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Original article

Novel indolizine derivatives lowers blood glucose levels in streptozotocin-induced diabetic rats: A histopathological approach

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ABSTRACT

Background: Diabetes mellitus is a deadly disorder in human which induce chronic complications. The streptozotocin (STZ)-induced diabetes in rat is the most common animal model of human diabetes. The present study investigated the effects of novel indolizine derivatives **(1–16)** on plasma blood glucose concentrations in STZ-diabetic rats.

Methods: In vitro experiments were performed on 1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide free radicals, α-glucosidase enzyme and *in vivo* studies on normal, oral glucose loaded and STZ-induced diabetic rats.

Results: Among all synthetic derivatives, compound **12** showed good inhibitory profile against DPPH, superoxide free radicals and α-glucosidase enzyme with half maximal inhibitory concentration (IC_{50}) values of 56.2, 33.5 and 26.5 μg/mL, respectively. The lethal dosage of indolizine derivatives was found to be above 1000 mg/kg body weight (b.w.). From the *in vivo* studies, it can be determined that the compound **12** depicted pronounced protective hypoglycemic effects in normal, glucose-loaded and STZ-induced diabetic rats with respect to the standard. Furthermore, 21 days of successive treatment with compound **12** in diabetic rats exhibited better recovery of body weight and considerable variations in biochemical parameters as that of the standard drug. Moreover, the histopathological section of pancreas and testes justifies the rehabilitation and regeneration of islets, acini and Sertoli cells in animals treated with compound **12**.

Conclusion: Our data suggest that the indolizine derivatives can be a benchmarks for designing potent oral antidiabetic agents.

Introduction

Diabetes mellitus is a metabolic disorder characterized by elevated levels of plasma blood glucose, resulting from absence/inadequate pancreatic insulin [1]. According to World Health Organization, it is estimated that about 3% of the world's population have diabetes and the prevalence is expected to double by 2025. A wide variety of pharmacological drugs are being used for the treatment of diabetes, but several limitations like toxicity, obesity, hyperandrogenemia, less tissue penetration and high index of adverse effects limit their use [1,2]. In order to overcome all these aspects, there is a great need to design and synthesize novel antidiabetic drugs.

From decades, the chemistry of indolizines (isosteric analogues of indolesare) captivated importance as these analogues have been found to exhibit several pharmacological activities such as hypoglycemic [3], antidiabetic [4,5], anti-acetylcholine [6,7], CNS depressant [6], antihistaminic [6,7], anti-5-hydroxytrypatamine [7], monoamine oxidase inhibitor [8], substance P and neurokinin-A receptor antagonist [9], antiallergic [10], phospholipase A2 inhibitory [11], anti-inflammatory [11], phosphodiesterase [12] and phosphatase inhibitory [13], HIV-1 viral infectivity factor inhibitory [14], anti-tubercular [15], aromatase inhibitory [16], antimicrobial [17,18], antimutagenic [18], antioxidant [19], ACE inhibitory [20], anti-leishmanial [21], herbicidal [22], analgesic

Abbreviations: BM, basement membrance; DA, damaged acini; DI, damaged islets; DILD, damaged interlobular duct; DSC, damaged; DSG, damaged spermatogonia; NI, normal islets; NSC, normal sertoli cells; NSG, normal spermatogonia; RA, recovered acini; RI, recovered islets; RILD, recovered interlobular duct; RLD, recovered intralobular duct; RSC, recovered sertoli cells; RSG, recovered spermatogonia.

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[23], anticancer [24] and uterotrophic [25] activities. Possessing such diverse range of biological activities, these molecules are subject of interest to many synthetic researchers to develop fused derivatives. In recent times, our group have synthesized and reported a series of novel pyrrolo[1,2-*a*]pyrazine incorporated indolizine derivatives [26]. Now, these series of analogues (Fig. 1) were evaluated for their antioxidant, α-glucosidase inhibitory, hypoglycaemic and anti-hyperglycaemic activities using standard protocols.

The aim of the study was two-fold. First aim was to perform the *in vitro* antioxidant and enzyme inhibitory assays, to identify the most active compounds in the series of synthesized indolizine analogues. Second aim was to analyze the hypoglycemic and anti-hyperglycemic effects of indolizine derivatives, in type 2 diabetic rats and to compare them with healthy albino rats.

