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FULL ARTICLE

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Quercetin modulates hyperglycemia by improving the pancreatic antioxidant status and enzymes activities linked with glucose metabolism in type 2 diabetes model of rats: In silico studies of molecular interaction of quercetin with hexokinase and catalase

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Abstract

Quercetin was assessed for its antihyperglycemic effect in fructose-streptozotocin (STZ) induced diabetic rats. The oral administration of quercetin at the dosage of 25 and 50 mg/kg for 28 days remarkably reduced the level of blood glucose, glycosylated hemoglobin (Hb), and hepatic glycogen but enhanced plasma Hb concentration. The altered activities of glucose-6-phosphatase and hexokinase in diabetic rats were significantly improved upon quercetin treatment. Furthermore, the antioxidant activity of pancreatic superoxide dismutase, catalase (CAT), and reduced glutathione was effectively increased while the value for thiobarbituric acid reactive species was decreased. A significant reduction of glycemia was observed in the glucose tolerance test, 120 min after the glucose pulse. Also, the damage caused by fructose-STZ in the liver and pancreas of diabetic animals were restored to near normal. Molecular docking of quercetin showed a high affinity for hexokinase and CAT with a binding energy of −7.82 and −9.83 kcal/mol, respectively, more elevated than the standard drugs.

Practical applications

Functional foods and nutraceuticals have increasingly interested the consumers in terms of health benefits and have started focussing on the prevention or cure of disease by the foods and their health-enhancing phytochemicals. Quercetin is one of the most potent naturally occurring antioxidants within the flavonoid subclasses, mostly distributed as a secondary metabolite in fruits, vegetables, and black tea. Based on the results exhibited in the present study, we proposed that the consumption of foods rich in quercetin could be a cheap and affordable nutraceutical that can be developed for the treatment of T2DM and its complications. Further studies on the safety aspects of quercetin in long-term usage are strongly recommended before implementing for the treatment of human diseases.

KEYWORDS
anti-hyperglycaemic, antioxidant, glucose metabolism, histopathology, molecular docking, Quercetin

1 | INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of multiple etiologies characterized by hyperglycemia as a result of pancreatic β-cell dysfunction and insulin resistance in the liver, skeletal muscle, and adipose tissue (Pessin & Saltiel, 2000). It is a global epidemic affecting about 425 million adults worldwide, and the prevalence has risen faster in middle and developing countries (IDF, 2015). Type 2 diabetes (T2D) is the most common form of diabetes, afflicting 85%–95% of all diabetic individuals over 40 years of age due to a rapid increase in obesity, high consumption of calorie-rich diets, sedentary lifestyle, and ageing population (Salas-Salvado, Martinez-Gonzalez, Bullo, & Ros, 2011). In T2D, the pancreatic β-cell function continues to deteriorate due to oxidative stress-related DNA damage in the islet that triggers necrosis via activation of poly ADP ribose polymerase. This phenomenon occurs as a consequence of low antioxidants to scavenge the reactive oxygen species (ROS) (Tiwari, Pandey, Abidi, & Rizvi, 2013).

Several pharmacological drugs such as sulfonylureas, biguanides, peroxisome proliferator-activated receptor-γ (PPAR γ), glucagon-like peptide-1 (GLP-1) analogues, and dipeptidyl peptidase IV (DPPIV) have been in use for the management of diabetes, but they tend to lose their effectiveness in the long-term use (DeFronzo et al., 2004). One of the most recent therapies for T2D, Byetta, targets GLP-1 receptors on β-cells to stimulate insulin secretion, and studies in mice suggest that it also increases β-cell mass. However, it has been reported to cause acute pancreatitis and kidney failure in some patients (Hu et al., 2018). Therefore, there is a need for the development of novel therapies with minimal side effects that can enhance pancreatic β-cell function and β-cell mass for glucose utilization. These drugs are expensive, especially in poor and developing countries, and their adverse effects are unbearable to patients. Thus, an increasing number of diabetic patients have resorted to seeking alternative therapies with less severe or no side effects to better their health conditions (VanDeKoppel, Choe, & Sweet, 2008). Plant compounds are potential sources of novel molecules that control insulin imbalance by acting as insulin secretagogues and improving insulin sensitivity (Oh, 2015). Some clinical studies carried out in recent years have shown improved blood glucose control by the plant-derived products in human diabetic subjects (Bhatta, Thomas, & Joghee, 2012; Knop et al., 2013).

Quercetin (pentahydroxy flavones) is one of the most potent naturally occurring antioxidants within the flavonoid subclasses found in fruits, vegetables, and black tea. It is an essential antioxidant agent that scavenges oxygen radicals responsible for the neurodegenerative diseases, membrane peroxidation, and necrosis (Tiwari et al., 2013). Previous studies have shown that quercetin inhibits xanthine oxidases, lipid peroxidation, and serum lipid profiles, via regulation of lipogenic genes and regeneration of vitamin E (Kobori, Matsumoto, Akimoto, & Oike, 2011; Vessal, Hemmati, & Vasei, 2003). So far, several animal models have been used to investigate the antidiabetic effects of quercetin, but no scientific data reported on a model that closely mirrors the natural history of T2D from insulin resistance to cell dysfunction along with metabolic characteristics of human T2D. For example, Vessal et al. (2003) reported that quercetin reduced plasma glucose levels through the regeneration of the pancreatic β-cells in STZ-induced diabetic rats. In additional experiments, oral administration of 0.08% quercetin increase plasma adiponectin and High Density Lipoprotein (HDL)-cholesterol and also enhances antioxidant enzyme activities in the liver of C57BL/KsJ-db/db mice (Jeong et al., 2016).

Recent studies conducted by Roslan, Nelli Giribabu, Karim, and Salleh (2017) demonstrated the ameliorative effect of quercetin against oxidative stress, inflammation, and apoptosis in the heart of STZ-nicotinamide-induced diabetic rats. The cotreatment of quercetin with resveratrol showed a promising antidiabetic potential by maintaining the hepatic glucose metabolic enzymes activity and structure of pancreatic β-cells in STZ-induced diabetic rats (Yang & Kang, 2018). Most of the studies carried out on quercetin reported its antidiabetic effect in a high-fat diet or STZ-induced diabetic rats but not in a model that mimics etiological and pathophysiological conditions of T2DM found in man. A lower dose of STZ at 40 mg/kg via intraperitoneal injection has been indicated to be less effective in inducing T2D while the dosage at 50 mg/kg is known to cause pathogenesis of type 1 diabetes in experimental rats (Wilson & Islam, 2012). Additionally, STZ is unable to establish insulin resistance in animals but induces hyperglycemia via pancreatic β-cell damage.

The present study, therefore, aimed to investigate whether quercetin improves glucose tolerance, pancreatic antioxidant status, glucose-6-phosphatase, and hexokinase activities in a different type 2 diabetic animal model, a model that mimic etiological and pathophysiological conditions found in man. The histopathological examinations of the liver and pancreas of diabetic treated rats and in silico molecular docking study of quercetin interaction with hexokinase and CAT were also determined.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

The compounds 3,5,7,3,4, pentahydroxy flavones (quercetin), STZ, potassium hydroxide (KOH), cyanmethemoglobin, trichloroacetic acid (TCA), sulfuric acid (H_2SO_4), HPLC grade ethanol, potassium chloride (KCl), ethylenediaminetetraacetic acid (EDTA), ammonium molybdate {$(NH4)_6Mo_7O_{24}$}, ammonium acetate ($NH_4CH_3CO_2$),

amino naphthol sulfonic acid ($C_{10}H_9NO_4S$), Tris-HCl buffer, citrate buffer, sucrose, glucose, and Drabkin reagents purchased from Merck Chemicals (Pty) Ltd., Bellville, South Africa. All other chemicals and reagents were of analytical grade and procured from Sigma-Aldrich Chemical Co. (Johannesburg-SA).

2.2 | Experimental animals and diets

Twenty-five male Wistar Albino rats with an average weight of 200 g were procured from the Department of Veterinary Medicine, the Michael Okpara University of Agriculture, Umudike (MOUAU), Abia State, Nigeria. The experimental animals are kept in the animal room under standard laboratory conditions of temperature (22 ± 2°C), humidity-measured environment (50 ± 5%) for 12-hr light and dark cycle. All animals have free access to tap water and received a standard chow diet ad libitum containing 9.2% water, 22.1% crude protein, 5.1% crude fat, 5.2% crude ash, 4.12% crude fiber, 50% nitrogen-free extract, 1.24% calcium, 0.92% phosphorous, 1.34% lysine, and 0.78% methionine and cysteine, for 28 days. These studies were carried out under the approval and guidelines of the animal ethics committee of the Michael Okpara University of Agriculture, Umudike, Nigeria.

2.3 | Experimental design and induction of type 2 diabetes (T2D)

Experimental rats were randomly divided into five groups (n = 5 for each group): normal control (NC), diabetic control (DBC), quercetin low dose group (DQL, 25 mg/kg), quercetin high dose group (DQH, 50 mg/kg), and metformin-treated group (DMF, 10 mg/kg). The quercetin dosages used in this study was chosen due to the previous report of Ruiz et al. (2006) who reported non-pathologic changes in hepatocytes or kidney tubules, glomeruli, and pancreatic islets after treatment of normal and diabetic groups (Swiss mice) with a maximum dose of 3,000 mg/kg for 28 days. After a week adaptation period and subsequent overnight fasting, the animals in DBC, DQL, DQH, and DMF groups were given a 10% v/v fructose solution ad libitum for 2 weeks to induce insulin resistance (Chukwuma et al., 2018). After 2 weeks, a single intraperitoneal injection of STZ (40 mg/kg) prepared in citrate buffer (pH 4.5) was administered to the animals to induce a nongenetic T2D animal model and partial pancreatic β-cell dysfunction (Wilson & Islam, 2012). The animal model was closely related to natural human T2D. The rats in the NC group were supplied with ordinary drinking water and injected with citrate buffer instead of 10% fructose and STZ injection. One week after STZ injection, the non-fasting blood glucose (NFBG) of all animals measured in the blood collected from the tail vein by using a portable glucometer (Bayer Healthcare, Japan). Animals with an NFBG level > 180 mg/dl were considered diabetic and used for the study. After diabetes confirmation, quercetin or metformin was administered orally throughout the experimental period using a gastric gavage needle while the rats in NC and DBC groups treated with a similar volume of the vehicle. The feed and water intake were measured every morning, weekly body weight, and NFBG levels were measured. The oral glucose tolerance test (OGTT) was performed toward the last week of the experiment after oral administration of glucose solution (2 g/kg BW), followed by blood glucose level measurement using glucometer at 0, 30, 60, 90, and 120 min.

