Antihyperglycemic activity with DPP-IV inhibition of Prunus amygdalus extract: investigation via experimental validation and molecular docking

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Abstract

Background and Purpose: Prunus amygdalus (PA) is a popular invasive seed utilized in the management of diabetes in Jammu and Kashmir, India. The current study scrutinizes the antihyperglycemic effect of PA on Streptozotocin (STZ) induced diabetic rats. Experimental approach: BBD was performed to determine the effect of PA powder on methanol, extraction time and extraction temperature on DPPH and ABTS free radical scavenging activity of decoction. Oral glucose tolerance test (OGTT) executed for the estimation of glucose utilization by PA extract. Type II diabetes mellitus was initiated by single intraperitoneal injection of STZ. The Blood Glucose Level (BGL) and body weight were estimated at regular interval of time. Different biochemical parameters; hepatic parameters; antioxidant parameters and lipid parameters were estimated followed by histopathological observation. of pancreas Key Results: The variation observed were DPPH antiradical scavenging activity 40.0-90.0% and ABTS antiradical scavenging activity 34-82%, respectively. OGTT study, PA treatment significantly ($P<0.001$) down-regulated the BGL level 170, 142.66 and 100.36 mg/dL at 50, 100 and 200 mg/kg, respectively. PA significantly ($P<0.001$) altered the biochemical, hepatic and antioxidant parameters in a dose-dependent manner. Further alteration of antioxidant parameters, hepatic and renal parameters were also observed. Histopathological examination demonstrated the increased mass of β-cells in the pancreas. Conclusions & Implications: Consequently, we can say that response surface methodologies successfully carry out the extraction of Prunus amygdalus with the highest antioxidant effect and PA treatment would be helpful in the preclusion of diabetes and its complications.

Abbreviation

STZ=Streptozotocin, PA=Prunus *amygdalus* , LC-MS= Liquid chromatography-mass spectroscopy, BGL=Blood Glucose Level, SGOT=serum glutamic oxalo acetic transaminase, SGPT=serum glutamic pyruvic transaminase, ALP=alkaline phosphatise, SOD=superoxide dismutase, LPO=lipid peroxidation, CAT=catalase, GSH=glutathione, HDL=high-density lipoprotein, TG=triglyceride, TC=total cholesterol, LDL=low-density lipoprotein, VLDL=very low density lipoprotein, OGTT= oral glucose tolerance test, DM= Diabetes mellitus, WHO= World Health Organization, GLP-1=glucagon-like peptide-1, GIP=glucose-dependent insulinotropic polypeptide, NAFLD=non-alcoholic fatty liver disease,ROS= reactive oxygen species.

Introduction

Diabetes mellitus (DM) constitute various forms of metabolic disorders, categorized via increase BGL with a reduction in the plasma insulin level and serve as hyperglycemia condition that affects diverse organs of the

human body (Drouin et al., 2009). Nowadays, without any doubt, DM has considered as the biggest global health and economic problem Present with high obesity incidence, the prevalence of diabetes is increasing day by day. 6% of global population is affected by it or by its complications. Among total diabetic patient, 90-95% patient suffers from the type II diabetes and rest from type I. According to WHO report, 366 million suffer from DM in 2011 and 422 million people suffer from diabetes in 2014, the figures were quadrupled in 1980 (Shaw et al., 2010; Ramachandran et al., 2012). The development of the DM incidence occurs mainly in the middle-income countries. Based on the recent review, the cost of the DM medicine approx US$ 827 billion, annually. In the modern world, the choice of the treatment of DM is a synthetic drug, but with the limitation of side effects in long-term treatment. The treatment of diabetes without any side effects is still a challenge and dream. Health professionals have moved towards the herbal-based drug therapy due to the more beneficial effect on the DM with fewer side effects (Pannala et al., 2008; Kavishankar et al., 2011). According to WHO, more than 150 plants have already been used for the treatment of DM and the study of hypoglycaemic plants are under consideration. Indian system of medicine is one of the richest medicinal systems in the world. Consequently, there is still an urgent need for novel Indian medicinal plant that can normalize the hyperglycemia and revolutionizes oxidative stress for the preclusion of diabetes and its interconnected and hidden complications (Bisht et al., 2010; Kavishankar et al., 2011).

Optimization of methanolic extraction of plant material has been widely scrutinized on a variety of food materials such as dry seeds, plant materials, and herbs. Generally, the most significant parameters are extraction time, solvent concentration, temperature and powder mass to methanol concentration. In particular extraction of antioxidant principles from the plant materials has been shown to depend upon such factors, but the interaction effects of these factors may depend on the type of matrix and this has not been examined on *Prunus amygdalus*

Various researchers suggested the correlation between the oxidative stress and hyperglycemia. During DM, increased BGL and generation of reactive oxygen species (ROS) via various pathways such as amplification of advanced glycation products, protein kinase C activation, dysregulation of redox equilibrium and overproduction of superoxides have been well studied. The overproduction of superoxide anions that start the production of hydroxyl radical via Haber Weiss reaction, resulting in peroxidation of protein glycation and lipids membrane inducing oxidative damage to cell membranes can be easily correlated to above statement (Rolo and Palmeira, 2006; Giacco and Brownlee, 2010).

Various researchers have even targeted the incretin hormones for the treatment of DM. According to them, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) is the major hormone, secreted by the intestinal cells. GLP-1 plays a significant role to maintain the BGL via promoting the insulin secretion, β-cell masses, reduction of glucagon secretion and altering the gastric emptying rate. Researchers suggest that the GLP-1 have a short life about 1-2 min, approximately. Conversely, GLP-1 is quickly metabolized via a DPP-IV enzyme. Inhibition of DPP-IV maintains the level of endogenous GLP-1 and prolongs its half-life. Now, the researchers have shifted their focus and attention on the inhibition of DPP-IV for the treatment of DM (Chyan, 2007; Seino et al., 2010).

The available treatment for the DM is not effective for the progression of the disease and also it's producing the various side effects. The researchers focus on the alternative therapy for the treatment of DM due to its specific action, low cost and easily available. Several phytoconstituents/compound/plant extract/herbal formulations are already in the market for the applications in the field of agriculture, human disease and veterinary (Rubin, 2005). *Prunus amygdalus*(Amygdalaceae) commonly known as badam is widely cultivated in India, America, Africa and other tropical countries. The whole plant is used for treating the various diseases like AIDS, cancer to name few through its etc (Pandey et al., 2017), antioxidant and anti-inflammatory properties (Sang et al., 2002). Therefore, due to the presence of antioxidant phytoconstituents, we try to explore the anti-diabetic potential of *Prunus amygdalus* in experimental rats.

Materials and methods

2.1 Chemicals

All the chemicals were procured from the Sigma Aldrich (St. Louis, MO, USA), Hi-media, and Sisco Research Laboratories (Mumbai, India).

2.2 Extraction of *Prunus amygdalus* seed coat

Fresh *Prunus amygdalus* were collected from the Jammu & Kashmir, India and the plant were authenticated by the Dr. Imran Kazmi (Pharmacognosist), Department of Pharmaceutical Sciences, Glocal University, India and one specimen voucher (VK/2017/04/0032) submitted in the department for further reference.