Materials and methods

Chemicals

All the chemicals used in the present experiment were of analytical grade. *p*-Nitrophenyl-α-D-glucopyranoside, rat intestinal acetone powder, sucrose, acarbose, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide dismutase were from Sigma Aldrich (Mumbai, India); streptozotocin (STZ) was from the Himedia Laboratories Pvt. Ltd. (Mumbai, India); ascorbic acid and glibenclamide from the Avantis Pharma Ltd. (Mumbai, India), and rat feed from the Hindustan Lever Ltd. (Mumbai, India).

Antioxidant activity

DPPH assay

The antioxidant activity was assessed by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay [27] in triplet. Initially, 100 μL of 100 μM of DPPH in ethanol was prepared and reacted with known concentrations (25, 50, 75 and 100 μg/mL) of the test and standard (ascorbic acid) samples, incubated for 30 min. The absorbance was noted at 517 nm on UV–vis spectrometer (Electron 420 series spectrophotometer). The percentage inhibition was calculated using the below formula. Simultaneously, the IC_{50} was calculated using linear graph section between percentage inhibition and concentration of tested sample.

Superoxide radical scavenging assay

In the radical method [28], the superoxide radicals generated from non-enzymatic phenazine methosulfate/nicotinamide adenine dinucleotide (PMS/NADH) reduces nitro blue tetrazolium (NBT) to a purple formazan. To 1 mL of reaction mixture contained 20 mM phosphate buffer (pH 7.4), 73 μM NADH, 50 μM NBT, 15 μM PMS added various concentrations (25, 50, 75 and 100 μg/mL) of test/ascorbic acid and incubated for 10 min at room temperature and the absorbance was noted at 562 nm against blank and the