2.4 | Collection of blood and tissue sample

At the end of the experimental period, animals were fasted overnight and euthanized using ketamine anesthesia (100 mg/kg). The blood sample was collected via cardiac puncture into sample bottles with EDTA and immediately preserved in a refrigerator at 4°C for biochemical analysis. Serum was obtained by centrifuging blood at a 3,000 rpm for 15 min. The aliquot was collected and stored in the fridge at 4°C for the determination of glycosylated hemoglobin (HbA1c) and Hb concentration. The liver tissue was dissected out, washed in 0.9% saline, and weighed. Ten-percent (10%) of the liver or pancreas was homogenized with pre-chilled mortar and pestle at 10,000 rpm for 10 min in 25 M sucrose solution. The supernatant was collected and used to determine glycogen level, hexokinase, and glucose-6-phosphatase activity of rats in each group. The pancreatic tissue was chopped and homogenized in saline solution at 10,000 rpm for 15 min at 4°C. The supernatant was collected and used immediately to determine the level of thiobarbituric reactive species (TBARS), reduced glutathione (GSH), CAT, and superoxide dismutase (SOD).

2.5 | Determination of Hb and HbA1c

The level of Hb in the blood samples was determined according to the cyanmethemoglobin method of Drabkin and Austin (1932) modified by Jayaprasad, Sharavanan, and Sivaraj (2016). In brief, the reaction mixture in a volume of 5.02 ml consisted of 5 ml of Drabkin's reagent and 0.02 ml of the blood sample. The reaction mixture was kept at room temperature for 5 min to ensure the completion of the reaction. The solution was read at 540 nm, together with the standard cyanmethemoglobin. The level of HbA1c in the blood of experimental rats was estimated according to the method described by Nayak and Pattabiraman (1981). To 0.02 ml of the lysed blood was added 4 ml of oxalate hydrochloric solution and heated at 100°C for 4 hr. The resulting solution was allowed to cool down and precipitated with 2 ml of 40% w/v TCA. After centrifugation, 0.5 ml of the supernatant was collected and added to 0.05 ml of 80% v/v phenol and 3.0 ml of concentrated sulfuric acid, and the color developed was read at 480 nm after 30 min of incubation at room temperature.

2.6 | Determination of hepatic glycogen content

For this assay, 100 mg of the liver tissue was subjected to alkali digestion (5 ml of 30%w/v KOH) in a boiling water bath for 20 min. The samples were allowed to cool down to room temperature and then

added 3.0 ml of absolute ethanol, followed by a drop of $NH_4CH_3CO_2$. The resulting mixture was centrifuged at 3,000× *g* for 10 min to collect the precipitate. An aliquot of the precipitated glycogen sample was reacted with 4 ml of anthrone reagent heated in a boiling water bath for 20 min. The hepatic glycogen content (green color) was measured at 640 nm and expressed as mg/g wet tissue.

2.7 | Determination of hexokinase (HK) activity

For the determination of hexokinase activity, an enzyme for glucose metabolism in the liver was assayed according to the method of Brandstrup, Kirk, and Bruni (1957) modified by Jayaprasad et al. (2016). The reaction mixture in a total volume of 5.3 ml consisting of glucose (1 ml, 5 mM), Adenosine triphosphate (ATP) (0.5 ml, 4 mM), magnesium chloride (0.1 ml, 100 mM), potassium dihydrogen phosphate (0.4 ml), KCl (0.4 ml, 7 mM), sodium fluoride (0.4 ml), and 50 mM Tris-HCl buffer (2.5 ml, pH 8.0). The reaction mixture was preincubated at 37°C for 5 min, followed by the addition of liver homogenate (2 ml) to start the reaction. A volume of 1 ml was transferred immediately to the tubes containing TCA (10% w/v, 1 ml) and then incubated for 30 min at 37°C. The residual glucose content in the supernatant was measured at 340 nm after centrifugation to estimate hexokinase activity.

2.8 | Determination of Glucose-6-phosphatase (G-6-Pase) activity

The glucose-6-phosphatase activity was assayed by estimating the inorganic phosphate (Pi) liberated from glucose 6-phosphate (G6P) according to the method of Jayaprasad et al. (2016). Briefly, 1.5 ml of 2 mM glucose-6-phosphate added to the 0.5 ml of hepatic tissue homogenate mixed and incubated for 30 min at 37°C. The G-6-Pase activity in the reaction mixture terminated by adding 1 ml of 10% TCA to the reaction tubes. The suspension was centrifuged, and the amount of Pi content in the supernatant of liver tissue homogenate (0.6 ml) was estimated by adding 1 ml of $(NH4)_6Mo_7O_{24}$ (0.25%) and 0.4 ml of ANSA (amino naphthol sulfonic acid) reagent. After 20 min, the absorbance was read at 680 nm against the blank (200 µl of 10% TCA). The standard curve was determined using 0.1 ml of different concentrations of K_2HPO_4 (0.5– 4 mM). The G-6-Pase activity was expressed as unit/mg of protein. One unit of glucose-6-phosphatase activity defined as the amount of phosphorous liberated/min at 37°C under the assay condition.

2.9 | Determination of pancreatic antioxidant status

Superoxide dismutase activity in the pancreatic tissue was estimated by the method of Kakkar, Das, and Viswanathan (1984). CAT activity was assayed by the method of Sinha (1972) and GSH by Ellman (1959). Pancreatic lipid peroxidation was determined by measuring the level of malondialdehyde (MDA), an index of lipid peroxidation following the method of Nichans and Samuelson (1968).

2.10 | Histopathological analysis of liver and pancreas

After 28 days of quercetin and metformin dosing, the animals from each group were euthanized, and the liver and pancreas were dissected out, washed in 0.9% saline, and then fixed in 10%v/v of formalin solution. For histological studies, the liver and pancreas sections were sliced and transferred immediately into 70% ethanol and then dehydrated via series of graded ethanol (30%, 50%, 70%, 90%, and 100%). After dehydration, the tissues were transferred into xylene and then embedded in paraffin wax and cut into 5-micron sections and then stained with hematoxylin and eosin (H&E). A microscopic examination was performed at magnification 400×.

2.11 | Molecular docking

For the molecular docking study, 3D structures of desired target enzymes were analyzed. Unfortunately, any X-ray or NMR crystallographic 3D structures were not available for docking study. Therefore, the 3D structures of hexokinase and CAT sequences of *Rattus norvegicus*, the most promising activity exhibited by quercetin, were retrieved from the UniProtKB database with individual reference IDs, P04762, and P27881, respectively. The particular homology models/theoretical 3D structure was generated using ideal template structures from the BlastP search. The generated structures stability was validated by the Ramachandran plots (Swain, Paidesetty, & Padhy, 2017a). Furthermore, the intermolecular interactions between quercetin with targeted enzymes were studied using molecular docking study and visualized using the software, AutoDock Vina, and Discovery Studio Visualizer, respectively (Swain, Paidesetty, & Padhy, 2017b).

2.12 | Statistics

Data analysis was done on Microsoft Excel to obtain descriptive statistics. Means values separated by the Duncan multiple tests using Statistical Analysis Software (SAS). The different levels of significance within the groups were analyzed using a one way analysis of variance (ANOVA). The values were considered significant at $p < .05$.

3 | RESULTS AND DISCUSSION

The high consumption of fructose rich-foods associated with the development of obesity, hyperinsulinemia, and fatty liver diseases, which is an underlying cause of insulin resistance responsible for the global epidemic and prevalence of T2D (Wilson & Islam, 2012). A substantial increase in the dietary fructose consumption to 85–100 g per day has been reported to rapidly stimulate lipogenesis and triglyceride accumulation leading to reduced insulin sensitivity to target tissues and hepatic insulin resistance (Shulman, 2000). One of the consequences of fructose-induced insulin resistance is impaired glucose tolerance

attributed to a defect in insulin binding, post-receptor, and signal transduction mostly characterized in humans with T2D (Shulman, 2000). The significant increased of fasting blood glucose, glucose intolerance, body weight gain, and MDA levels observed in our studies confirmed the development of insulin resistance in the experimental animal (Chukwuma et al., 2018; Ibrahim & Islam, 2017).

3.1 | Effect of quercetin on animal body weight, feed, and water intake

Table 1 shows a significant reduction ($p < .05$) in the body weights of untreated diabetic rats (DBC) at the end of the experiment while the bodyweight of quercetin-treated diabetic rats (DQL and DQH) was significantly improved as compared to the DMF and NC groups (Figure 1). There was a significant decreased in feed and water intake in the DBC group but reverted in diabetic-treated rats with metformin and quercetin, suggesting their proper glycaemic control (data not showed). The body weight loss seen in DBC may be attributed to the loss of appetite as a result of excessive catabolism of protein to provide amino acids for gluconeogenesis (Ramesh et al., 2010). Previous studies of Coskun, Kanter, Korkmaz, and Oter (2005) reported a similar effect of quercetin in STZ-induced diabetic rats.

3.2 | Effect of quercetin on the organ/body weight ratio of liver and pancreas

The pancreas is an essential organ of carbohydrate metabolism that maintains glucose homeostasis via insulin biosynthesis. Intraperitoneal injection of STZ, a selective toxic agent, causes pancreatic β-cell damage and thus affects insulin secretion through inhibition of β-cell O-GlcNAcase (Fadillioglu, Kurcer,

TABLE 1 Effect of oral treatment of quercetin and metformin on the weight and relative organ-body weight of liver and pancreas in normal and diabetic rats

			Liver	Pancreas
Animal group	Liver (g)	Pancreas (g)	Organ/body weight (%)	
NC	10.21 ± 1.22[a]	1.52 ± 0.08[a]	4.32 ± 0.22[a]	0.64 ± 0.02[a]
DBC	8.46 ± 1.84[c]	1.24 ± 0.13[c]	4.80 ± 0.64[c]	0.70 ± 0.03[c]
DQL	8.95 ± 1.21[b]	1.38 ± 0.26[b]	4.08 ± 0.52[b]	0.67 ± 0.06[b]
DQH	9.56 ± 0.72[b]	1.46 ± 0.34[b]	4.17 ± 0.83[b]	0.65 ± 0.07[b]
DMF	9.25 ± 1.28[b]	1.50 ± 0.11[b]	4.38 ± 1.28[a]	0.73 ± 0.02[b]

Note: Data are presented as the mean ± *SD* ($n = 5$).

[a–c]Values with different letters are significantly different from each other.

Abbreviations: DBC, diabetic control; DMF, diabetic metformin (10 mg/kg); DQH, diabetic quercetin high dose (50 mg/kg); DQL, diabetic quercetin low dose (25 mg/kg); NC: normal control.