2.3 Extraction procedure

The primary aim to scrutinize the appropriate extraction system for plant material and find out the possible system having maximum antioxidant activity. For the extraction system, solvent nature (methanol), solvent concentration (50-100%), extraction temperature (0.5-4h) and sample to the solvent ratio (1/25-1/100) were estimated, respectively and the result was used for optimise the antioxidant extraction with using RSM methodology. The seed coats were removed and shade dry and were further crushed to form the coarse powder. This coarse powder was used for the extraction by methanol. The extracted

crude extract was dry under the vacuum to form a viscous mass.

2.4 Experimental design

Box Behnken Design (BBD) was used to experimental design purpose that includes the variables and three factorial levels. The independent variable used in the study were methanol concentration (X_1, %, v/v), extraction temperature (x_2, °C) and time (x3, min) while response variable was DPPH and ABTS free radical scavenging activity. Supplementary table 1 exhibited the coded and uncoded levels of independent variables. The center point of variable stands for coded value (0) and repeated experimental error. Factorial points were coded as ± 1.

2.5 Insilico study

Insilico docking studies were performed for understanding binding character and mode of rutin at the molecular level. Maestro (Schrodinger software suite, version 9.4) was used for docking stimulation of ruin. The ligprep application was further employed d for preparation of ligand 3D format via using build panel. Protein was obtained from protein data bank (PDB ID: 5EQG, 2G63) this study n was prepared via eliminating the hydrogen, solvent, and minimization of prepared legend. Validation was made by the standard drug (glibenclamide) to re-docked at the protein catalytic site.

2.6 α-γλυϲοσιδασε αϲτιιτψ ασσαψ

The α-glucosidase inhibitory effect of *PA* extract was determined as described by Toma et al., with minor modification (Toma et al., 2014). For the estimation of α-glucosidase inhibitory effect, different concentration (10-200 μg/ml) of *PA* extract was prepared and 5 Mm solution of the substrate were incubated in the 96 well microplate and the mixture was subjected to at 37°C for 10 min. After 10 min, 0.8 U/ml α-glucosidase (prepared in 0.01 MPBS) was added and again incubated for 15 min. After 15 min, the reaction was terminated by addition of Na_2CO_3 (0.2M) prepared in PBS with the determination of absorbance at 405 nm. All the data presented as % inhibition from the following formula:

$$\%\ \text{in}h\text{ibition} = \frac{(\text{Absorbance of Control} - \text{ Absorbance of Test})}{\text{Absorbance of Control}} \times 100$$

2.7 α-αμψλασε αϲτιιτψ

A modified method of Lordon et al. was used for the determination of α-amylase enzymes activity (Lordan et al., 2013). Briefly, (acarbose and*PA*) was added in the sodium phosphate and incubated at room temperature for 5 min further 1 ml of salicylic acid in both samples were added and boiled for 5 min. The reaction mixture was cool in an ice bath and deionized water was added to calculate the absorbance at 540 nm. The percentage inhibition was estimated as per above formula.

2.8 Assay for DPP-4 inhibition

96 microwell plates were used to performe the DPPIV assay. Pre-incubation solution 50 ml contained in Tris-HCl buffer (35 ml) and DPP-IV (15 ml) at various dilution and different concentration of extract and standard (6.25 – 100 mg/ml) were incubated. The prepared sample was re-incubated at room temperature (37°C) for 15 min, followed by addition of substrate (gly-propnitroanilide).The reaction mixture was re-incubated at 37°C for 30 min and the absorbance was estimated by using the microplate readers (Lamont and Drucker, 2008).

2.9 Animals

The male Swiss albino Wistar rats (2-3 month; 170-200 g) were obtained from Central Animal House Facility. The rats were kept in the single polyethylene cage with standard experimental condition (25±2°C; relative humidity 75±5 with 12h/12h dark and light cycle). The rats received the pellet feed and water *ad libitum* . All the experimental study was performed according to the Institutional guidelines.

2.10 Oral glucose tolerance test

Oral glucose tolerance test was performed according to the reported method of Kumar et al., The rats were divided into five groups of six rats in each group. Gp I served as control and received distilled water only; Gp II, Gp III, Gp IV, Gp V received the oral dose of *PA* 50, 100 and 200 mg/kg and glibenclamide (2.5 mg/kg), respectively. All the group rats received the 2 gm/kg glucose and after 60 min oral dose administration of pre-determined treatment. For the estimation of BGL, blood samples were collected via puncturing the retro-orbital plexus (0h) and every 30 min interval received the BGL of all group rats till 120 min (Ahmed et al., 2013, 2014c, 2015; Kumar et al., 2014).

2.11 Experimental induction of diabetes

The overnight fasted rats treated with the single intraperitoneal injection of STZ (55 mg/kg, b.w.), freshly prepared in citrate buffer (0.1 M, pH=4.5). The rats received glucose solution (20%) for 24 h to avoid the preliminary drug-induced hypoglycemic mortality. The rats displayed enormous hyperglycemia and glycosuria within few days. The diabetes mellitus was confirmed via estimation the BGL and rats having the BGL more than 250 mg/dL considered as the diabetic and were further exploited for experimental study (Kumar et al., 2013, 2014).

The rats were randomly divided into 7 groups and each group contains the 6 rats. The rats divided into following groups: Gp I: served as normal control and received vehicle only; Gp II served as normal control and received *PA* (200 mg/kg, b.w.); Gp III served as diabetic control (received vehicle only); Gp IV served as diabetic control and received*PA* (50 mg/kg, b.w.); Gp V served as diabetic control and received*PA* (100 mg/kg, b.w.); Gp VI served as diabetic control and received *PA* (200 mg/kg, b.w.) and Gp VII served as diabetic control and received glibenclamide (2.5 mg/kg, b.w.), respectively. All group rats received the oral administration of single dose of pre-determined treatment till 28) . The food and water intakes were estimated at regular interval. At the end of the experimental study, all group rats fasted overnight and the blood samples of rats were collected via puncturing the retro-orbital plexus. After that, rats were sacrificed via cervical decapitation. The organ-like pancreas immediately removed and washed with ice-cold saline and stored for further biochemical and histopathological observation (Kumar et al., 2013, 2014).

2.12 Biochemical analysis

The BGL was determined by glucoseoxidase–peroxidase (GOD-POD) method by blood glucose level kits. The plasma insulin level was estimated by standard kits. The biological parameters such as glucose-6-phosphatase, hexokinase and fructose-16-bisphosphatase were determined as per the reported method of Kumar et al., Ahmed et al., Hepatic parameters such as Serum glutamic oxalo acetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT) and Alkaline Phosphatase (ALP) were estimated by diagnostic kits (Span diagnostic). Lipid profiles such as high-density lipoprotein (HDL), triglyceride (TG), total cholesterol (TC),

were estimated by the diagnostic kits (Span diagnostic kits) and very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) were estimated via using the following formula.

The coronary risk and atherogenic index were estimated via using the following formula

$$\text{At}h\text{erogenic index} = \frac{\text{Low density lipoprotein } ch\text{olesterol}}{h\text{igh} - \text{density lipoprotein } ch\text{olesterol}}$$

$$\text{Coronary risk index} = \frac{\text{Total } ch\text{olesterol}}{h\text{igh} - \text{density lipoprotein } ch\text{olesterol}}$$

HOMA IR and HOMA-β were estimated via using the previously reported protocol of Ahmed et al., with slight modification (Ahmed et al., 2014a, 2015).

