/kg, p.o.), and their combination for 4 weeks. Twenty-four hours after the last treatment, the pharmacodynamic interaction of PJ and TOL was evaluated by antinociceptive activity, electrocardiographic parameters, serum glucose, biomarkers, and lipid profile values. The influence of PJ on the pharmacokinetics of TOL was studied using the high performance liquid chromatography method.

Results: The combination of PJ and TOL resulted in a significant improvement against diabetic complications compared to the group treated with TOL alone. The combination group was found to be the best protective group by significant improvement of antinociceptive activity, restoration of electrocardiographic parameters, serum glucose, biomarkers, and lipid profile compared to the group treated with TOL alone. Results of the pharmacokinetic study revealed that PJ increases bioavailability and half-life, along with a decrease in clearance and elimination rate of TOL.

Conclusion: From this study, it can be concluded that the combination of PJ and TOL exhibited profound protection compared to TOL alone against diabetic complications. The findings of pharmacokinetic interaction justified the results of pharmacodynamic interaction.

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1. Introduction

Diabetes mellitus is a chronic metabolic disorder associated with macro- and microvascular complications. The pathological conditions associated with diabetes are responsible for the impairment of numerous organs and functions of the organism resulting in chronic deterioration of the organs and premature morbidity and mortality.[1]

Since ancient times, in many societies, herbs and herb-based therapy have been used to treat different diseases and improve quality of life. In a recent trend, herb–drug interaction studies are getting more popular, wherein each may influence the pharmacokinetic and pharmacodynamic profile of the other, and can mimic, magnify, or oppose the action of the other.[2]

Punica granatam, popularly known as pomegranate, is a member of Punicaceae family, which is a large deciduous shrub or small tree, and has a rich history of traditional use in medicine. Pomegranate juice (PJ) has been shown to exert significant anticancer, anti-inflammatory, antidiabetic, antimicrobial, antioxidant, antiatherosclerotic, and antihypertensive effects.[3,4] Some studies have indicated that the significant antidiabetic effect associated with pomegranate may be attributable to the presence of oleanolic, ursolic, and gallic acids as chief chemical constituents. No attempts have been taken to demonstrate the beneficial effect of pomegranate in diabetes-induced secondary complications.[5,6]

Sulfonylurea, an insulin secretagogue, is a popular class of antidiabetic therapy. Most sulfonylureas are extensively metabolized in the liver, primarily by cytochrome P450 (CYP) 2C9 isoenzyme.[7] Among the sulfonylureas, tolbutamide (TOL) is one of the popular oral hypoglycemic agents, but poor bioavailability associated with TOL is a matter of medical concern.[8]

Thus, it is worthwhile to make an attempt to increase the bioavailability of TOL. One of the interesting findings regarding PJ is its ability to inhibit CYP2C9 activity by which it can potentiate the effect of those drugs that are metabolized by the same pathway. It has been reported that pomegranate at a dose of 3 mL [per os (p.o.)] is responsible for the increased potency of TOL by inhibiting CYP2C9 activity. Pomegranate is also responsible for the inhibition of uptake of solute carrier transporters (SCTs), which are membrane proteins responsible for the cellular influx of various substances including drugs and xenobiotics.[9,10]

So the present study was designed to evaluate the effect of pomegranate alone and in combination with TOL against alloxan-induced diabetic complications.

2. Methods

2.1. Chemicals

All chemicals used were of analytical grade and purchased from standard companies such as R L Fine Chemicals (Bengaluru, India) and Rankem (Mumbai, India). Biochemical kits were procured from Crest Biosystems (Goa, India). A pure sample of TOL was gifted by the Bangalore Test House (Bangalore, India).

2.2. Experimental animals

Healthy adult Wistar albino rats of either sex weighing 175–250 g were housed in polypropylene cages and maintained under standardized conditions (12-hour light/dark cycles, $25 \pm 5\,^{\circ}C$) with paddy husk bedding at the Central Animal House, Shree Devi College of Pharmacy, Mangalore, India. They were provided with standard pellet food and had free access to purified drinking water. The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India, were followed and prior permission was sought from the Institutional Animal Ethics Committee for conducting the study (SDCP/IAEC13/2013-14).

2.3. Plant material

Fresh pomegranate fruits were purchased in June 2015 from the local market of Mangalore. Authentication was performed by Dr Neoline J. Pinto (Head of the Department, Department of Botany, St. Agnes College, Mangalore, India; SAC/MNG/SMP/Drug/2015-06/52.