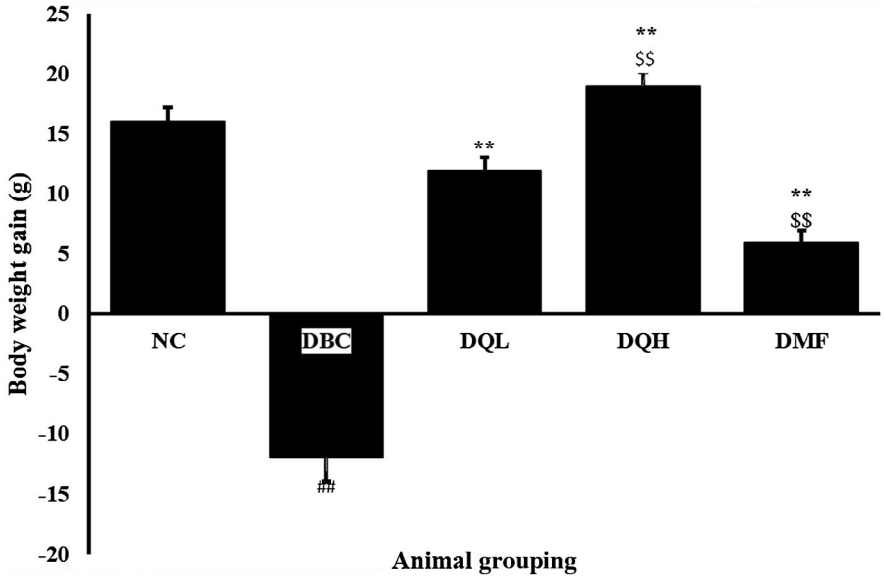

FIGURE 1 Effect of oral treatment of quercetin and metformin on the weight and relative organ-body weight of liver and pancreas in normal and diabetic rats. Data are presented as the mean ± *SD* ($n = 5$). DBC, diabetic control; DMF, diabetic metformin (10 mg/kg); DQH, diabetic quercetin high dose (50 mg/kg); DQL, diabetic quercetin low dose (25 mg/kg); NC: normal control. Multiple comparisons were made using one way ANOVA. $^{\#\#}p < .01$ versus control; $^{**}p < .01$ versus DBC; $^{\$\$}p < .01$ versus metformin groups

Parlakpinar, Iraz, & Gursul, 2008). However, the liver, as a primary site of endogenous glucose production, has a high capacity to neutralized toxic substance produced by STZ that causes pancreatic β-cell damage. The significant increase ($p < .05$) in the relative organ body weights of the liver in the DBC group (Table 1) despite a decreased liver weight is a useful indicator of hepatic damage. Conceivably due to triglyceride accumulation as a consequence of hyperinsulinemia and deficit of Apo-lipoprotein B synthesis (Zafar et al., 2009). Also, the induction of diabetes with STZ decreases nicotinamide-adenine dinucleotide (NAD) in pancreatic β-cells leading to a significant reduction of the pancreatic mass (Li et al., 2007). However, the continued oral administration of quercetin and metformin at the dosages investigated for 28 days restored the organs to near normal. A similar observation was reported by Vessal et al. (2003), who observed normalization of pancreas and liver in STZ-induced diabetic rats treated with quercetin at 15 mg/kg for 7 days.

3.3 | Effect of quercetin on plasma blood glucose and OGTT

The oral fructose feeding and intraperitoneal administration of STZ selectively destroyed some population of pancreatic β-cells in experimental animals, causing hyperglycemia as compared with the NC rats (Table 2). Oral administration of quercetin and metformin caused significant reduction ($p < .05$) of the blood glucose level within the range of 27.8% and 46.3%. Our results corroborate with the findings of Srinivasan, Meadows, and Maxwell (2018), who reported a similar antidiabetic activity of quercetin in STZ-induced diabetic rats through enhanced insulin secretion from pancreatic β-cells. Eid and Haddad (2017) also attributed the antidiabetic effect of quercetin to the inhibition of intestinal glucose absorption and improved glucose utilization in peripheral tissues. In the OGTT, the blood glucose levels in NC, DBC, DQL, DQH, and DMF groups significantly increased within 30 min of loading the animals with 2 g/kg glucose solution (Figure 2).

TABLE 2 Effect of oral treatment of quercetin and metformin on the blood glucose level (mg/dl) in normal and type 2 diabetes model of rats

Animal groups	0 week	1st week	2nd week	3rd week	4th week
NC	98.62 ± 5.00^{a}	94.62 ± 6.72^{a}	101.4 ± 10.2^{a}	96.00 ± 6.53^{a}	94.44 ± 7.22^{a}
DBC	447.4 ± 20.11^{c}	462.4 ± 32.12^{c}	470.3 ± 31.55^{e}	473.8 ± 30.09^{e}	462.5 ± 36.05^{d}
DQL	395.5 ± 27.12^{b}	368.3 ± 22.13^{b}	332.1 ± 21.34^{d}	308.2 ± 28.42^{d}	285.6 ± 30.02^{c}
DQH	410.2 ± 42.06^{b}	365.2 ± 34.03^{b}	310.1 ± 22.91^{c}	256.2 ± 25.77^{c}	220.4 ± 18.20^{c}
DMF	462.0 ± 28.08^{b}	408.0 ± 27.32^{b}	364.2 ± 32.00^{b}	273.3 ± 28.52^{b}	269.1 ± 31.51^{b}

Note: Data are presented as the mean ± *SD* ($n = 5$).

$^{a-e}$Values with different letters are significantly different from each other.

Abbreviations: DBC, diabetic control; DMF, diabetic metformin (10 mg/kg); DQH, diabetic quercetin high dose (50 mg/kg); DQL, diabetic quercetin low dose (25 mg/kg); NC, normal control.

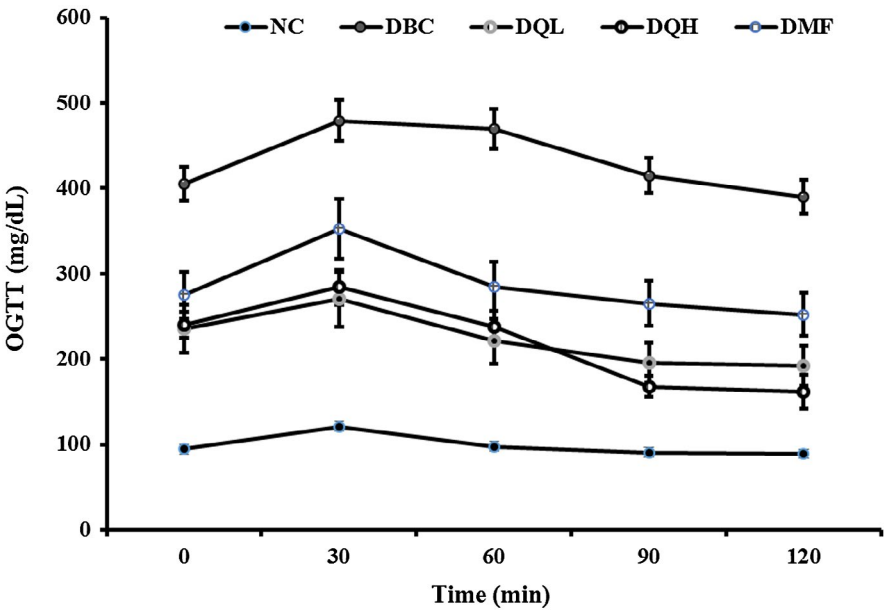

FIGURE 2 Oral glucose tolerance test (OGTT) for all groups of animals in the last week of the experimental period. Data are presented as the mean ± *SD* ($n = 5$). DBC, diabetic control; DMF, diabetic metformin (10 mg/kg); DQH, diabetic quercetin high dose (50 mg/kg); DQL, diabetic quercetin low dose (25 mg/kg); NC, normal control

The DBC group maintained a persistent significant increased in the blood glucose level for 120 min but remarkably declined in DQL, DQH, DMF, and NC rats.

3.4 | Effect of quercetin on Hb and HbA1C

Glycosylated Hb is an excellent marker mostly used to measure how much glucose molecules irreversibly bind with the Hb over the lifespan of the red blood cell (Daisy & Rajathi, 2009). It is caused by the non-enzymatic reaction of glucose and the Hb to form Amadori product. The rearrangement of this product leads to the formation of advanced glycation end-products that play an essential role in the pathogenesis of diabetic complications (Daisy & Rajathi, 2009). During diabetes conditions, the elevated blood glucose, if not treated, reacts with Hb to form HbA1c causing a significant reduction in Hb level (Kiho et al., 2000). The DBC rats showed a higher level of HbA1c (0.72 ± 0.12) but a lower level of total Hb (8.22 ± 1.05) as compared to the NC

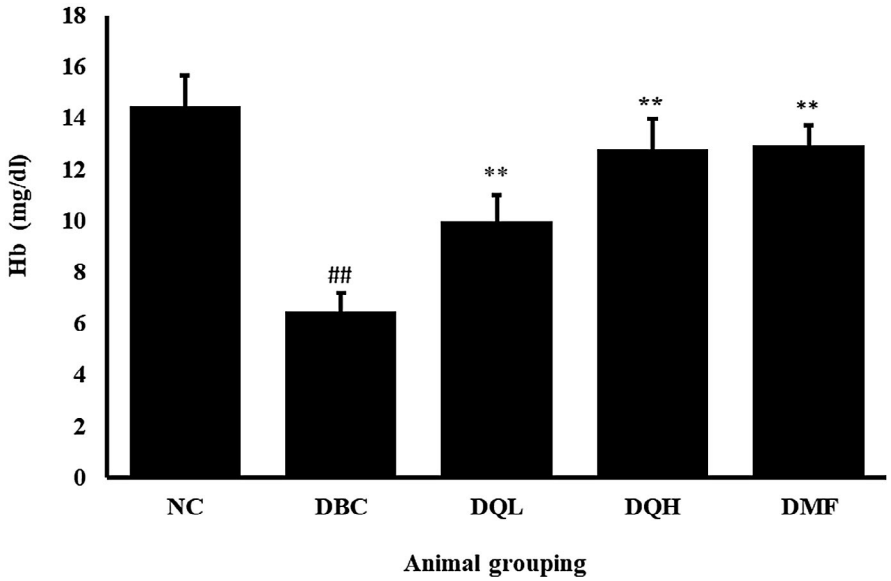

FIGURE 3 Effect of oral treatment of quercetin or metformin on the serum hemoglobin (Hb) level in the normal and type 2 diabetes model of rats. Data are presented as the mean ± *SD* (*n* = 6). DBC, diabetic control; DMF, diabetic metformin (10 mg/kg); DQH, diabetic quercetin high dose (50 mg/kg); DQL, diabetic quercetin low dose (25 mg/kg); NC, normal control. Multiple comparisons were made using one way ANOVA. $^{\#\#}p < .01$ versus control; $^{**}p < .01$ versus DBC; $^{\$\$}p < .01$ versus metformin groups