2.13 Statically analysis

All the data presented in the mean ± SEM and analysis of variance (ANOVA) via using the Graph Pad Prism software version 5.0 (U.S.A). The values were considered to be significant when the P value was $p < 0.05$, $p<0.01$ more significant and $p<0.001$ most significant.

Result & discussion

Analysis of response surfaces

The response surface was performed to scrutinize the various parameters for extraction of phyto-constituent from the plant extract. The mathematical model generated the experimental data with the quadratic polynomial equation for each response. Mathematical equation 1 and 2 summaries the relationship between the studied factors on the response variables.

$$\text{DPPH} = 69.60+9.38\times A+8.75\times B+7.88\times C+2.75\times AB+0.5000\times AC+1.25\times BC+0.7000\times A^2-4.55\times B^2-3.30\times C^2 \quad 1$$

$$\text{ABTS} = 61.60+10.13\times A+8.38\times B+7.50\times C+3.00\times AB+0.7500\times AC+0.7500\times BC-0.8000\times A^2-6.30\times B^2-3.55\times C^2 \quad 2$$

The surface response plots are the best way to represents the effect of any independent on the free radicals via generating the surface response plot of the model, which were generated via differentiation of two variable within the experimental range under investigation and holding the other variable at its central level (0 level). Supplementary figure 1 exhibited the three-dimensional plot, which represents the effect of concentration solvent and temp on the scavenging the DPPH free radicals on the extract of PA. On the resultant, we found that the solvent concentration having the higher effect on the extraction of the PA extract as a comparison with time effect. The supplementary figure 1A and 1B represent that the increase the concentration of solvent increase the scavenging activity of DPPH free radical. Because enhance the solvent concentration encouraged the cell membrane break-down that boosting the permeability of solvent into the solid matrix. On the other hand, a high concentration of methanol, the start was appropriate for the extraction of free radicals scavengers from the PA extract. In the supplementary figure, 1C and 1D exhibited that increase the time for extraction of plant extract increase the antioxidant activity via scavenging the DPPH free radical. Supplementary figure 1E and 1F demonstrated the increase the solvent concentration up-regulate the extraction of phyto-constituents from the plant extract and the same result was observed in the up-regulation in the time for the extraction process. According to the supplementary figure 1E and 1F, it was found that both temp and time influenced concurrently free radical scavenging capacity. The extraction time plays a considerable role, which influenced the PA extraction and hence free radical scavenging effect. The mass transfer increase with increasing the time until the maximum of extraction was achieved. Several research exhibited that the increase the time extraction, its start the decomposition of active phyto-constituents. A similar trend was observed in supplementary figure 2, which exhibited the effect of solvent concentration, temperature and

time on the scavenging activity of PA extract. On the resultant, we observed that the solvent concentration and temp play a crucial role in the extraction of plant extract.

Insilico activity

Molecular modeling studies using the automated docking protocol into the ATP-binding site of amyloid beta protein at 2.0 A° resolution (PDB code: 2g63), following a flexible rigid/ligand-receptor docking protocol was performed. Maestro 9.4 was used for estimation of binding pose presented in supplementary figure 3. As can be seen, the docking program positioned compounds rutin makes a hydrogen bond with ser209, glu205, glu206, tyr662, tyr547, and arg125 and also makes pi-pi stacking with tyr547 and arg125against backbone of DPP4 protein. Herein, rutin gives better interaction with a backbone of DPP4protein due to its hydrophilic interaction and pi-pi interaction with the amino acid residue of the DPP4 protein(Chen and Zhi, 2001; Gray et al., 2003). We further validate the interaction by superimposing our ligand (rutin) with glibenclamide ligand and found that the binding orientation of our ligand and glibenclamide was similar as above. The binding affinity of ruin with the DPPIV protein was found -11.25 kcal/mol and glibenclamide showed the docking affinity -6.053 kcal/mol, respectively (Supplementary table 3).

As can be seen, the docking program positioned compounds rutin makes a hydrogen bond with thr137, glu380, asn415, asn288 and asn411 against the backbone of GLUT1 protein (Supplementary figure 4). Herein, rutin gives better interaction with a backbone of GLUT1 protein because it is exhibiting hydrophilic interaction and pi-pi interaction with the amino acid residue of the GLUT1 protein. We further validate the interaction by superimposed our ligand (rutin) with glibenclamide ligand and observed that binding orientation of our ligand was similar to the ruin. The binding affinity of rutin with the GLUT1 protein was found -13.101 kcal/mol and glibenclamide showed the docking affinity -8.29 kcal/mol, respectively (Supplementary table 3).

As can be seen, the docking program positioned compounds rutin makes a hydrogen bond with glu259 against the backbone of PPARY protein. Herein, rutin gives better interaction with a backbone of PPARY protein because it is exhibiting hydrophilic interaction and pi-pi interaction with the amino acid residue of PPARY protein (Supplementary figure 5). We further validate the interaction by superimposed our ligand (rutin) with glibenclamide ligand and observed that binding orientation of our ligand was similar to the rutin. The binding affinity of rutin with the DPPIV protein was found -12.849 kcal/mol and glibenclamide showed the docking affinity -6.732 kcal/mol, respectively (Supplementary table 3).

Effect of *PA* extract on enzyme inhibitory effect

Supplementary table 4 explains the effect of the *PA* extract on the different enzymes. *PA* showed the IC_{50} 56.4 µg/mL against α-amylase, IC_{50} 220.5 µg/mL against α- glucosidase and IC_{50} 162.9 µg/mL against DPPIV, respectively.

DPP-IV is a member of peptidase family, with 766 amino acids and is distributed in various tissues. It is considered as the hydrolase enzyme and is also present in the circulating. DPP-IV showed their biological effect via two mechanisms, first, by its binding to adenosine deaminase and conveys the independent signal to its enzymatic function via alteration of intracellular singling pathway (Wang et al., 2013). Secondly, its catalytic activity is exhibited by spanning membrane form of a molecule. Various researchers suggest that the DPP-IV mediated inactivation of GLP-1 is the key mediator for the GIP activity that can be targeted by inhibition of DPP-IV as the novel approach for the treatment of DM (Gupta et al., 2009). The inhibition of DPP-IV prevents the degradation of GLP-1 and alters the clearance of GLP-1 from the renal tissue. Normally, GLP-1 takes part in the promotion of insulin secretion from the β-cells, down-regulation of glucogen secretion, enhancing masses of β-cells, inducing satiety and diminution of gastric emptying rate, which helps to maintain the normal level of BGL in type II diabetes mellitus (Seino et al., 2010; Meier, 2012).

α-amylase cleavage the glycosidic bond. Glycoside with glycosidic bond linkage can change the role of starch substrate. According to this mechanism, starch is not converted into disaccharide in the body and help to

promote the action of glucosidase, which converts the disaccharide into monosaccharides and maintain the glucose level in the body (Perić-Hassler et al., 2010).