The fruit was preserved at $4\,^{\circ}C$ until use. PJ was isolated by squeezing the edible portion of the pomegranate and filtering it to remove the residue. All samples were used within 1 hour after they were squeezed and filtered.[9]

2.4. Dose selection

Based on an earlier literature review, the therapeutic dose of TOL is 20 mg/kg. It was found that PJ at a dose of 3 mL/rat was able to inhibit the CYP2C9 enzyme, which was a metabolic enzyme responsible for the metabolism of TOL.[9]

2.5. Experimental protocol

The animals were divided into five different treatment groups of eight animals each. Group I and Group II received saline and were termed as normal control and diabetic control, respectively; Groups III and IV received TOL (20 mg/kg) and PJ (3 mL/rat); Group V received the combination of TOL and PJ. All treatments were given for 4 weeks through the oral route. Apart from the normal control group, for all the other groups diabetic rats were used.

2.6. Induction of diabetes

Diabetes was induced via an intraperitoneal (i.p.) injection of a freshly prepared aqueous solution of alloxan monohydrate (150 mg/kg body weight)[6] in normal saline. Seventy-two hours after alloxan administration, blood was withdrawn from the overnight fasted rats through the tail vein for glucose analysis, and rats with fasting glucose ranging from 210 to 220 mg/dL, showing clear signs of polyuria, polyphagia, and polydipsia, were considered diabetic. Animals with fasting blood glucose less than 200 mg/dL were not used for experimentation.

2.7. Estimation of serum glucose level

Twenty-four hours after the last treatment, serum glucose level was estimated using commercial kits with the help of a semiautoanalyzer.[6]

2.8. Effect on fasting insulin level

Twenty-four hours after the last treatment, blood was collected and serum was separated by centrifugation. Insulin levels were measured using rat insulin enzyme-linked immunosorbent assay kit (Mercodia, Uppsala, Sweden) in serum.[11]

2.9. Serum biomarker levels

Twenty-four hours after the last treatment, blood was collected by retro-orbital puncture, and the serum was separated by centrifugation. Isolated serum was analyzed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP), creatine kinase MB (CK-MB), creatine kinase NAC (CK-NAC), lactate dehydrogenase (LDH), creatinine and albumin. Estimation of different biomarkers was performed using commercial kits with the help of a semiautoanalyzer.[12]

2.10. Antinociceptive activity

Twenty-four hours after the last treatment, antinociceptive activity was measured using hot plate and tail immersion tests.[13]

2.11. Electrocardiographic studies

Twenty-four hours after the last treatment, the animals were anesthetized with the combination of ketamine (75 mg/kg, i.p.) and xylazine (8 mg/kg, i.p.). Leads were attached to the dermal layer of both the front paws and hind legs, and recordings were made with the help of a digital physiograph (model no-DI-2; INCO, Ambala City, India). The changes in heart rate, QRS interval, QT interval, and RR interval were noted.[12]

2.12. Lipid profile assay

Serum cholesterol and triglyceride levels were measured using commercial kits with the help of a semiautoanalyzer.[12]

2.13. Statistical analysis

Results are expressed as mean ± standard error. Statistical significance was assessed using one-way analysis of variance followed by Tukey–Karmer multiple comparison tests. A $p < 0.05$ was considered statistically significant.

2.14. Pharmacokinetic interaction

Animals were divided into two groups consisting of eight animals each. Group I received TOL (20 mg/kg; single dose) and Group II received PJ (3 mL/rat) for 4 weeks + TOL (20 mg/kg; single dose). All treatments were administered through the oral route. Immediately after the administration of TOL, 0.5-mL blood samples were withdrawn at each time interval over 24 hours (0, 1, 2, 4, 8, 16, and 24 hours) by puncturing the retro-orbital vein under partial ether anesthesia and subjected to analysis. The hypovolemia is prevented by an i.p. administration of 0.5 mL of normal saline immediately after each withdrawal of blood. The concentration of TOL was determined by high performance liquid chromatography. A Waters 6000 pumping system (Waters Associates, Singapore) coupled to a spectroflow SF 770 variable wavelength detector (Schoeffel Instrument Corp., Singapore) was used for this study.