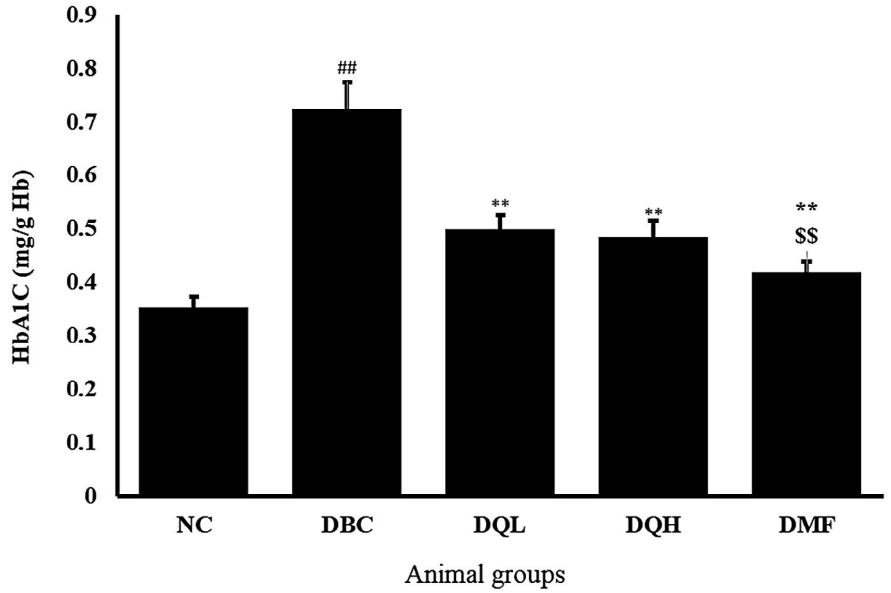

FIGURE 4 Effects of oral treatment of quercetin or metformin on the serum glycosylated hemoglobin (HbA_{1C}) level in the normal and T2DM model of rats. Data are presented as the mean ± *SD* (*n* = 5). DBC, diabetic control; DMF, diabetic metformin (10 mg/kg); DQH, diabetic quercetin high dose (50 mg/kg); DQL, diabetic quercetin low dose (25 mg/kg); NC, normal control. Multiple comparisons were made using one way ANOVA. $^{\#\#}p < .01$ versus control; $^{**}p < .01$ versus DBC; $^{\$\$}p < .01$ versus metformin groups

rats (Hb; 0.352 ± 0.02 vs. HbA1c; 13.6 ± 2.21) as shown in Figures 3 and 4. However, the repeated dose of quercetin for 28 days significantly decreased ($p < .05$) the level of HbA1c while the total Hb was markedly increased in a dose-dependent manner indicating better glycaemic control comparable to that of metformin and healthy rats. Our results are consistent with the findings of Peng et al. (2017), who demonstrated an equivalent effect of quercetin at the dosage of 10 and 50 mg/kg in STZ-induced diabetic rats. The present data may perhaps due to the ability of quercetin to improve glucose metabolism through its potent antioxidant capacity since glycosylation of Hb triggered by the oxidation reaction (Abdelmoaty, Ibrahim, Ahmed, & Abdelaziz, 2010).

3.5 | Effect of quercetin on hepatic glycogen

Glycogen is the primary intracellular storable form of glucose in the liver and skeletal muscles as a readily available source of glucose for energy production. Krasak et al. (2004) reported low glycogen storage concentration in diabetic patients due to impaired insulin-stimulated glycogen synthesis and inhibition of glycogen metabolism. In the diabetic state, the liver loses its capacity to activate or synthesize glycogen synthase to increase postprandial glycogen storage leading to a decreased level of glycogen, which is also corresponding to their food intake (Krasak et al., 2004). As shown in Figure 5, the untreated diabetic rats showed a significant reduction in glycogen content, but significantly improved after treatment with quercetin. The effect of quercetin at a maximal dose of 50 mg/kg comparable with the metformin-treated group and healthy rats, perhaps due to increased insulin secretion, as reflected by the improved function of pancreatic tissue in the treated diabetic rats.

3.6 | Effect of quercetin on liver hexokinase

Hexokinase is a rate-limiting enzyme in the glycolytic pathway, responsible for the first phosphorylation of glucose during glycolysis. It plays a prominent role in hepatic glucose homeostasis and the development of DM (Zhang et al., 2009). Postic, Shiota, and Magnuson (2001) indicated that restoration of hepatic hexokinase activity provides a possible therapeutic strategy for diabetes treatment through enhanced glucose utilization and glycogen synthesis. In the present study, hexokinase activity in the liver of diabetic rats was significantly reduced, eliciting the diminished use of glucose and the increased amount of blood glucose (Zhang et al., 2009). However, the treatment of diabetic rats with daily doses of quercetin produced a substantial increased in the hexokinase activity hence suggest increased glucose uptake from the blood by the liver cells. The obtained data at the maximal dose of quercetin corroborated with that of Vessal et al. (2003) and compared favorably well with healthy rats and standard metformin. Quercetin at the dosages investigated improved the hexokinase activity by 1.2 and 1.8- fold increased for DQL and DQH, respectively, as compared with the untreated diabetic group (Figure 6). The effect produced by quercetin, a naturally occurring antioxidant, could be attributed to its potent antioxidant capacities against oxidative damages in the hepatic tissues caused by STZ (Johnson et al., 2013).

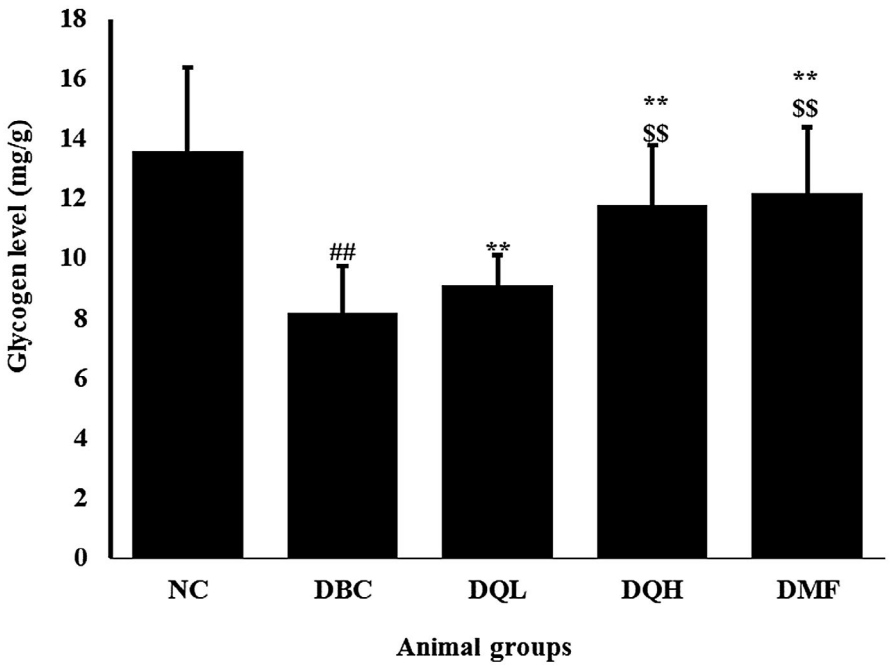

FIGURE 5 Effects of quercetin or metformin on the glycogen level in the liver of the normal and type 2 diabetes model of rats. Data are presented as the mean ± *SD* ($n = 5$). DBC, diabetic control; DMF, diabetic metformin (10 mg/kg); DQH, diabetic quercetin high dose (50 mg/kg); DQL, diabetic quercetin low dose (25 mg/kg); NC, normal control. Multiple comparisons were made using one way ANOVA. $^{\#\#}p < .01$ versus control; $^{**}p < .01$ versus DBC; $^{\$\$}p < .01$ versus metformin groups

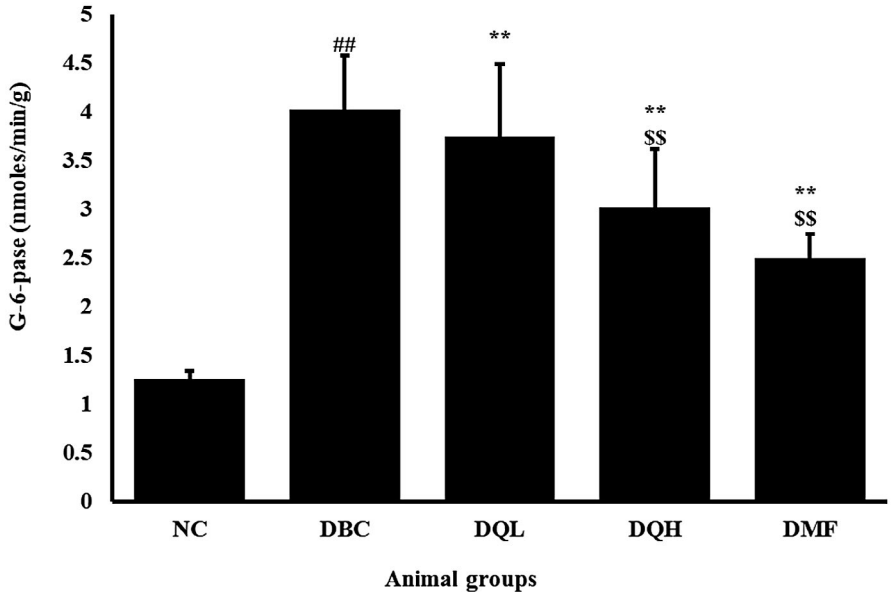

FIGURE 6 Effect of oral treatment of quercetin or metformin on the glucose-6-phosphatase activity in the liver of the normal and type 2 diabetes model of rats. Data are presented as the mean ± *SD* (*n* = 5). DBC, diabetic control; DMF, diabetic metformin (10 mg/kg); DQH, diabetic quercetin high dose (50 mg/kg); DQL, diabetic quercetin low dose (25 mg/kg); NC, normal control. Multiple comparisons were made using one way ANOVA. $^{\#\#}p < .01$ versus control; $^{**}p < .01$ versus DBC; $^{\$\$}p < .01$ versus metformin groups

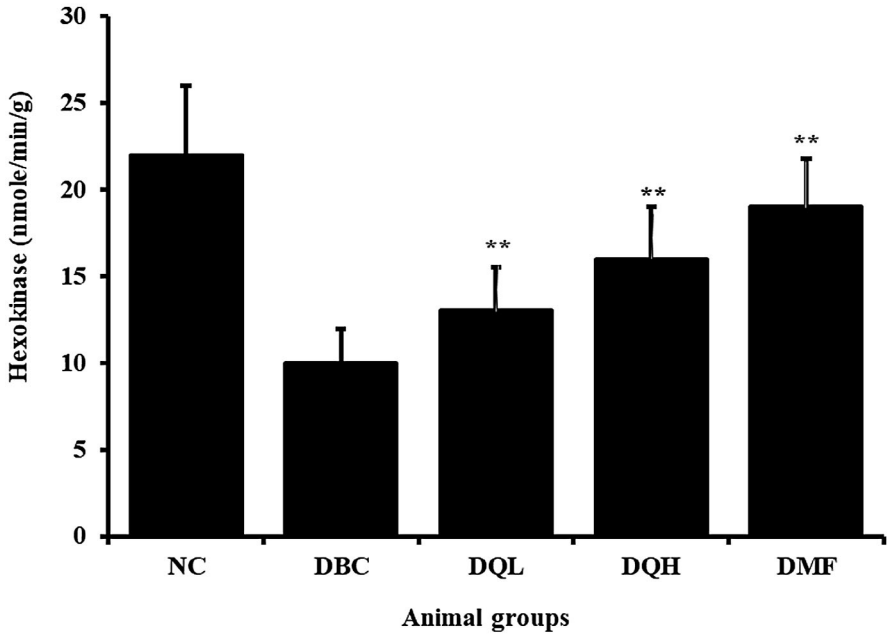

FIGURE 7 Effect of quercetin or metformin on the hexokinase activity in the liver of the normal and type 2 diabetes model of rats. Data are presented as the mean ± *SD* (*n* = 6). DBC, diabetic control; DMF, diabetic metformin (10 mg/kg); DQH, diabetic quercetin high dose (50 mg/kg); DQL, diabetic quercetin low dose (25 mg/kg); NC, normal control. Multiple comparisons were made using one way ANOVA. $^{**}p < .01$ versus DBC

3.7 | Effect of quercetin on liver glucose-6-phosphatases

Glucose-6-phosphatase (G6Pase) is a gluconeogenic enzyme that catalyzes the final step in gluconeogenic and glycogenolytic pathways. It liberates inorganic phosphate from glucose-6-phosphate to release glucose into the blood circulation (Smith, Marks, & Lieberman, 2005). Enhanced G6Pase activity is a well-known factor that exacerbates the symptoms associated with hyperglycemia in the diabetic condition through a decrease of glycolytic flux. In Figure 7, there was a notable increased in G6Pase action in diabetic rats due to increased G6P dephosphorylation that leads to elevated hepatic glucose production,

impaired suppression of fructose-1,6-bisphosphatase and insulin deficiency (Bopanna, Kannan, Godgil, Balaraman, & Rathod, 1997). However, a significant reduction in the G6Pase activity was observed after treatment with quercetin at 25 and 50 mg/kg, as well as metformin. Therefore, the considerable decrease in the blood glucose level recorded in DQL and DQH may be related to decreased G6Pase activity as a result of AMPK activation (Eid & Haddad, 2017).