3.2 Effect of *PA*extract on oral glucose tolerance test (OGTT)

Figure 1 depicts the effect of the *PA* extract and glucose control group rats. The figure 1 shows that the normal and*PA* (200 mg/kg) group rats showed almost similar BGL at end of the experimental study. Glucose control group rats showed the upregulation of BGL after the glucose administration and it reached maximum level (130 mg/Dl) after 30 min. After that, the BGL slightly decreased at end of the experimental study and came to 111 mg/dL. *PA* received group rats showed the reduction of BGL 100.66 and 96.33 and 81 mg/dL at a dose of 50, 100 and 200 mg/kg, respectively. On the other hand, standard drug (glibenclamide) showed the reduction in the BGL at 73.66 mg/dL.

Figure 1b demonstrates the effect of the *PA* administration on the OGTT. After glucose load, we observe that the NC and NC received*PA* (200 mg/kg) showed the almost similar $AUC_{glucose}$ value. On the other hand, glucose control group showed the increase $AUC_{glucose}$ as a compared to other groups of rats. *PA* treatment showed the diminution of $AUC_{glucose}$ at end of the OGTT study. The same results were observed in the glibenclamide treated group rats.

Oral glucose tolerance test (OGTT) was performed for the recognizing the modulation of carbohydrate metabolism during the post glucose treatment (Ahmed et al., 2013, 2014c; Kumar et al., 2014). Glucose control group rats showed the increased BGL at end of the experimental study and glucose control group rats showed the reduction in the BGL after treatment with the *PA* at dose-dependently. *PA* showed the marked reduction 7.69%, 29.49% and 37.69% in the BGL at dose 50, 100 and 200 mg/kg, respectively. On the other hand, glibenclamide showed the marked 41.28% reduction in BGL. The result clearly indicates the significant reduction in the BGL and suggests the better utilization capacity. *PA* showed the decreased BGL may be due to insulin secretion from β-cells and improved the utilization of via glucose consumption.

3.3 Effect of *PA* extract on BGL

BGL of the normal and normal received *PA* (200 mg/kg) is almost same at the end of the experimental study. STZ induced DC rats showed continues increase in BGL from 272.66 mg/dL to 388.76 mg/dL at end of the experimental study and confirm the expansion of diabetes. DC rats treated with *PA* (50 mg/kg) demonstrated the reduction in the BGL 266.43 mg/dL to 170 mg/dL; *PA* (100 mg/kg) reveals the reduction from 265 mg/dL to 142.66 mg/dL and *PA* (200 mg/kg) illustrates the 256.36 mg/dL to 100.36 mg/dL, respectively. Glibenclamide treated group rats showed the decreased BGL from 262 mg/dL to 93.37 mg/dL. The result suggests the anti-diabetic effect of *PA* by reduction of BGL.

Figure 2b shows the percentage of reduction in the BGL. *PA*demonstrated 37.05, 46.58 and 61.42% diminution in the BGL at a dose of 50, 100 and 200 mg/kg, respectively. On the country, glibenclamide showed the 64.25% reduction in the BGL as a comparison to DC group rats.

Diabetes is categorized via hyperglycemia with a down-regulation of insulin secretion and its function. It is well-documented fact that hyperglycemia produces an excess amount of ROS, which further causes the oxidative stress in the system. Several published research suggests that the antioxidant play a significant role in the reduction of l free radicals generation (Valko et al., 2007; Rains and Jain, 2011). As on date market is flooded by synthetic drugs for the treatment of DM, but they account for limitation due to side effects. From our current knowledge till date no work is published on the effect of *PA* as an anti-diabetic seed. We already suggest the possible role of antioxidant therapy against the DM. In the continuation of our research, we explore the possible phytoconstituents present in the extract and scrutinize against DM. Glibenclamide was marked as the reference (standard) drug for its sulfonylurea moiety and can improve the glucose control via acting on the insulin action and its secretion.

Streptozotocin (STZ), a commonly used nitrosourea obtained from the soil bacteria *Streptomyces achromogenes* , for induction of diabetes by increasing e the reactive oxygen species (ROS) load. The low dose of the STZ induces the type I DM and a high dose of STZ to induce the type II DM. Its penetrate the β-cells via

glucose transporter and induce the breakdown the DNA in β-cells inducing the reduction of insulin secretion (Szkudelski, 2012). The breakage of DNA strand leads to increase in glucose concentration in blood. In the rodent model, the STZ initiates within 2 hours high level of insulin in blood followed by hyperglycemia within 6 hours suggesting hyperglycemia develops with a high level of insulin. As STZ destroys the Beta Cell of panaceas the insulin level goes down and hyperglycemia is maintained (Esposito et al., 2002; Alipio et al., 2010).

3.4 Effect of *PA* extract on body weight

Another parameter to estimate the presence of diabetes is a body weight. Due to an expansion of diabetes, reduction in body weight commonly observed due to loss of muscle. The same results were observed in the DC control group rats, the initial body weight of the DC group rats was 161±2.34 g, which was further estimated at end of the experimental study and was found to be 148.5±4.29 g. The result suggests that the expansion of diabetes decreases the body weight. The rats treated with the*PA* showed the increased in the body weight. The *PA* -treated rats showed the initial body weight of 159.5±2.87, 162±3.72 and 160.5±4.12 g, at a dose of 50, 100 and 200 mg/kg, which was significantly ($p<0.001$) enhanced at the end of the experimental period. The final body weight was observed 183.5±3.84, 195.4±4.94 and 204±2.03 g, respectively (figure 2a). An almost similar pattern of results was observed in the glibenclamide group rats, the initial body weight was found to be 161±2.09 g and final body weight was 203±2.93.

The NC and NC treated group rats showed the almost similar pattern 22.03% in increased body weight at end of the study. DC group rats showed the reduction in the body weight 8.19%, which suggests diabetes, is indirectly estimated by a reduction in the body weight. *PA*treatment showed the 13.02, 17.04 and 21.12% enhancement of body weight (figure 2b). Glibenclamide showed the 21. 22% increase in body weight.

STZ induced DM rats showed the decrease BW throughout the experimental study. Several researchers suggest that the reduction of body weight of STZ induced DM rats is due to gluconeogenesis. During the DM condition, there is the degradation of structural proteins and fats that lead to enhanced muscle destruction (Ahmed et al., 2013, 2014b; Kumar et al., 2014). STZ induced DM rats treated with *PA* showed the increased body weight and suggesting the anti-diabetic effect by inhibition of degradation of muscle proteins and fat.

3.5 Effect of *PA* extract on biochemical parameters

Plasma insulin is one of the essential factors for estimation sugar associated with diabetes. Several researchers suggested that the plasma insulin level reduces during the expansion of diabetes due to break down of pancreatic β-cells. The plasma insulin level was almost same in normal and *PA* (200 mg/kg) group rats. STZ induced DM rats showed the reduction in plasma insulin that may be attributed to dysfunction of pancreatic β-cells. DM rats treated with *PA* showed the significantly ($p<0.001$) enhancement of plasma insulin level in dose-dependent manner. STZ group rats demonstrated the plasma insulin 3.1±0.32 (μU/mL), which was less than 4 times as compared to normal and*PA* (200 mg/kg) group rats. *PA* treatment showed the insulin level as 5.05±0.93, 8.13±0.56 and 12.54±0.85 (μU/mL), respectively (Supplementary table 5). The results suggest that PA improved the plasma insulin and decrease the BGL and confirms its anti-diabetic effect.

A similar style was observed in case of hexokinase content. The hexokinase level was decreased in the STZ induced DM group rats and does depend on the treatment of *PA* significantly ($p<0.001$) (Supplementary table 5).