The data were represented in a plasma level–time curve from where the area under time curve ($AUC_{0-24\,h}$) was calculated using the trapezoid rule. The maximum concentration (C_{max}) and maximum time (T_{max}) were obtained directly from the generated data. The elimination constant (K_e) and half-life ($T_{1/2}$) were determined from the semilog plot of the data. The clearance (CL) and apparent volume of distribution (V_d) of the drug in the animals were calculated using the equation: $CL = V_d \times K_e$, where V_d denotes the administered dose of drug/initial plasma concentration of drug obtained at the intercept of semilog plot of plasma drug sample. AUC total = $AUC_{0-24\,h} + C_{24\,h}/K_e$. The results were analyzed statistically using Student *t* test.[14]

3. Results

3.1. Effect on serum glucose level

The diabetic control group showed a significant increase in serum glucose level compared to the normal control group. All other treatment groups such as TOL, PJ alone, and the combination group of TOL and PJ reported significant decreases in blood glucose level compared to the diabetic control group. The combination group of TOL and PJ demonstrated a significant reduction in blood glucose level compared to the group treated with TOL alone (Table 1).

3.2. Effect on serum insulin level

The diabetic control group showed a significant decrease in serum insulin level compared to the normal control group. All treatment groups witnessed a significant increase in insulin level compared to the diabetic control group. The combination group of TOL and PJ reported a significant increase in serum insulin level compared to the group treated with TOL alone (Table 1).

3.3. Effect on antinociceptive property

In both tests, the diabetic control group exhibited significant increase in reaction time compared to the normal control group. All treatment groups witnessed significant improvement in reaction time compared to the diabetic control group. The TOL and PJ combination group showed a significant restoration of reaction time compared to the group treated with TOL alone (Table 1).

Table 1 – Effect on serum glucose, insulin, biomarkers, and antinociceptive activities against alloxan induced diabetic complications.

Treatment	Glucose (mmol/L)	Insulin level (μu/mL)	LDH (IU/L)	CK-MB (IU/L)	CK-NAC (IU/L)	Antinociceptive activity	
						Hot plate test (s)	Tail immersion test (seconds)
Normal control	95.57 ± 0.57	27.56 ± 0.62	96.64 ± 0.57	209.53 ± 1.15	73.22 ± 0.57	6.33 ± 0.28	3.66 ± 0.28
Diabetic control	189.22 ± 0.15^{c}	9.21 ± 0.24^{c}	277.1 ± 1.15^{c}	428.5 ± 2.88^{c}	175.52 ± 1.15^{c}	13.66 ± 0.57^{c}	13.33 ± 0.57^{c}
TOL	138.89 ± 0.57^{c,e}	19.84 ± 0.59^{b,e}	137.05 ± 1.15^{c,e}	342.46 ± 1.73^{c,e}	125.66 ± 0.57^{c,e}	9.57 ± 0.28^{b,e}	8.45 ± 0.57^{c,e}
PJ	143.57 ± 0.57^{c,e}	15.39 ± 0.73^{c,e}	146.99 ± 1.1^{c,e}	375.37 ± 2.30^{c,e}	142.81 ± 1.15^{c,e}	10.66 ± 0.57^{c,d}	10.33 ± 0.17^{c,d}
TOL + PJ	114.25 ± 0.28^{c,e,h}	23.83 ± 0.19^{a,e,g}	128.63 ± 0.57^{c,e,h}	240.37 ± 2.30^{c,e,h}	80.21 ± 0.28^{c,e,h}	7.52 ± 0.17^{e,f}	6.01 ± 0.26^{a,e,f}

All values are mean ± SEM, $n = 8$.
$^{a}p < 0.05$, $^{b}p < 0.01$, and $^{c}p < 0.001$, when compared to normal control; $^{d}p < 0.01$, and $^{e}p < 0.001$ compared to diabetic control; and $^{f}p < 0.05$, $^{g}p < 0.01$, and $^{h}p < 0.001$ when compared to TOL alone treated group.
CK-MB, creatinine kinase MB; CK-NAC = creatinine kinase NAC; LDH, lactate dehydrogenase; PJ, pomegranate juice; SEM, standard error of the mean; TOL, tolbutamide.

Table 2 – Effect on electrocardiographic parameters against alloxan-induced diabetic complications.