3.8 | Effect of quercetin on pancreatic antioxidant enzymes and TBARS

Persistent hyperglycemia remains the primary cause of diabetes complications and oxidative stress resulting from the increased generation of ROS. The chronic exposure of pancreatic islets cells to ROS reduces insulin gene expression and insulin secretion due to the low level of antioxidant enzymes such as SOD, CAT, and Glutathione peroxidase (GPx) to detoxify free radicals toxicity (Oguri, Motegi, & Endo, 2003; Oyedemi, Yakubu, & Afolayan, 2011). SOD is a first-line antioxidant enzyme that catalyzes the dismutation reaction of superoxide anion to oxygen and hydrogen peroxide, which are further detoxified to water and oxygen by the action of CAT. The GSH is a nonenzymatic antioxidant with the capacity to catalyze the reduction of hydrogen peroxide into the water, thus preserve cellular protein from free radicals toxicity (Wang et al., 2006). The effect of quercetin on MDA, SOD, CAT, and GSH in the pancreas of diabetic rats and normal rats are presented in Table 3. In diabetic rats, the level of MDA an excellent marker of lipid peroxidation was significantly ($p < .05$) elevated

TABLE 3 Effect of oral treatment of quercetin or metformin on the pancreatic SOD, CAT, GSH, and MDA in the normal and type 2 diabetes model of rats

Animal groups	MDA (mmol/g)	CAT (nmol/min/ml)	SOD (U/ml)	GSH (µmol/ml)
NC	0.62 ± 0.00[a]	52.42 ± 2.21[a]	5.00 ± 1.00[a]	2.44 ± 0.72[a]
DBC	2.41 ± 0.12[c]	12.34 ± 1.51[e]	1.82 ± 0.09[e]	0.52 ± 0.05[d]
DQL	1.53 ± 0.08[b]	38.13 ± 1.34[d]	2.8 ± 0.42[d]	1.00 ± 0.02[c]
DQH	1.22 ± 0.06[b]	45.21 ± 2.91[c]	3.6 ± 0.77[c]	1.54 ± 0.20[c]
DMF	1.02 ± 0.08[b]	48.22 ± 3.00[b]	4.1 ± 0.52[b]	2.04 ± 0.51[b]

Note: Data are presented as the mean ± *SD* ($n = 5$).

[a–e]Values with different letters are significantly different from each other.

Abbreviations: CAT, catalase; DBC, diabetic control; DMF, diabetic metformin (10 mg/kg); DQH, diabetic quercetin high dose (50 mg/kg); DQL, diabetic quercetin low dose (25 mg/kg); GSH, reduced glutathione; MDA, malondialdehyde; NC, normal control; SOD, superoxide dismutase.

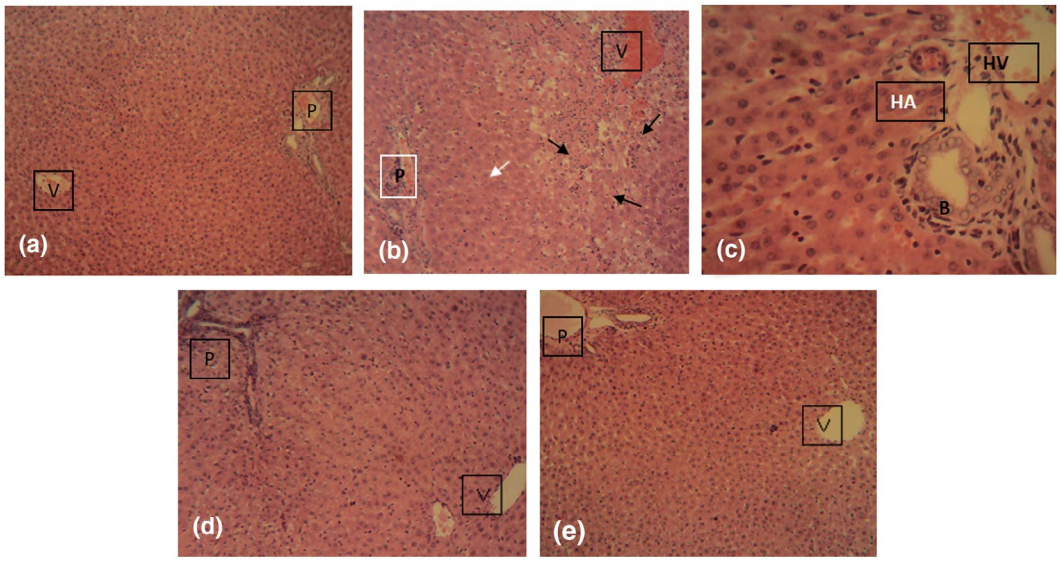

FIGURE 8 Photomicrographs of a section of the liver tissue of rats. Group NC (a) showed hepatic lobules consisting of normal hepatocytes arranged in interconnecting radiating cords around the central veins (V). The radiating cords terminate at the periphery of the hepatic lobules, where they make contact with the structures of the hepatic artery (HA), hepatic vein (HV), and bile duct (B) embedded in loose fibrous connective tissue. Group DBC (b) showed severe, coagulative necrosis (black arrow) of the centrilobular and mid-zonal hepatocytes with moderate infiltration of phagocytic mononuclear leukocytes. Central vein (V); Portal area (P); normal hepatocytes (white arrow). Groups DQL (c) showed hepatic lobules consisting of almost normal hepatocytes arranged in interconnecting radiating cords around the central veins (V). The radiating cords terminate at the periphery of the hepatic lobules, where they make contact with the structures of the hepatic triads. Groups DQH and DMF (d and e) show active and healthy hepatocytes with active vesicular nuclei like that of the NC group and the absence of any degenerative change. All the treated groups reversed the damaging effect of the low dose of STZ. All sections were stained with H and E stain and viewed with a light microscope (400× magnification)

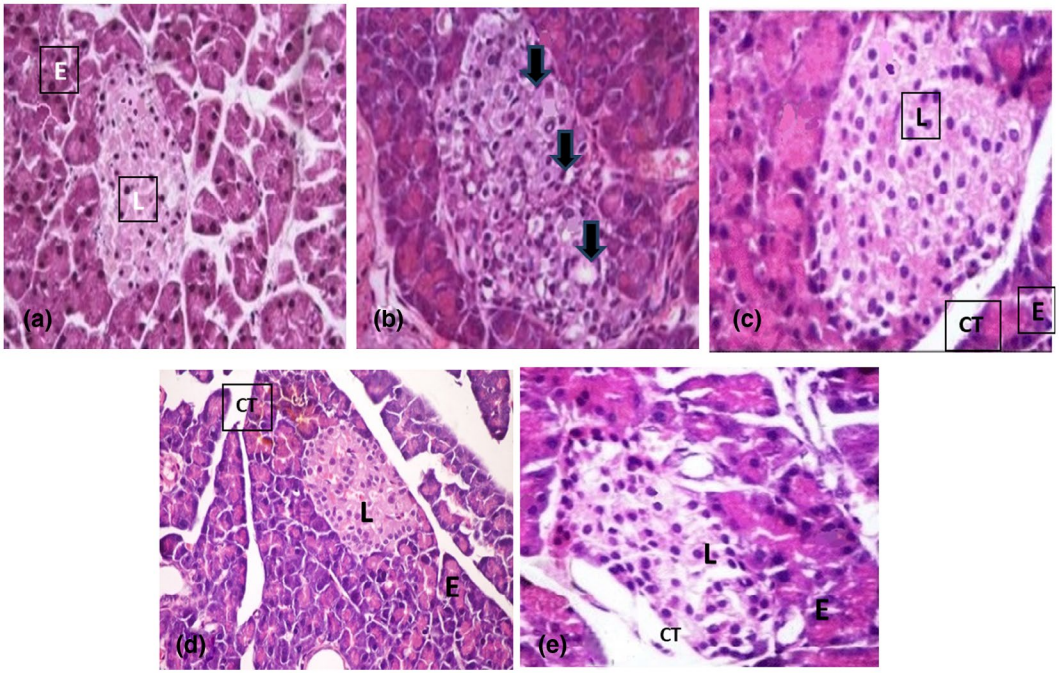

FIGURE 9 Photomicrographs of a section of the endocrine pancreatic tissue of rats: (Group NC: a) sections show normal islets of Langerhans (L) surrounded by exocrine portion (E) of pancreatic tissues with a healthy cellular population. Group DBC (b) shows the distortion of endocrine cells with the presence of necrotic cells and inflammatory cells disorganized islets of Langerhans. Group DQL (c) indicates almost normal islets of Langerhans and blood sinusoids. (Group DQH: d) shows a normal exocrine pancreatic acinar architecture (e) and pancreatic islets of Langerhans (L) and interlobular connective tissue (CT) with a healthy cellular population. Group DMF (E) shows a normal islet of Langerhans with a healthy population of β-cells and the absence of any degenerative change similar to the NC group. All the treated groups reversed the damaging effect of the low dose of STZ. All sections were stained with H and E stain and viewed with a light microscope (400× magnification)

in the pancreas by 3.88-fold over the healthy rats. However, the elevated MDA in the pancreas of diabetic rats effectively declined after daily treatment with quercetin by 1.58, 1.98, and 2.36-folds for DQL, DQH, and DMF groups, respectively. Also, the treatment of diabetic rats with quercetin enhanced CAT activity by 68% and 73% in DQL and DQH, respectively. The oral administration of quercetin and metformin significantly ($p < .05$) increased the SOD activity in the pancreas of diabetic rats by 1.54, 1.98, and 2.25-folds for DQL, DQH, and DMF, respectively. We also observed a significant reduction ($p < .05$) in the level of GSH in the pancreas of diabetic rats compared to the NC rats. The GSH levels were dose-dependent after treatment with quercetin for 28 days. Our results further confirmed the robust antioxidant potential of quercetin in vivo as a promising scavenger of free radicals generated in the pancreas of diabetic rats by STZ induction (Atalia, Fuentes, Wehrhahn, & Speisky, 2017). The present data, therefore, substantiate the effect of quercetin on glycaemic control, possibly due to improved pancreatic mass and enhanced insulin secretion from remnant pancreatic β-cells.