The level of glycated Haemoglobin, fructose-1-6-biphosphatase, and glucose-6-Phosphatase was increased in the STZ induced DM group rats, which was significantly ($p<0.001$) down-regulated by the*PA* *in* t a dose-dependent manner (Supplementary table 5).

Supplementary table 6 illustrates the effect of the *PA* on the HOMA IR and HOMA β. HOMA IR level of DC control group rats increased and the level of HOMA β reduced in the DC group rats, the dose-dependent treatment of *PA* altered the level of HOMA IR and HOMA β a resemblance with glibenclamide treated group.

The liver tissue plays a crucial role in the circulation of glucose in various pathological and physiological states especially in case of diabetes. Several drugs, compounds, and many more products are detoxified and metabolized in the liver by a different mechanism (Ahmed et al., 2013, 2014b; Kumar et al., 2014). Existing literature suggests that diabetes directly linked to the variety of hepatic abnormalities such as fibrosis, glycogen deposition, increased non-alcoholic fatty liver disease (NAFLD), liver enzymes, Hepatocellular carcinomas, cirrhosis, viral hepatitis, acute liver disease and fibrosis to name few. One important role by the liver is well associated with control of postprandial hyperglycemia and glycogen synthesis. The liver enzymes such as fructose-1-6-biphosphatase, Hexokinase and glucose-6-phosphate play a vital role during the conversion of glucose to required energy, glycogen synthesis and glucose utilization (Tappy and Lê, 2012). Several research suggests that the reduced level of hexokinase increase the glucose level into the circulating blood via inhibiting the conversion of glucose into glucose-6-phosphate (Newsholme et al., 1968). Our results of STZ induced DM group rats and *PA* -treated group rats showed the increased level of hexokinase, responsible for its anti-diabetic effects. glucose-6-phosphate is regulated during glucose metabolism with the help of hexokinase. Increase level of glucose-6-phosphate, can be related to its increase gluconeogenetic enzyme activity and boost the production of fats and in renal and hepatic tissue (Ahmed et al., 2013, 2014c, 2015; Kumar et al., 2013, 2014). Fructose-1,6 biphosphatase and glucose-6-phosphatase, both play a key role in the gluconeogenic pathway, in diabetes, there is increased synthesis of both these enzymes, responsible for increasing the glucose production by the tissue. STZ induced DM rats showed the increased activity of both enzymes and dose-dependent treatment of *PA*showed the reduction of the activity in fructose-1,6 biphosphatase and glucose-6-phosphatase. These results confirm and suggest the anti-diabetic effect of *PA* . The possible mechanism may be due to the reduction of the glyconeogenesis and increase glycolysis.

3.6 Effect of *PA* extract on hepatic parameters

The hepatic parameters were estimated in term of SGOT, ALP, and SGPT. The level of hepatic parameters such as SGOT, ALP, and SGPT were almost similar in the normal and normal received *PA* (200 mg/kg). STZ induced DM group rats showed the boosted level of these parameters, which reach almost double as compared to normal level. Dose-dependent manner treatment of *PA* (200 mg/kg) significantly ($p<0.001$) down-regulated and suggest the hepatic protective effect of the extract. A similar result was observed in the glibenclamide treated group rats (Supplementary table 7).

3.7 Effect of *PA* extract on antioxidant parameters

Supplementary table 8 illustrates the effect of *PA* and glibenclamide on the antioxidant parameter in the STZ induced DM rats. The antioxidant parameters such as LPO, CAT, SOD, and GPx were scrutinized in all experimental animals. The level of antioxidant parameters was same in normal and normal received *PA* (200 mg/kg) group rats. STZ induced DM group revealed the increased level of LPO (15.5±1.84 µmole of MDA/mg protein) and reduce the level of SOD (14.54±3.01 units/mg protein), CAT (43.57±4.37 µmole of H_2O_2 consumed/min/mg of protein) and GPx (13.34±2.14 µmole of GSH/mg protein), respectively. STZ induced DM group treated with *PA* (200 mg/kg) showed the increase level of LPO (7.81±1.45 µmole of MDA/mg protein) and reduction in the level of SOD (43.65±2.05 units/mg protein), CAT (67.98±3.04 µmole of H_2O_2 consumed/min/mg of protein) and GPx (35±1.76 µmole of GSH/mg protein). STZ induced DM group treated with the glibenclamide demonstrate the increased level of LPO (7.98±1.23 µmole of MDA/mg protein) and reduction in the level of SOD (42.98±2.65 units/mg protein), CAT (66.71±2.93 µmole of H_2O_2consumed/min/mg of protein) and GPx (34.06±1.34 µmole of GSH/mg protein).

In STZ induced type II DM, destroy the pancreatic β-cells are destroyed as a result of, increase reactive oxygen species (ROS) and continuous generation of ROS that initiate the damage of tissues and react with the all cell membrane component and biological system, that lead to increased lipid peroxidation (LPO) reaction. The enhanced level of LPO modulate the membrane-bound enzymes activity and reduce the membrane fluidity, which results in damage to membrane fluidity (Ahmed et al., 2013, 2014c, 2015; Kumar et al., 2013, 2014). The increased level of LPO and decrease level of SOD, CAT, GPx were observed in the STZ induced DM group rats. Several researchers suggest that regular intake of antioxidant-rich food, increase antioxidant content reduces the LPO and enhanced the SOD, CAT, and GPx. SOD and CAT

(Kumar et al., 2013, 2014; Ahmed et al., 2014c, 2015). SOD looks for and removes superoxide component from liver and converts to hydrogen peroxide CAT scavenges Hydrogen peroxide and detoxify the cells from free radical generators.

3.8 Effect of *PA* extract on lipid parameters

Estimation of the antihyperlipidemic effect of *PA* was scrutinized through lipid profile of the STZ induced DM group rats. Several researchers suggest that the hyperlipidemia is the result of secondary complication after expansion of DM. The normal and normal received*PA* (200 mg/kg) showed the normal lipid profile, but the STZ induced DM group rats showed the alteration of lipid profile in terms of TG (130.1±3.78 mg/dL), LDL (89.58±2.58 mg/dL), TC (83.4±2.94 mg/dL), HDL (20.1±2.54 mg/dL) and VLDL (26.02±1.83 mg/dL), the values were almost double as compared to normal and normal received *PA* (200 mg/kg) group rats. STZ induced DM treated with *PA* (200 mg/kg) showed the significantly ($p<0.001$) modulation of TG (86.9±2.43 mg/dL), LDL (23.53±1.45 mg/dL), TC (83.4±2.94 mg/dL), HDL (42.5±1.39 mg/dL) and VLDL (17.38±1.03 mg/dL). STZ induced DM treated with glibenclamide showed the significantly ($p<0.001$) modulation of TG (87.3±2.06 mg/dL), LDL (25.84±1.76 mg/dL), TC (85.2±3.54 mg/dL), HDL (41.9±1.79 mg/dL) and VLDL (17.46±1.11 mg/dL), respectively.