Treatment	Heart rate (beats/min)	QRS duration (ms)	QT interval (ms)	RR interval (ms)	ST interval (ms)
Normal control	183.12 ± 2.30	120.93 ± 1.73	120.78 ± 1.73	192.2 ± 2.30	57.01 ± 1.15
Diabetic control	247.77 ± 2.88^{b}	203.49 ± 2.30^{b}	244.49 ± 2.88^{b}	314.83 ± 2.88^{b}	92.34 ± 1.73^{b}
TOL	232.7 ± 2.30^{b,c}	164.01 ± 2.30^{b,e}	164.23 ± 1.73^{b,e}	248.15 ± 2.30^{b,e}	76.59 ± 1.45^{b,e}
PJ	221.51 ± 2.88^{b,e}	181.77 ± 1.73^{b,e}	155.95 ± 1.73^{b,e}	291.27 ± 2.80^{b,e}	82.25 ± 1.73^{b,d}
TOL + PJ	200.42 ± 2.30^{a,e,f}	129.14 ± 1.73^{e,f}	128.45 ± 1.73^{e,f}	224.26 ± 2.30^{b,e,f}	61.56 ± 1.73^{e,f}

All values are mean ± SEM, $n = 8$.
$^{a}p < 0.01$ and $^{b}p < 0.001$, when compared to normal control; $^{c}p < 0.05$, $^{d}p < 0.01$, and $^{e}p < 0.001$ when compared to diabetic control; and $^{f}p < 0.001$ when compared to the group treated with TOL alone.
PJ, pomegranate juice; QRS, ; QT, ; RR, ; SEM = standard error of the mean; ST, ; TOL, tolbutamide.

Table 3 – Effect on serum biomarkers and lipid profile against alloxan-induced diabetic complications.

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Creatinine	Albumin	Total cholesterol (units/mg)	Triglycerides (units/mg)
Normal control	92.26 ± 0.57	234.34 ± 1.15	144.18 ± 0.57	1.52 ± 0.11	2.11 ± 0.17	141.40 ± 1.15	41.88 ± 0.28
Diabetic control	207.21 ± 1.15^{c}	496.97 ± 2.30^{c}	296.80 ± 2.30^{c}	7.55 ± 0.28^{c}	5.9 ± 0.28^{c}	213.97 ± 1.73^{c}	91.81 ± 0.57^{c}
TOL	154.85 ± 0.57^{c,d}	298.05 ± 2.30^{c,d}	238.58 ± 1.45^{c,d}	3.78 ± 0.057^{c,d}	3.51 ± 0.115^{b,d}	167.03 ± 0.57^{c,d}	52.85 ± 0.28^{c,d}
PJ	184.58 ± 1.73^{c,d}	346.13 ± 2.8^{c,d}	255.89 ± 1.73^{c,d}	3.75 ± 0.17^{c,d}	3.92 ± 0.14^{c,d}	181.68 ± 1.15^{c,d}	81.47 ± 0.57^{c,d}
TOL + PJ	124.75 ± 0.57^{c,d,f}	251.23 ± 1.15^{c,d,f}	191.69 ± 4.37^{c,d,f}	2.34 ± 0.11^{a,d,f}	2.57 ± 0.11^{d,e}	149.76 ± 1.15^{b,d,f}	48.34 ± 0.28^{c,d,f}

All values are mean ± SEM, $n = 8$.
$^{a}p < 0.05$, $^{b}p < 0.01$, and $^{c}p < 0.001$, when compared to normal control; $^{d}p < 0.001$ compared to diabetic control; $^{e}p < 0.05$, and $^{f}p < 0.001$ when compared to the group treated with TOL alone.
ALP, alkaline phosphate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; PJ, pomegranate juice; SEM, standard error of the mean; TOL, tolbutamide.

3.4. *Effect on electrocardiographic parameters*

The diabetic control group demonstrated a significant increase in heart rate, QT segment, RR interval, and QRS interval compared to the normal control group. All treatment groups showed significant improvement in ECG (Electrocardiogram) parameters compared to the diabetic control group. The combination group of TOL and PJ restored ECG parameters significantly compared to the group treated with TOL alone (Table 2).

3.5. *Effect on serum biomarkers*

The diabetic control group showed a significant increase in AST, ALT, ALP, CK-MB, CK-NAC, LDH, creatinine, and albumin levels compared to the normal control group. All treatment groups exhibited significant improvement in biomarker levels compared to the diabetic control group. The combination group of TOL and PJ demonstrated significant decrease in biomarker levels compared to the group treated with TOL alone (Tables 1 and 2).