3.9 | Histopathology of liver tissue

Figure 8 showed the liver tissue of non-diabetic (NC), untreated diabetic (DBC), and diabetic treated rats with quercetin (DQL and DQH) or standard metformin (DMF). The observation of the hepatic tissue of non-diabetic rats showed norsmal histo-architecture of hepatic lobules and normal hepatocytes arranged in interconnecting radiating cords around the central veins (V). The radiating cords terminate at the periphery of the hepatic lobules, where they make contact with the structures of the hepatic triads; hepatic artery (HA), hepatic vein (HV) and bile duct (B) embedded in loose fibrous connective tissue (Figure 8a). The section of the hepatic tissue of untreated diabetic control (DBC) rats showed severe coagulative necrosis of the centrilobular and mid-zonal hepatocytes with moderate infiltration of phagocytic mononuclear leukocytes (Figure 8b). Upon treatment of diabetic rats with a DQL, the hepatic tissue showed near normal hepatocytes radially arranged around the central vein and terminate at the periphery of the hepatic lobules (Figure 8c). The liver tissues from the DQH group showed almost normal hepatocytes radially arranged around the central vein without the appearance of apoptotic cells (Figure 8d). The

TABLE 4 Docking scores (kcal/mol) of quercetin and standard drugs against newly modelled hexokinase and catalase enzymes of *Rattus norvegicus* (rats)

Chemical/drug	Hexokinase	CAT
Quercetin	−7.82	−9.83
Metformin	−5.43	ND
Ascorbic acid	ND	−6.90

Abbreviation: ND, not done.

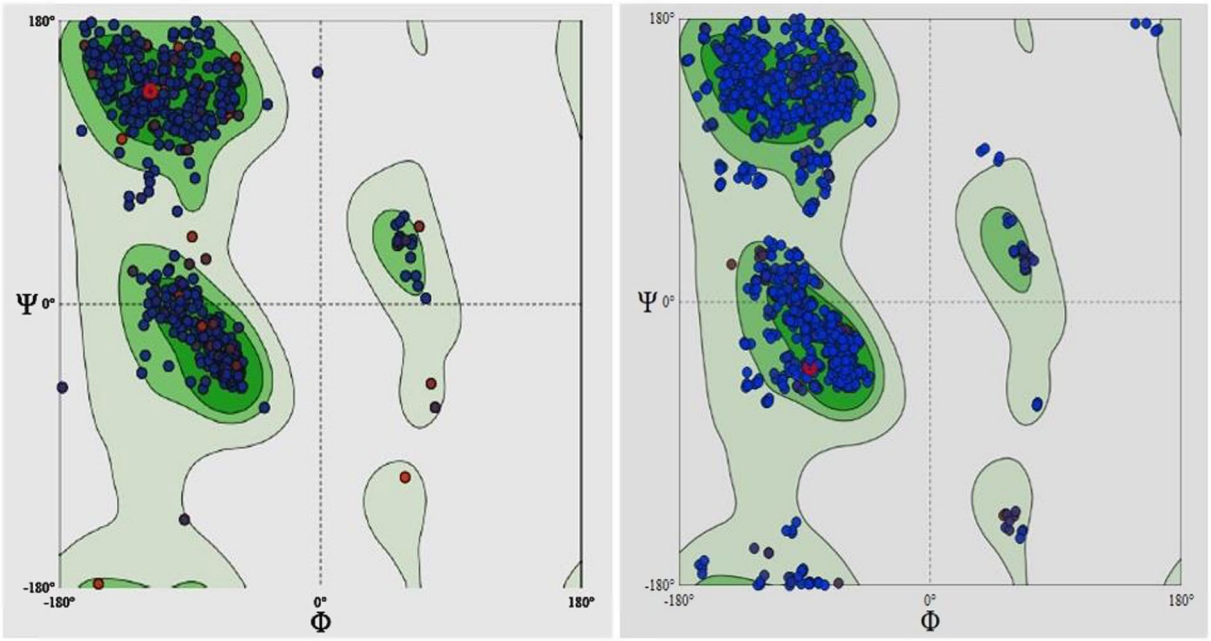

FIGURE 10 The Ramachandran plot in right side represented for hexokinase model with 97.54% of amino acids were found in favorable regions (in dark blue color spots), and the left side figure for catalase (CAT) with 96.32% of amino acids were found in the desirable areas

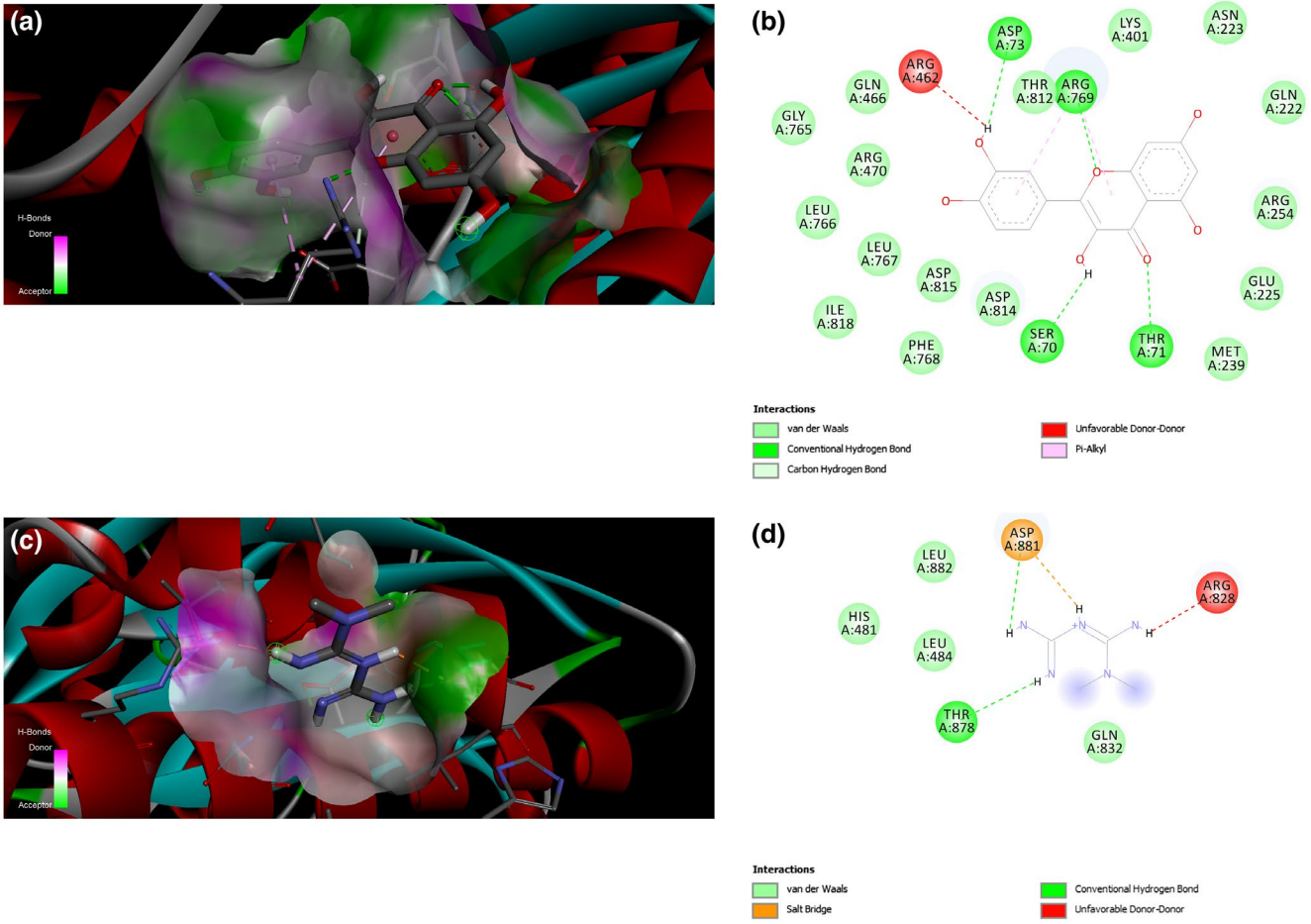

FIGURE 11 Protein-ligand interactions during docking study of quercetin and metformin against hexokinase; (a and b) represent two different cartoon interaction views of quercetin and (c and d) represents two different cartoon interaction views of metformin against hexokinase of *R. norvegicus*

group treated with standard metformin caused a significant reversal of coagulative necrosis observed in DBC by showing normal hepatocytes arranged in interconnecting radiating cords around the V, which terminate at the periphery of the hepatic lobules, where they make contact with the structures of the hepatic triads. Our observations on the hepatic tissues of treated diabetic rats with quercetin comparable with that of Selvakumar et al. (2013), who reported a protective effect of quercetin supplementation at the dosage of 50 mg/kg on histological changes in the liver of polychlorinated biphenyls-induced adult male Wistar rats.