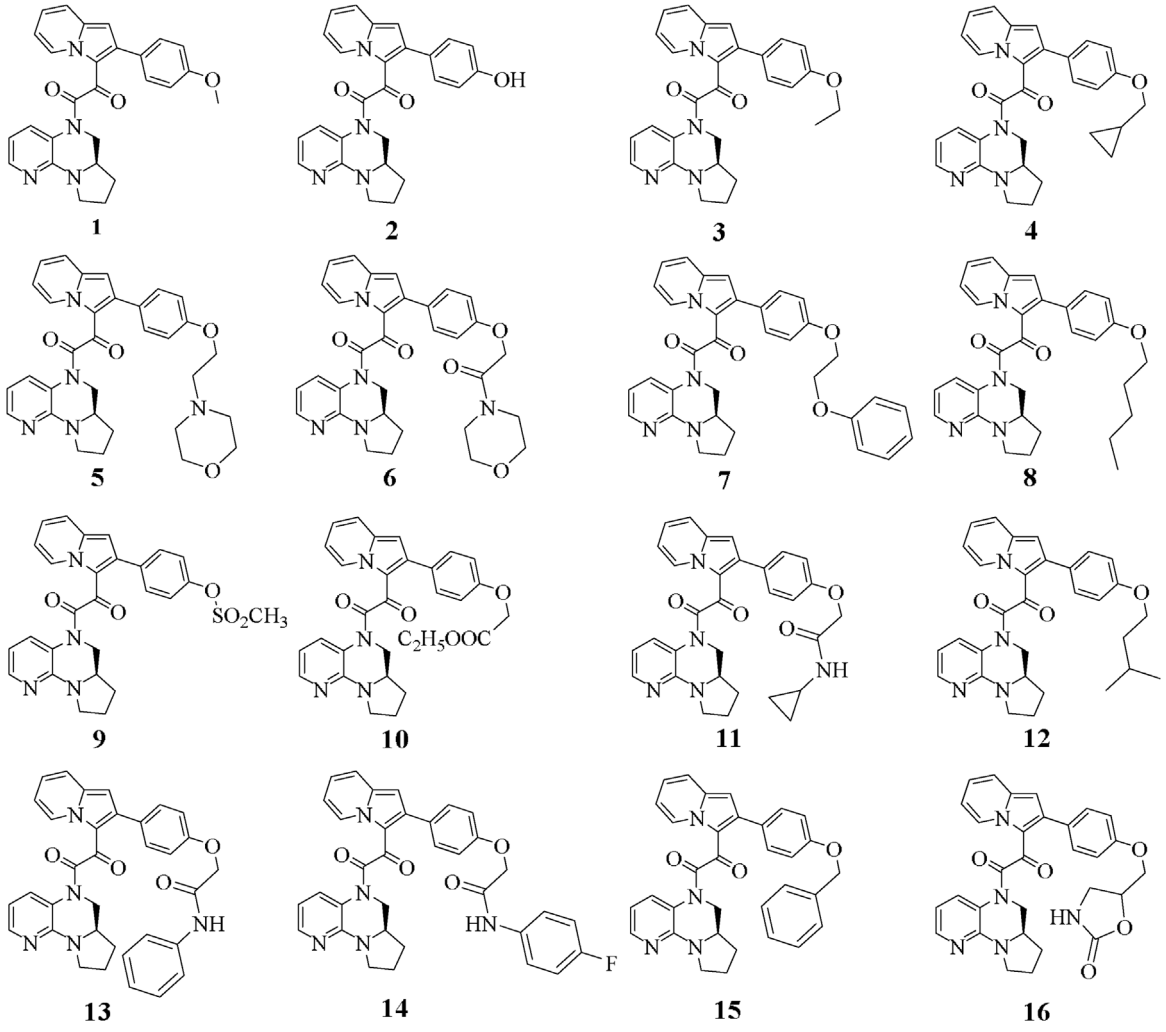

Fig. 1. Structures of pyrrolo[1,2-*a*]pyrazine incorporated indolizine derivatives **(1–16).**

experiment was triplicated and the data was data was expressed as percentage inhibition.

Percentage inhibition (%) = $(A_c - A_s)/A_c \times 100$

where A_c is the absorbance of the control. A_s is the absorbance of sample.

α-Glucosidase inhibitory assay

The assay of α-glucosidase inhibitory activity was estimated by using modified procedures of Shind group [29] in triplet (n = 3). 2.0 μL of α-glucosidase from rat intestine acetone powder solution (a stock solution of 1.0 mg/mL in 10 mM phosphate buffer, pH 6.8, diluted 40-fold with same buffer) was mixed with 20 μL of the samples at different concentrations (25, 50, 75 and 100 μL) and added 100 μL of 50 mM phosphate buffer (pH 6.8) in 96 well microplate and incubated for 5 min at 37 °C. After incubation, 50 μL of substrate (5 mM of *p*-nitrophenyl-α-D-glucopyranoside prepared in 50 mM of phosphate buffer, pH 6.8) were added and the entire reaction mixture was again incubated for 20 min at 37 °C. Thereafter the reaction was terminated by adding 50 μL of Na_2CO_3 (1 M) and made up the final volume to 150 μL. The amount of *p*-nitrophenol released from substrate was noted at 405 nm spectrophotometrically (Spectra MAX plus 384, Molecular Devices Corporation, Sunnyvale, CA, USA). DMSO and acarbose were used as control and standard, respectively. The percentage of enzyme inhibition was calculated by using below formula

Percentage of inhibition (%) = (C–S)/C × 100

where C is the absorbance of the control, S is the absorbance of sample. IC_{50} values of the samples were determined by plotting percentage inhibition against concentrations.

Animals

A total of 210 healthy albino wistar rats weighing between 180–200 g of either sex were maintained on standard rat feed were utilized for this study. The research experiments were approved and conducted according to the Organization for Economic Co-operation and Development (OECD) guidelines and regulations of Institutional Ethical Committee (Regd No. 516/PO/c/01/CPCSEA). For ethical reasons, each animal was used only once and all animals were sacrificed at the end of the study.

Toxicity studies

The toxicity of indolizine derivatives were performed as per the OECD guidelines. The male albino rats were divided into four groups (n = 6), were allowed free access to water and diet under room temperature for one week before the experiment. The compound **1** at 100, 200, 500 and 1000 mg/kg body weight (b.w.) were administered orally and the rats were observed at regular intervals of time (1, 2, 4, 8, 12 and 24 h) for aggressiveness, morbidity, oral secretions, respiratory movements, sensitivity and their mortality [30].