Table 1 represents the coronary risk index and atherogenic index from a different group of rats. The level of the coronary risk and atherogenic index almost equal to normal and normal rats received *PA* (200 mg/kg). STZ induced DM rats showed the atherogenic index (4.4±0.53) and coronary risk index (6.75±1.02), which increased more than 5 times more as compared to normal and normal rats received *PA* (200 mg/kg). On the other hand, STZ induced DM rats treated with *PA* (200 mg/kg) showed the atherogenic index (0.55±0.09) and coronary risk index (1.96±0.68), which was reduced more than 5 times as comparison STZ induced DM rats. Glibenclamide treated rats showed the atherogenic index (0.62±0.08) and coronary risk index (2.03±0.53), respectively.

During the normal condition, insulin activates the lipolytic hormones and acts on the peripheral fat depots, hydrolyzes triglycerides and avoid mobilization of free fatty acids. The reduced level of HDL and increase level of TC, TG, LDL, VLDL are considered as a hyperlipidemic condition. Hyperlipidemia condition majorly contributed to the expansion of cardiovascular disease (Takahashi et al., 2004; Lionetti et al., 2009). STZ induced DM group rats to present the same condition and shows the hyperlipidemia in the rats. During the DM condition, insulin deficiency is observed, which inactivates the lipoprotein lipase enzyme and promotes the conversion of free fatty acid into cholesterol and phospholipids and finally discharge into the blood. This results in an increase of cholesterol and phospholipids level into the blood (Geethan and Prince, 2008). *PA* treatment significantly ($P<0.001$) altered the lipid profile near to normal control group rats in a dose-dependent manner (table 2). The alteration of lipid profile may be due to the insulin-like effect of *PA* that down-regulates the lipid metabolism thus supporting its beneficial effects in DM and its related complications.

3.9 Effect of *PA* on pancreas histopathology

Figure 3 showed the histopathology of normal and treated group rats. The normal control and normal control received *PA* (200 mg/kg) demonstrated the normal articulature of pancreas histopathology. STZ induced DM rats pancreas histopathology showed the destruction of pancreatic β-cells. *PA* -treated rats showed the enlargement of the pancreatic β-cells at dose-dependent manner. An almost similar effect was observed in the glibenclamide treated group rats.

Conclusion

The extraction condition of plant material have been a significant effect on the antioxidant and hypoglycemic effect of *Prunus amygdalus* extract, but the behavior varied from one response variable to another. The result obtained from the experimental study showed the antihyperglycemic effect and in-vitro inhibition of DPPIV explained the effectiveness of *PA* in regulating diabetes. The docking study showed the potent DPPIV activity of *PA* extract (rutin) as compared to marketed drug vildagliptin. Several previous studies showed that the standard drug such as glibenclamide has several effects such as a headache, nausea, low blood sugar

level when taken in higher amount etc. Base on the result we can be concluded that the *PA* extract might be useful in the treatment and prevention of diabetes and its complication such as insulin resistance and spoiled glucose tolerance with DPP-IV inhibitory effect.

Conflict of interest

All authors declare none conflict of interest

Author contribution

VK was performed the experimental study. KC, RS, FAA and FA interpretate the biochemical and histopathological data. All authors equally contributed in proof reading.

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Figure legends

Figure 1: showed the effect on the blood glucose level of studies of *Prunus amygdalus* extract and glibenclamide in Oral Glucose Tolerance Test (OGTT). **a:** showed the blood glucose level at different time interval and b: Area under curve (AUC) on the different time interval.

Figure 2: showed the effect on the blood glucose level of*Prunus amygdalus* extract and glibenclamide in STZ induced type II Diabetic mellitus rats. **a:** showed the blood glucose level at end of the experimental period and **b:** showed the effect on the blood glucose level (% inhibition). The comparisons were made by ANOVA followed by Dunnett's test. $^{z}P < 0.05$ is considered significant, $^{y}P < 0.01$ is considered very significant, $^{x}P < 0.001$ is considered extremely significant.

Figure 3: showed the effect on the body weight effect of*Prunus amygdalus* extract and glibenclamide in STZ induced type II Diabetic mellitus rats. **a:** showed the body weight at end of the experimental period and **b:** showed the effect on the body weight (% inhibition). The comparisons were made by ANOVA followed by Dunnett's test. $^{z}P < 0.05$ is considered significant, $^{y}P < 0.01$ is considered very significant, $^{x}P < 0.001$ is considered extremely significant.

Figure 4: showed the pancreas histopathology of normal and treated group rats. **a:** showed the normal group rat histopathology, **b:** showed the histopathology of normal group rat received *PA* (200 mg/kg), **c:** showed the STZ induced type II Diabetic mellitus rat histopathology, **d:** showed the histopathology of STZ induced type II Diabetic mellitus received*PA* (50 mg/kg), **e:** showed the histopathology of STZ induced type II Diabetic mellitus received *PA* (100 mg/kg),**f:** showed the histopathology of STZ induced type II Diabetic mellitus received *PA* (200 mg/kg) and showed the histopathology of STZ induced type II Diabetic mellitus received glibenclamide (2.5 mg/kg)

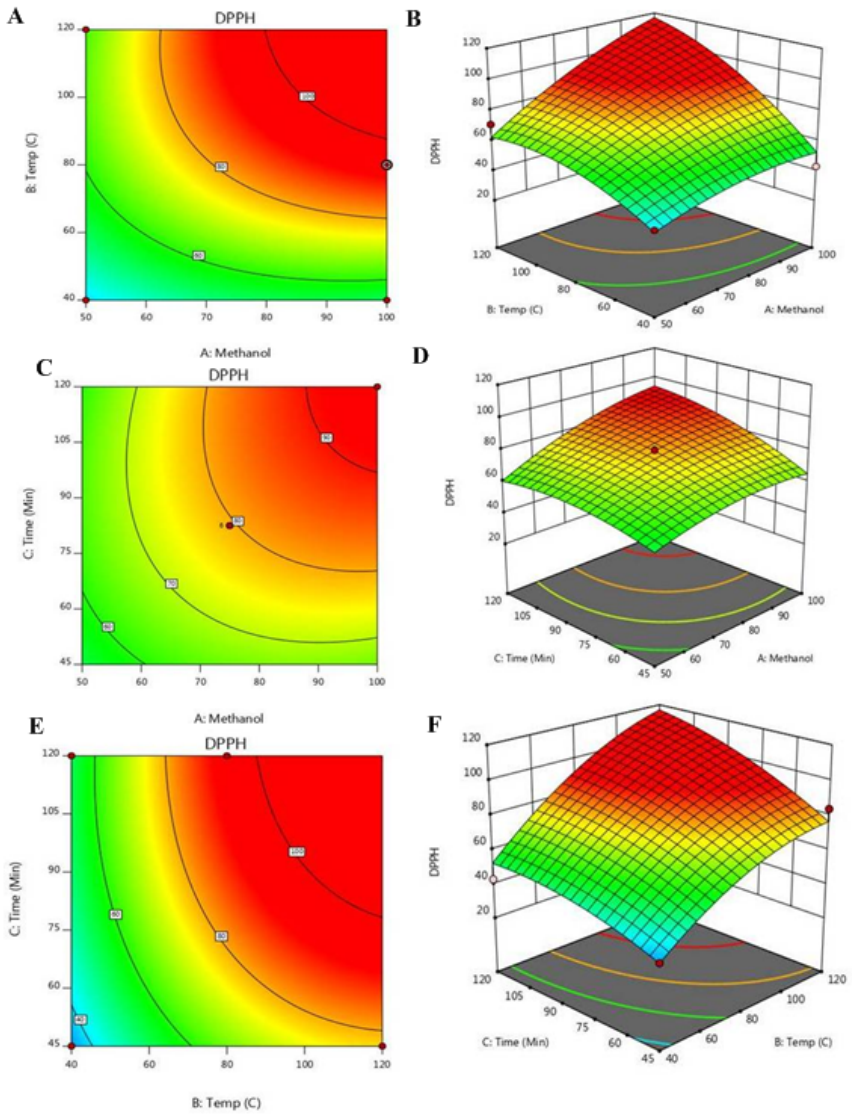
Supplementary figure 1: Effect of extraction temperature, time and solvent concentration (A), (B) and ratio (C) on DPPH free radical scavenging activity of *Prunus amygdalus* extract.