3.10 | Histopathology of pancreatic tissue

Figure 9 showed the effect of quercetin on the pancreatic tissue of rats as compared with the standard metformin, diabetic control, and non-diabetic groups. The section of the pancreas from the non-diabetic group showed the normal pancreatic histo-architecture for laboratory rats. The slide sections showed normal islets of Langerhans surrounded by the exocrine portion of pancreatic tissues with a healthy cellular population (Figure 9a). Part of the pancreas collected from the diabetic rat showed distortion of endocrine cells, disorganized islets of Langerhans with the presence of necrotic, and a cluster of inflammatory cells (Figure 9b). The light microscopy examination of the pancreatic tissue of the diabetic rats treated with quercetin and metformin showed the normal exocrine pancreatic acinar architecture and pancreatic islets of Langerhans (Figure 9c-e). Also, there is interlobular connective tissue with a healthy cellular population, and the islets of Langerhans have a normal-looking population of β-cells. At least in part, our observations substantiated the previous report on the regeneration of the pancreatic islets in STZ-induced diabetic rats after 7 days of treatment with quercetin at the dosage of 15 mg/kg (Vessal et al., 2003). It is possible to suggest that the remarkable improvement in the blood glucose level and enzymatic activity linked with diabetes

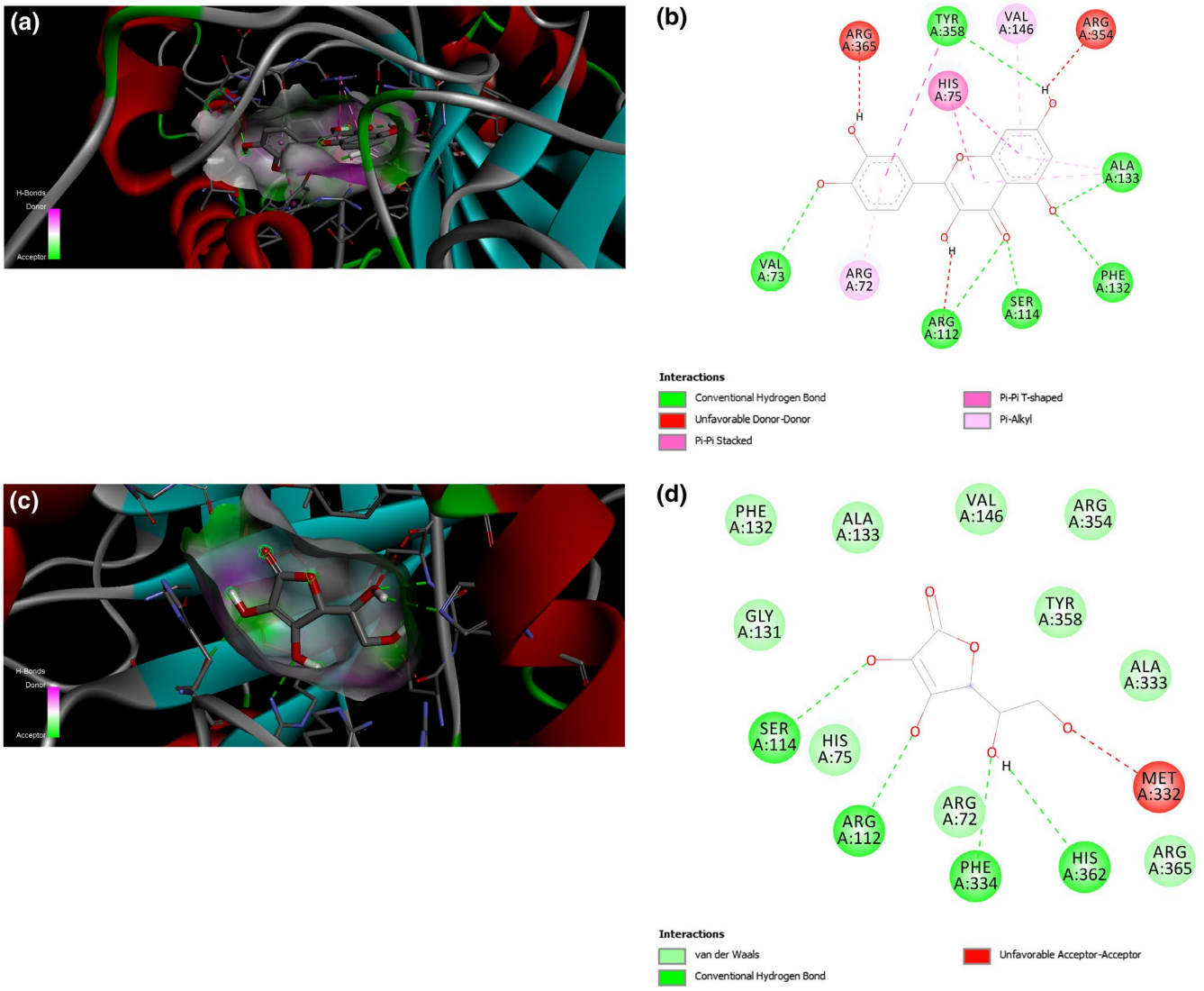

FIGURE 12 Protein-ligand interactions during docking study of quercetin and ascorbic acid against CAT; (a and b) represent two different cartoon interaction views of quercetin and (c and d) represents two different cartoon interaction views of ascorbic acid against CAT from *R. norvegicus*

control could be attributed to a reversal of deteriorated liver and pancreatic β-cells.

3.11 | Molecular docking of quercetin interaction with hexokinase

Based on the structural similarity and percentage of identity, the crystal structure of human hexokinase 2 (PBD ID: 5HEX and the crystal structure of *Bos taurous* [2J7B]) was used as an ideal template. Here, hexokinase was chosen as a target protein because of the promising activity exhibited by quercetin. Hexokinase is a rate-limiting enzyme in the glycolytic pathway that plays a significant role in hepatic glucose homeostasis (Zhang et al., 2009). The restoration of hexokinase activity provides a possible therapeutic strategy for diabetes treatment via improved glucose utilization and glycogen synthesis (Postic et al., 2001). The Ramachandran plot statistic showed 97.54% of amino acids in the favorable regions for hexokinase binding activity (Table 4; Figure 10). Moreover, the advanced molecular docking of quercetin interactions with hexokinase as a diabetic drug target enzyme in man showed the docking score −7.82 kcal/mol higher than the standard metformin with docking score of −5.43 kcal/mol (Table 4). Based on the docking score generated by AutoDock Vina software, more in negative scores represent a more effective agent against the docked enzyme. ASP 73, SER 70, ARG 769, and THR 71 residues which are present in the active site of hexokinase formed a strong polar interaction with the quercetin and strong hydrophobic interactions with GLY 765, GLN 466, ARG 470, LEU 766, LEU 767, ILE 818, ASP 814, ASP 815, PHE 768, MET 239, GLU 225, ARG 254, GLN 222, ASN 223, LYS 401, and THR 812 (Figure 11a,b).However, metformin had a strong polar interaction with THR 878 and strong hydrophobic interactions with LEU 484, HIS 481, LEU 882, and GLN 832 (Figure 11c,d).

3.12 | Molecular docking of quercetin interaction with CAT

The deficient level of CAT, a hydrogen peroxide detoxifying enzyme, has been reported to contribute significantly to the pancreatic β-cells susceptibility to free radical-induced damage (Tiedge, Lortz, Drinkgern, & Lenzen, 1997). In this study, about 96% of amino acids were found in favorable regions for CAT (Table 4; Figure 10). The advanced molecular docking showed that quercetin had a valid docking score of −9.83 kcal/mol higher than the standard ascorbic acid with a docking score of −6.90 kcal/mol with target CAT (Table 4). Based on the molecular interaction, quercetin formed six hydrogen bonds with VAL 73, ARG 112, SER 114, PHE 132, ALA 133, and TYR 358 residues in the active site of CAT without hydrophobic interaction (Figure 12a,b). The molecular interaction of ascorbic acid with CAT established four strong polar interactions with SER 114, ARG 112, PHE 334 and HIS 362 with ten strong hydrophobic interactions with PHE 132, GLY 131, HIS 75, ARG 72, ARG 365, ALA 333, TYR 358, ARG 354, VAL 146 and ALA 133 residues in the catalytic sites of CAT (Figure 12c,d). The intermolecular interaction of quercetin comparatively stronger than the standard drugs hence substantiated in vivo results.

4 | CONCLUSION

We have shown that quercetin at the dosages investigated substantially reversed the high blood glucose, enhanced glycogen content, and enhanced Hb as well as improved the pancreatic antioxidant status. Quercetin could improve or prevent secondary complications associated with Hb glycosylation during chronic hyperglycemia. The normalization of organ body weight ratio of the pancreas and liver suggests its potential to regenerate pancreatic β-cells and inhibits hypoinsulinemia as confirmed in increased hepatic hexokinase activity and decreased glucose-6-phosphatase activity as an indirect index of insulin release by pancreatic β-cells. Quercetin is a plant-derived product that represents a useful adjunct therapy that can be developed as an oral anti-hyperglycemic drug candidate for diabetic patients based on the data obtained from this study. Thus, from the in vivo and molecular docking studies, we suggested that quercetin is a promising nutraceutical candidate that can be developed into a potent antidiabetic drug though further studies on the toxicity for its long-term usage are strongly recommended.

CONFLICT OF INTEREST

The authors declare that there are no commercial or financial relationships that could be construed as a potential conflict of interest as regarding the publication of this paper.

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REFERENCES

Abdelmoaty, M. A., Ibrahim, M. A., Ahmed, N. S., & Abdelaziz, M. A. (2010). Confirmatory studies on the antioxidant and antidiabetic effect of quercetin in rats. *Indian Journal of Clinical Biochemistry*, *25*, 188–192. https://doi.org/10.1007/s12291-010-0034-x

Atalia, E., Fuentes, J., Wehrhahn, M. J., & Speisky, H. (2017). Quercetin and related flavonoids conserve their antioxidant properties despite undergoing chemical or enzymatic oxidation. *Food Chemistry*, *234*, 479–485. https://doi.org/10.1016/j.foodchem.2017.05.023

Bhatta, J. K., Thomas, S., & Joghee, M. (2012). Resveratrol supplementation improves glycaemic control in type 2 diabetes mellitus. *Nutrition Research*, *32*, 537–541.

Bopanna, K. N., Kannan, J., Godgil, S., Balaraman, R., & Rathod, S. P. (1997). Antidiabetic and antihyperlipidemic effects of neem seed kernel powder on alloxan-diabetic rabbits. *Indian Journal of Pharmacology*, *29*, 162–172.

Brandstrup, N., Kirk, J. E., & Bruni, C. (1957). Determination of hexokinase in tissues. *Journal of Gerontology*, *12*, 166–171.

Chukwuma, C. I., Mopuri, R., Nagiah, S., Chuturgoon, A. A., & Islam, M. S. (2018). Erythritol reduces small intestinal glucose absorption, increases muscle glucose uptake, improves glucose metabolic enzymes activities and increases expression of Glut-4 and IRS-1 in type 2 diabetic rats. *European Journal of Nutrition*, *57*, 2431–2444. https://doi.org/10.1007/s00394-017-1516-x.

Coskun, O., Kanter, M., Korkmaz, A., & Oter, S. (2005). Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and beta-cell damage in rat pancreas. *Pharmacological Research*, *51*, 117–123.

Daisy, P., & Rajathi, M. (2009). Hypoglycemic Effects of *Clitoria ternatea* Linn. (Fabaceae) In Alloxan-induced Diabetes in Rats. *Tropical Journal of Pharmaceutical Research*, *8*, 393–398.

DeFronzo, R. A. (2004). Pathogenesis of type 2 diabetes mellitus. *Medical Clinics of North America*, *88*, 787–835. https://doi.org/10.1016/j.mcna.2004.04.013

Drabkin, D. L., & Austin, J. M. (1932). Spectrophotometric constants for common haemoglobin derivatives in human, dog and rabbit blood. *Journal of Biological Chemistry*, *98*, 719–733.

Eid, H. M., & Haddad, P. S. (2017). The antidiabetic potential of quercetin: Underlying mechanisms. *Current Medicinal Chemistry*, *24*, 355–364. https://doi.org/10.2174/0929867323666160909153707

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archive of Biochemistry and Biophysics*, *82*, 70–77. https://doi.org/10.1016/0003-9861(59)90090-6

Fadillioglu, E., Kurcer, Z., Parlakpinar, H., Iraz, M., & Gursul, C. (2008). Melatonin treatment against remote open injury induced by renal ischemia-reperfusion injury in diabetes mellitus. *Archive of Pharmaceutical Research*, *31*, 705–712.