Hypoglycemic activity

Effect on normal or oral glucose loaded rats

The hypoglycemic activity was performed by using oral glucose tolerance (OGT) test [31]. The plasma blood glucose levels of overnight fasted normal animals were noted using *i.e.* tail vein puncture and glucometer method [32]. These animals were grouped (n = 6) orderly and treated with control (only 0.5% CMC in distilled water), synthesized compounds (5 and 10 mg/kg b.w.) and glibenclamide (standard, 10 mg/kg b.w), which were deliquesced in 0.5% carboxymethylcellulose (CMC) in distilled water. By tail vein puncture method, the blood glucose levels were determined at 0, 30 and 60 min. After obtaining the reading at 60 min, all the animals were administered orally 15% glucose solution in distilled water (1.5 g/kg) using polyethylene gastric tube, then the blood glucose levels were noted at 120 and 240 min using above method.

Anti-hyperglycemic activity

Induction of diabetes

Diabetes was inducted to the animals (overnight fasted) by injected intraperitoneally the freshly prepared solution of Streptozotocin (STZ, 55 mg/kg b.w.) in 0.1 M cold citrate buffer (pH 4.5). In order to overcome drug induced hypoglycemia the animals were allowed free access to drink 5% glucose solution overnight. The control group animals received only citrate buffer. The blood glucose values of induced animals were monitored for three successive days after STZ injection. The animals with above 250 mg/dL blood glucose values on third day were considered as diabetic and used for the treatment from fourth day onwards [33]. Only 4% animals did not develop diabetes by this induction method.

Antidiabetic activity

The diabetic animals were overnight fasted and treated with samples for one day and blood glucose levels were deliberated by using tail vein puncture and glucometer at regular intervals of time *i.e.* 0, 30, 60, 120 and 240 min. Sample - the selected compound (5 and 10 mg/kg b.w.) were deliquesced in 0.5% CMC in distilled water and administered orally to diabetic animals which were divided into group containing animals each (n = 6). First and second groups served as normal control and diabetic control, respectively, dosed with 0.5% CMC; third for standard, remaining for selected compounds. The percentage reduction of plasma blood glucose was calculated using the below formula.

% reduction in glycaemia = $(G_0 - G_x)/G_0 \times 100$

Where G_0 is initial glycaemia (0 h); G_x is glycaemia at 30, 60, 120, 240 and 360 min.

The best activity obtained compound was further studied for 21 days of *in vivo* antidiabetic activity. During 21 days of antidiabetic therapy, the blood glucose levels were scrutinized on fasting, 1, 7, 14 and 21 day. The effect of test samples on body weight of animals also monitored on these days. On 22th day, blood samples were collected from animals by puncturing of retro-orbital plexus under anesthesia (diethyl ether). These blood samples used for estimation of plasma total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), and low density lipoprotein (LDL) using Randox diagnostic kits [34]. The absorbance was determined and calculated using fully smart semiautomated analyzer and finally animals were sacrificed and then kidneys, pancreas, liver and testes were detached for measurement of lipid peroxidation (TBARS) and histopathological studies.

Lipid peroxidation (TBARS) in tissues

The isolated liver and kidneys were immediately excised and washed with 0.9% NaCl, wet tissue homogenized in 4.5 mL of 0.25 M sucrose using homogenizer. The cytosolic fraction was collected by a two-step centrifugation, first at 1000 × g for 10 min and then at 2000 × g for 30 min at 4 °C A volume of homogenate (0.2 mL) was transferred to a vial and was mixed with 0.2 mL of 8.1% (w/v) sodium dodecyl sulphate solution, 1.5 mL of a 20% acetic

acid solution (pH 3.5) and 1.5 mL of 0.8% (w/v) solution of thiobarbutiric acid and the final volume of solution was adjusted to 4.0 mL with distilled water and then vials were heated in boiling water bath for 60 min. After cooling, equal volumes of tissue blank or test sample and 10% trichloroacetic acid were transferred into a centrifuge tube and centrifuged at 1000 × g for 10 min and the absorbance of the supernatant fraction was measured at 532 nm [35].