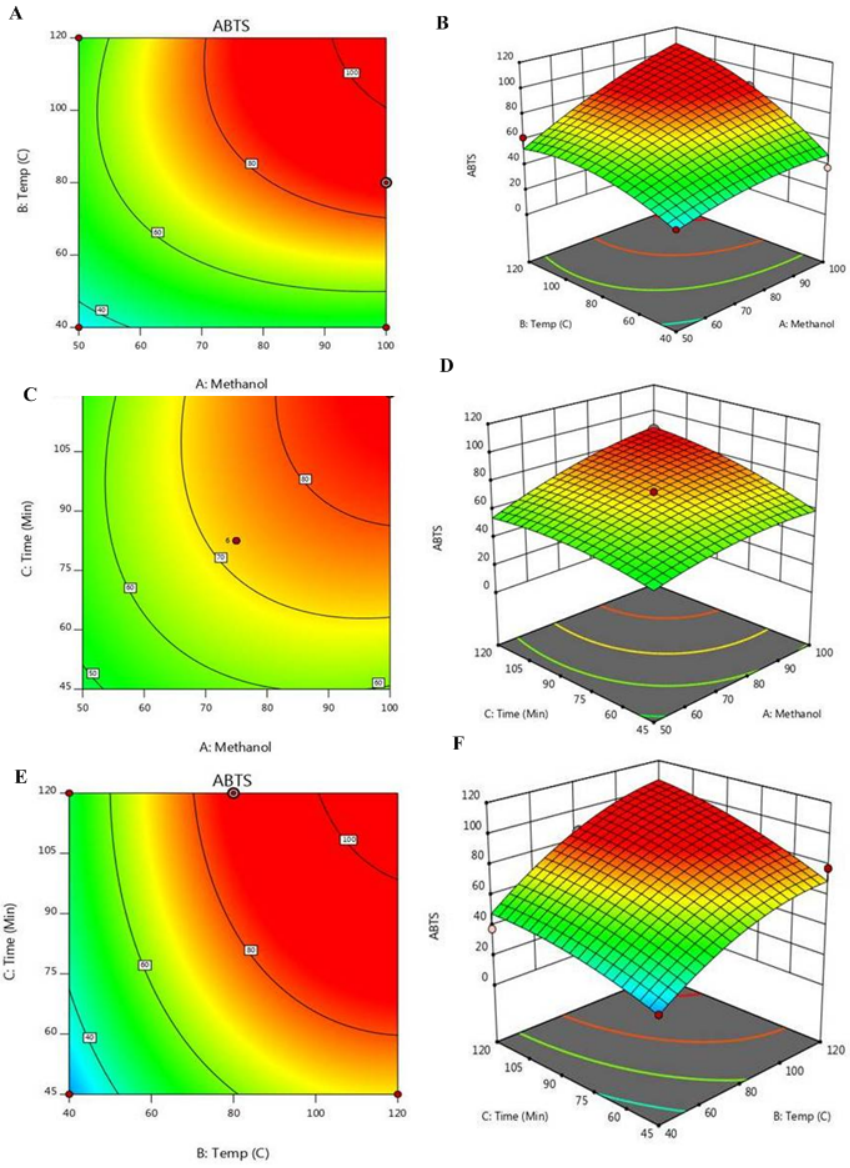
Supplementary figure 2: Effect of extraction temperature, time and solvent concentration (A), (B) and ratio (C) on ABTS free radical scavenging activity of *Prunus amygdalus* extract.

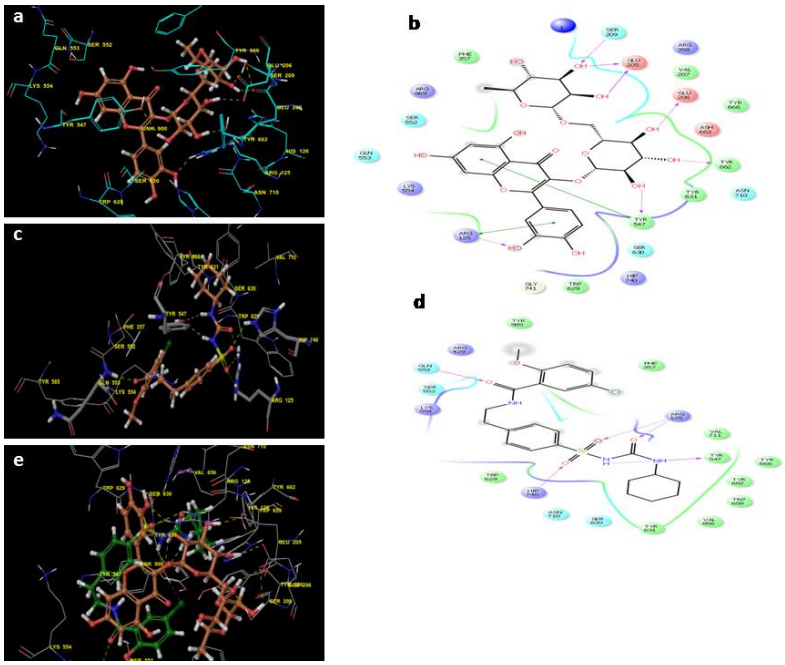
Supplementary figure 3: showed the molecular docking studies of*Prunus amygdalus* extract and glibenclamide against DPP4 catalytic site (PDBID-2G63). **a:** Binding interaction of Rutin against DPP4 catalytic site, b: Rutin against DPP4 catalytic site, c: Binding interaction of glibenclamide against DPP4 catalytic site, d: Ligplot of Glibenclamide against DPP4 and e: Superimposition of Rutin with glibenclamide against DPP4 catalytic domain.