3.6. *Effect on lipid profile*

The diabetic control group showed significant increase in serum cholesterol and triglyceride levels compared to the normal control group. All treatment groups exhibited significant decrease in lipid profile levels compared to the diabetic control group. The combination group of TOL and PJ demonstrated

Table 4 – Effect on serum biomarkers and lipid profile against alloxan-induced diabetic complications.

Parameters	TOL	TOL + PJ
C_{max} (μg/mL)	90.21 ± 2.49	135.46 ± 1.73[a]
T_{max} (h)	2.32 ± 0.12	1.55 ± 0.18[a]
AUC total (μg/h mL)	878.73 ± 5.23	1369.59 ± 6.46[b]
CL (mL/kg h)	65.69 ± 1.42	32.38 ± 1.21[c]
$T_{1/2}$ (h)	7.84 ± 0.82	15.92 ± 0.59[c]
V_d (mL/kg)	560.42 ± 7.34	595.69 ± 5.52
K_a (h^{-1})	0.77 ± 0.034	1.49 ± 0.11[b]

All values are mean ± standard error of the mean; $n = 8$.
[a] $p < 0.05$, [b] $p < 0.01$, and [c] $p < 0.001$, when compared to TOL.
AUC, area under time curve; CL, clearance; PJ, pomegranate juice; TOL, tolbutamide.

a significant reduction in lipid profile compared to the group treated with TOL alone (Table 3).

3.7. *Effect on pharmacokinetic parameters*

The TOL and PJ combination group showed significant increase in C_{max} and AUC_{total}. T_{max} was found to be almost the same for both groups, whereas C_{max} was remarkably high for TOL in the presence of PJ, indicating an enhanced extent of absorption. A significant prolongation of elimination half-life, $T_{1/2}$ (hour), was observed for the PJ and TOL combination group compared to the group treated with TOL alone. The combination group showed a significant decrease in clearance, but demonstrated a significant increase in the rate of absorption (K_a) compared to the group treated with TOL alone (Table 4).

4. Discussion

The aim of the present study was to elucidate the effect of PJ alone and the combination of PJ and TOL against alloxan-induced diabetic complications. Moreover, a pharmacokinetic study of PJ and TOL also has been designed that helped in the logical interpretation of the findings.

Alloxan is a toxic glucose analogue transported through the GLUT2 glucose transporter and majorly accumulates in pancreatic beta cells. Owing to the presence of intracellular thiols such as glutathione, alloxan generates reactive oxygen species with its reduction product, dialuric acid. Auto-oxidation of dialuric acid generates superoxide radicals, hydrogen peroxide, and—in a final iron-catalyzed reaction step—hydroxyl radicals. These hydroxyl radicals are ultimately responsible for the death of beta cells.[15]

In our study the group treated with alloxan alone also showed significant increase in blood sugar level compared to the normal control. The PJ- and TOL-treated group showed a significant decrease in blood sugar level compared to the diabetic control group. The combination of PJ and TOL demonstrated improved glycemic control compared to the group treated with TOL alone.

Diabetes mellitus is associated with damage of beta cells of the pancreas, which is responsible for the decreased insulin release. In our study also the diabetic control group showed a significant decrease in serum insulin level. Treatment with PJ, TOL, and their combination showed significant increase in insulin level compared to the diabetic control group. Among all the treatment groups, the combination group showed maximum insulin level.

It is a well-established fact that TOL appears to lower the blood glucose acutely by stimulating the release of insulin from the pancreas. In a recent study, it has been observed that antidiabetic activity associated with PJ may be attributable to strong free radical scavenging activity, which can provide protection against diabetes-induced oxidative stress. Moreover, antidiabetic activity associated with PJ is also attributed to agonistic activity on peroxisome proliferator-activated receptor-gamma binding and nitric oxide production.[16,17]

Diabetes is associated with different secondary complications such as diabetic neuropathy. It has been observed that there is loss of pain perception, and it is thought to be due to nerve damage and induction of peripheral neuropathy.[18]

In our study, the diabetic control group showed a significant increase in reaction time in hot plate and tail emersion tests. The PJ- and TOL- treated group showed significant protection whereas the combination group was found to be the most protective group in terms of antinociceptive activity.