Hu, S., Wang, L., Yang, D., Li, L., Togo, J., Wu, Y. … Spearman, J. R. (2018). Dietary fat, but not protein or carbohydrate, regulates energy intake and causes adiposity in mice. *Cell Metabolism*, *28*, 415–431.

Ibrahim, M. A., & Islam, M. S. (2017). Effects of butanol fraction of *Ziziphus mucronata* root ethanol extract on glucose homeostasis, serum insulin and other diabetes-related parameters in a murine model for type 2 diabetes. *Pharmaceutical Biology*, *55*, 416–422.

International Diabetes Federation. (2015). *IDF Diabetes atlas (2015)* (7th ed.). Brussels, Belgium: Author.

Jayaprasad, B., Sharavanan, P. S., & Sivaraj, R. (2016). Antidiabetic effect of *Chloroxylon swietenia* bark extracts in streptozotocin-induced diabetic rats. *Beni-Suef University Journal of Basic and Applied Sciences*, *5*, 61–69.

Jeong, Y., Han, H. S., Lee, H. D., Yang, J., Jeong, J., Choi, M. K., … Kim, S. T. (2016). A pilot study evaluating steroid-induced diabetes after antiemetic dexamethasone therapy in chemotherapy-treated cancer patients. *Cancer Research and Treatment*, *48*, 1429–1437. https://doi.org/10.4143/crt.2015.464

Johnson, R. J., Nakagawa, T., Sanchez-Lozada, L. G., Shafiu, M., Sundaram, S., Le, M., Ishimoto, T., Sautin, Y. Y., & Lanaspa, M. A. (2013). Sugar, uric acid, and the eatiology of diabetes and obesity. *Diabetes*, *62*, 3307–3315. https://doi.org/10.2337/db12-1814.

Kakkar, P., Das, B., & Viswanathan, P. N. (1984). A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry and Biophysics*, *21*, 130–132.

Kiho, T., Kobayashi, T., Morimoto, H., Usui, S., Ukai, S., Hirano, K., … Inakuma, T. (2000). Structural features of an anti-diabetic polysaccharide (TAP) from *Tremella aurantia*. *Chemical and Pharmaceutical Bulletin*, *48*(11), 1793–1795. (Tokyo).

Knop, F. K., Konings, E., Timmers, S., Schrauwen, P., Holst, J. J., & Black, E. E. (2013). Thirty days of resveratrol supplementation does not affect postprandial incretin hormone responses but suppresses postprandial glucagon in obese subjects. *Diabetic Medicine*, *30*, 1214–1218. https://doi.org/10.1111/dme.12231

Kobori, M., Matsumoto, S., Akimoto, Y., & Oike, H. (2011). Chronic dietary intake of quercetin alleviates hepatic fat accumulation associated with consumption of a Western-style diet in C57/BL6J mice. *Molecular Nutrition and Food Research*, *55*, 530–540. https://doi.org/10.1002/mnfr.201000392

Krssak, M., Brehm, A., Bernroider, E., Anderwald, C., Nowotny, P., Man, C. D., … Roden, M. (2004). Alterations in postprandial hepatic glycogen metabolism in type 2 diabetes. *Diabetes*, *53*, 3048–3056. https://doi.org/10.2337/diabetes.53.12.3048

Li, C., Heidt, D. G., Dalerba, P., Burant, C. F., Zhang, L., Adsay, V., … Simeone, D. M. (2007). Identification of pancreatic cancer stem cells. *Cancer Research*, *67*, 1030–1037. https://doi.org/10.1158/0008-5472.CAN-06-2030

Nayak, S. S., & Pattabiraman, T. N. (1981). A new colourimetric method for the estimation of glycosylated haemoglobin. *International Journal of Clinical Chemistry*, *109*, 267–274.

Nichans, W. G., & Samuelson, B. J. (1968). Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. *European Journal of Biochemistry*, *6*, 126–130.

Oguri, S., Motegi, K., & Endo, Y. (2003). Augmented lipopolysaccharide-induction of the histamine-forming enzyme in streptozotocin-induced diabetic mice. *Biochimica et Biophysica Acta*, *1637*, 83–90. https://doi.org/10.1016/S0925-4439(02)00217-X

Oh, Y. S. (2015). Plant-derived compounds targeting pancreatic beta cells for the treatment of diabetes. *Evidence-Based Complementary and Alternative Medicine*, *2015*, 1–12. https://doi.org/10.1155/2015/629863

Oyedemi, S. O., Yakubu, M. T., & Afolayan, A. J. (2011). Antidiabetic activities of aqueous leaves extract of *Leonotis leonurus* in streptozotocin-induced diabetic rats. *Journal of Medicinal Plant Research*, *5*, 119–125.

Peng, J., Li, Q., Li, K., Zhu, L. I., Lin, X., Lin, X., … Xie, X. I. (2017). Quercetin improves glucose and lipid metabolism of diabetic rats: Involvement of Akt signaling and SIRT1. *Journal of Diabetes Research*, *2017*, 1–10. https://doi.org/10.1155/2017/3417306

Pessin, J. E., & Saltiel, A. R. (2000). Signalling pathways in insulin action: Molecular targets of insulin resistance. *Journal of Clinical Investigation*, *106*, 165–169.

Postic, C., Shiota, M., & Magnuson, M. A. (2001). Cell-specific roles of glucokinase in glucose homeostasis. *Recent Progress in Hormone Research*, *56*, 195–217. https://doi.org/10.1210/rp.56.1.195

Ramesh, B. K., Maddirala, D. R., Vinay, K. K., Shaik, S. F., Tiruvenkata, K. E. G., Swapna, S., … Rao, C. A. (2010). Antihyperglycemic and antihyperlipidemic activities of methanol: Water (4:1) fraction isolated from an aqueous extract of *Syzygium alternifolium* seeds in streptozotocin-induced diabetic rats. *Food Chemistry and Toxicology*, *48*, 1078–1084.

Roslan, J., Nelli Giribabu, N., Karim, K., & Salleh, N. (2017). Quercetin ameliorates oxidative stress, inflammation and apoptosis in the heart of streptozotocin-nicotinamide-induced adult male diabetic rats. *Biomedicine and Pharmacotherapy*, *86*, 570–582. https://doi.org/10.1016/j.biopha.2016.12.044

Ruiz, M., Fernández-Franzón, M., Estela, J. M., Ansesi, M. A., Manes, J., & Pico, Y. (2006). Short-term oral toxicity of quercetin and pterostibene in Swiss mice. *Toxicology Letters*, *164*, S275–S276. https://doi.org/10.1016/j.toxlet.2006.07.232

Salas-Salvado, J., Martinez-Gonzalez, M. A., Bullo, M., & Ros, E. (2011). The role of diet in the prevention of type 2 diabetes. *Nutrition Metabolism and Cardiovascular Diseases*, *21*, B32–B48. https://doi.org/10.1016/j.numecd.2011.03.009

Selvakumar, K., Bavithra, S., Suganya, S., Bhat, F. A., Krishnamoorthy, G., & Arunakaran, J. (2013). Effect of quercetin on haematobiochemical and histological changes in the liver of polychlorinated biphenyls-induced adult male Wistar rats. *Journal of Biomarkers*, *2013*, 1–12, Article ID 960125. https://doi.org/10.1155/2013/960125

Shulman, G. I. (2000). Cellular mechanisms of insulin resistance. *The Journal of Clinical Investigation*, *106*, 171–176. https://doi.org/10.1172/JCI10583

Sinha, A. K. (1972). Colourimetric assay of catalase. *Annal Biochemistry*, *47*, 389–394.

Smith, C., Marks, A. D., & Lieberman, M. (2005). *Mark's basic medical biochemistry: A clinical approach* (2nd ed., pp. 120–136). Baltimore, Maryland: Lippincott Williams and Wilkins.

Srinivasan, M., Meadows, M. L., & Maxwell, L. (2018). Assessment of salivary adipokines resistin, visfatin, and ghrelin as type 2 diabetes

mellitus biomarkers. *Biochemistry Research International*. *2018*, 1–5. https://doi.org/10.1155/2018/7463796

Swain, S. S., Paidesetty, S. K., & Padhy, R. N. (2017a). Antibacterial activity, computational analysis, and host toxicity study of thymol-sulfonamide conjugates. *Biomedicine Pharmacotherapy*, *88*, 181–193. https://doi.org/10.1016/j.biopha.2017.01.036

Swain, S. S., Paidesetty, S. K., & Padhy, R. N. (2017b). Development of antibacterial conjugates using sulfamethoxazole with monocyclic terpenes: A systematic medicinal chemistry based computational approach. *Computer Method Programming Biomedicine*, *140*, 185–194. https://doi.org/10.1016/j.cmpb.2016.12.013

Tiedge, M., Lortz, S., Drinkgern, J., & Lenzen, S. (1997). The relation between antioxidant enzyme gene expression and antioxidative defence status of insulin-producing cells. *Diabetes*, *46*, 1733–1742.

Tiwari, B. K., Pandey, K. B., Abidi, A. B, & Rizvi, S. I. (2013). Markers of oxidative stress during diabetes mellitus. *Journal of Biomarkers*, *2013*, 1–8. https://doi.org/10.1155/2013/378790

VanDeKoppel, S., Choe, H. M., & Sweet, B. V. (2008). Managed care perspective on three new agents for type 2 diabetes. *Journal of Managed Care and Pharmacy*, *14*, 363–380. https://doi.org/10.18553/jmcp.2008.14.4.363

Vessal, M., Hemmati, M., & Vasei, M. (2003). Antidiabetic effects of quercetin in streptozotocin-induced diabetic rats. *Comparative Biochemistry and Physiology Part C*, *135*, 357–364.

Wang, L., Tu, Y. C., Lian, T. W., Hung, J. T., Yen, J. H., & Wu, M. J. (2006). Distinctive antioxidant and anti-inflammatory effects of flavonols. *Journal of Agricultural Food Chemistry*, *54*, 9798–9804. https://doi.org/10.1021/jf0620719

Wilson, R. D., & Islam, M. S. (2012). Fructose-fed streptozotocin- injected rat: An alternative model for type 2 diabetes. *Pharmacological Report*, *64*, 129–139. https://doi.org/10.1016/S1734-1140(12)70739-9

Yang, D. K., & Kang, H. S. (2018). Anti-diabetic effect of cotreatment with quercetin and resveratrol in streptozotocin-induced diabetic rats. *Biomolecules & Therapeutics*, *26*, 130–138. https://doi.org/10.4062/biomolther.2017.254

Zafar, M., Naeem-ul-Hassan, N. S., Ahmed, M., & KaimKhani, Z. A. (2009). Altered liver morphology and enzymes in streptozotocin-induced diabetic rats. *International Journal of Morphology*, *27*, 719–725.

Zhang, X., Liang, W., Mao, Y., Li, H., Yang, Y., & Tan, H. (2009). Hepatic glucokinase activity is the primary defect in alloxan-induced diabetes in mice. *Biomedical Pharmacotherapy*, *63*, 180–186.

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