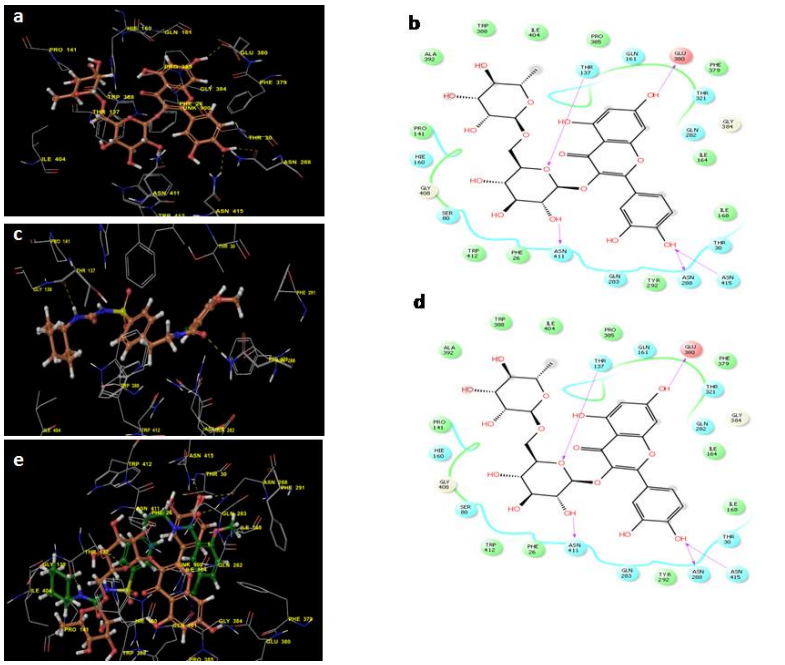
Supplementary figure 4: showed the molecular docking studies of*Prunus amygdalus* extract and glibenclamide against GLUT1 catalytic site (PDBID-5EQG). **a:** Binding interaction of Rutin against GLUT1 catalytic site, b: Ligplot Rutin against GLUT1 catalytic site, c: Binding interaction of glibenclamide against GLUT1 catalytic site, d: Ligplot of Glibenclamide against GLUT1 and e: Superimposition of rutin with glibenclamide against GLUT1 catalytic domain.

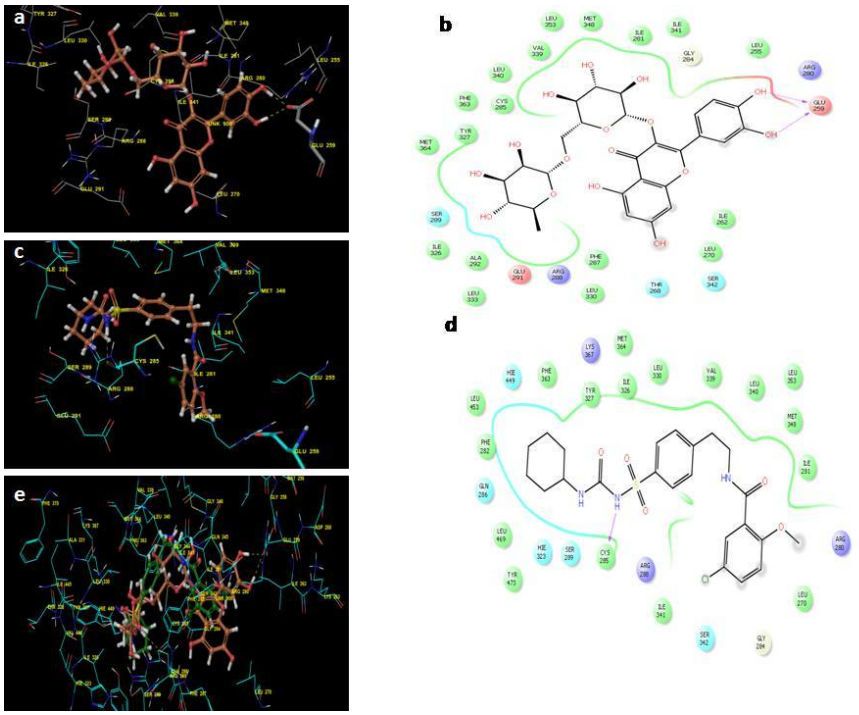
Supplementary figure 5: showed the molecular docking studies of*Prunus amygdalus* extract and glibenclamide against PPARY catalytic site (PDBID-2PRG). **a:** Binding interaction of Rutin against PPARY catalytic site, b: Rutin against PPARY catalytic site, c: Binding interaction of glibenclamide against PPARY catalytic site, d: Ligplot of Glibenclamide against PPARY and e: Superimposition of Rutin with glibenclamide against PPARY catalytic domain.

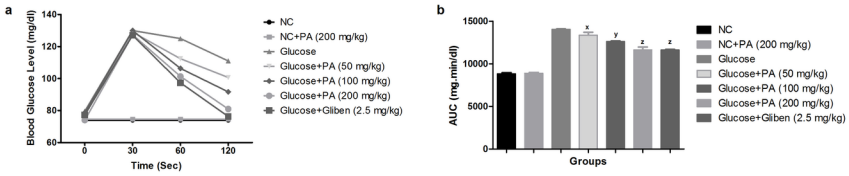


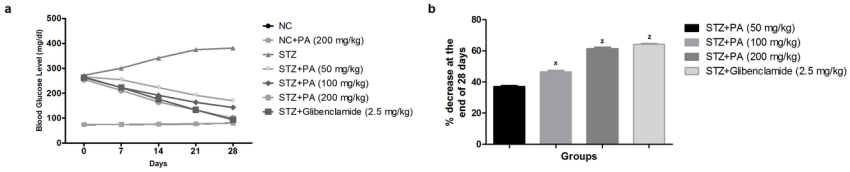


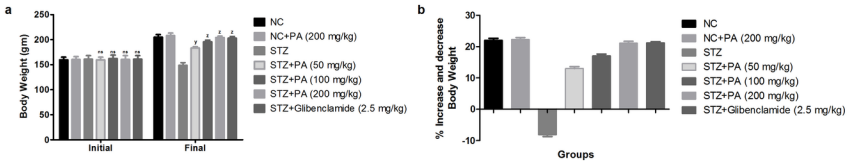


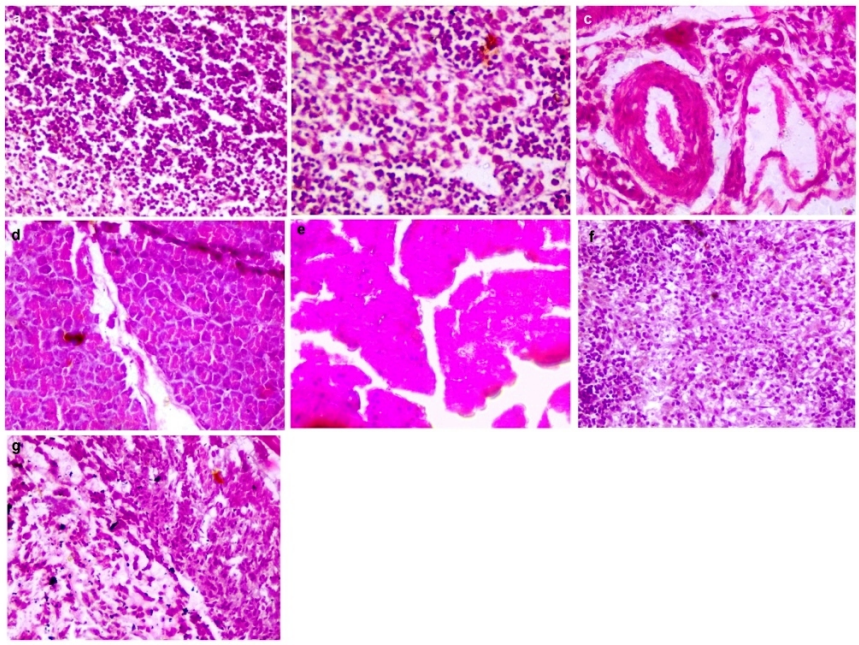












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