Antioxidant, anti-inflammatory, and aldose reductase inhibitory activities may be the prime factors behind the improvement in diabetic neuropathy condition. Aldose reductase is an enzyme involved in the etiology of diabetic complications.[19]

Chronic diabetes is associated with many pathological conditions of the cardiovascular system that can be determined by electrocardiographic study. Chronic high blood sugar level is responsible for prolongation of the QT segment by suppression of P-glycoprotein levels and I_{Kr} ion channel, which is the primary contributing ion channel to potassium extrusion and therefore repolarization.[20]

Ventricular arrhythmia is responsible for sudden cardiac death in patients with type 2 diabetes. Abnormality in conduction due to myocardial ischemia, ion channel dysfunction, increased adrenergic drive, and calcium overload are major contributory factors for induction of arrhythmias. Increase in PR interval and QRS interval are the early markers for induction of cardiac arrhythmia.[21]

Diabetes leads to cardiac autonomic neuropathy, which is associated with damage of both sympathetic and parasympathetic nervous system. Resting tachycardia can be observed, which is indicated by a fixed increased heart rate that is unresponsive to moderate exercise, stress, or sleep, indicating almost complete cardiac denervation.[22]

In this study, only the alloxan-treated group showed increase in heart rate, QRS interval, QT interval, and RR interval, which confirmed the induction of abnormal conditions in the heart.

PJ and TOL treatment demonstrated significant improvement in ECG studies majorly by glycemic control. Moreover, PJ can decrease or reverse the progression of ischemic lesion areas through a strong antioxidant capacity, enhanced biological actions of nitric oxide, decreased inflammation, and decreased angiotensin converting enzyme activity.[4]

The combination treatment showed better cardioprotective action in diabetic conditions.

Diabetes mellitus is responsible for the development of tremendous oxidative stress. Hyperglycemia causes severe tis-

sue damage due to those different organ-specific biomarkers that have been cited as responsible for the serum biomarker level.[12,23]

In the present study, the diabetic control group also showed significant increase in cardiac biomarkers such as LDH, CK-MB, and CK-NAC; hepatic biomarkers such as ALT, AST, ALP; and kidney biomarkers such as creatinine and albumin, which confirmed damage of these important organs.

The better protection observed in TOL- and PJ-treated groups may be attributable to their antidiabetic potential. PJ is able to neutralize the generated reactive oxygen species, increase certain antioxidant enzyme activities, and induce metal chelating activities, which are some of the contributory factors for PJ to show cellular protection. The combination group of TOL and PJ showed incremental cytoprotection.[24]

Patients with chronic untreated diabetes develop hyperlipidemia that may be attributable to decreased adipose tissue and muscle lipoprotein lipase activity.[25]

In our study, the diabetic control group showed significant increase in serum cholesterol and triglyceride levels. TOL and PJ treatment restored the lipid profile significantly. Resistin is an adipocyte-specific hormone believed to be the link between obesity, insulin resistance, and diabetes. PJ is able to inhibit resistin and can also cause LDL (Low density lipoprotein) receptor mRNA levels, which causes hepatic utilization of LDL.[24,26]

The combination group of TOL and PJ was found to be the most protective group.

In this study, we also observed that PJ- and TOL alone-treated groups showed significant protection against alloxan-induced diabetic complications. The combination group of PJ and TOL was found to be most beneficial group. From the present findings, it can be concluded that an advantageous pharmacodynamic interaction of PJ and TOL has been achieved.

Sulfonylureas such as TOL are metabolized by microsomal enzyme CYP2C9, where as PJ is an inhibitor of this enzyme. It is quite possible that by inhibiting metabolism, PJ can increase the potency of TOL. PJ is also responsible for the inhibition of uptake of SCTs. The rise in SCT level is responsible for increased influx of drug, drug deposition, and decreased clearance. The findings of our pharmacokinetic study justify these points by showing a significant increase in C_{max} and AUC_{total}, increase in the rate of absorption, as well as prolongation of elimination half-life $T_{1/2}$ (h).

From this study, it can be concluded that PJ can be very beneficial in the treatment of diabetes and related secondary complications. Sulfonylureas such as TOL and pomegranate, if given together, can potentiate the action by many folds. However, further study is still necessary to attain therapeutic optimization of PJ and drugs in the sulfonylurea class. Furthermore, this interesting finding requires further work to establish the fact clinically.

Conflicts of interest

None.

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