Histopathological studies

After the completing the compound treatment for 21 days, the group of animals were anaesthetized by the ether and abdomen of rats was dissected. Then the pancreas and testes were carefully dissected out and were kept in saline and then stored in 10% formalin solution. The organs were sliced to 5 μm thick sections using microtome and stained with hematoxylin and eosin and were used for histopathological examination.

Statistical analysis

The experimental values were calculated by using Prism 5 software given in mean ± SD in compounds, standard and control groups were compared by two-way ANOVA followed by Dunnett's test. The p value less than 0.05 were considered statistically significant.

Results

Antioxidant activity

The free radical-quenching assays of indolizine derivatives **(1–16)** against DPPH and superoxide radicals were illustrated in Fig. 2. The inferior IC_{50} values indicates superior inhibition of free radicals. From the antioxidant results it is confirmed that the compound **1, 2, 3, 6, 8, 9** and **12** depicted to have promising antiradical scavenging capacities.

Among all tested samples, the compound **6** and **12** showed better inhibition of DPPH free radicals with IC_{50} values of 53.5 and 56.5 μg/mL, respectively, whereas standard (ascorbic acid) with 27.0 μg/mL (Fig. 2). Moreover, the compound **1, 2, 3, 5, 7, 8, 9** and **10** depicted significant inhibition of DPPH radicals, while the compound **4, 10, 13, 14, 15** and **16** exhibited mild inhibition against DPPH free radicals (Fig. 2 and Table S1).

In superoxide radical assay, the compound **2** revealed equivalent inhibition as that of the standard drug (ascorbic acid) *i.e.* IC_{50} value of 32.0 μg/mL (Fig. 2). Furthermore, the compounds **1, 3, 6, 8, 9** and **12** showed significant inhibition of superoxide radical, whereas rest indolizine derivatives (*i.e.* compound **4, 5, 7, 10, 11, 13, 14, 15** and **16**) depicted lesser inhibition against superoxide free radicals (Fig. 2 and Table S2).

α-Glucosidase inhibitory assay

The *in vitro* antidiabetic activity was assessed by α-glucosidase inhibitory assay using *p*-nitrophenyl-α-D-glucopyranoside and acarbose as substrate and standard, respectively, and the results as IC_{50} values were presented in Fig. 2. From the assay, it was estimated that the compound **12** and **1** exhibited prominent inhibition of α-glucosidase enzyme with IC_{50} values of 26.5 and 29.2 μg/mL, respectively, whereas standard (acarbose) with 29.5 μg/mL (Fig. 2). In addition, the 50% concentration needed for compound **11, 8, 9, 7, 3, 4, 10, 5, 2** and **6** to inhibit α-glucosidase enzyme were determined to be 34.5, 44.0, 49.0, 71.6, 75.3, 81.0, 85.0, 92.6 and 99.5 μg/mL, respectively (Fig. 2).

Mortality

The acute toxicity studies of compound **1** exhibited no mortality or symptoms of toxicity up to 1000 mg/kg b.w. Hence the LD_{50} value of compound **1** was found to be above 1000 mg/kg b.w. (Table 1). Moreover, based on the Smith model of analysis the dosage of *in vivo* studies for indolizine derivatives were determined as 5 and 10 mg/kg b.w.

Biochemical analysis

Based on the result of antioxidant and α-glucosidase inhibitory assays, we further extended the study to evaluate the *in vivo*

Table 1
Blood glucose levels of STZ-induced diabetic rats administered with compound **12** observed within 21 days of treatment.

Sample	Plasma glucose levels (mg/dL) (% reduction)			
	Day 1	Day 7	Day 14	Day 21
Normal control	82.5 ± 3.50	89.17 ± 3.13 (−8.09%)	93.84 ± 9.26 (−13.75%)	91.67 ± 7.82 **(−11.12%)**
Diabetes control	354.5 ± 4.23	377.84 ± 5.15 (−6.58%)	386.67 ± 8.89 (−9.08%)	403.34 ± 5.19 (−13.78%)
12 (5 mg)	301.5 ± 3.29	270.33 ± 9.83 (+10.34%)	202.84 ± 7.25* (+32.72%)	185.67 ± 7.87* (+38.42%)
12 (10 mg)	325.34 ± 6.83	289.34 ± 9.50 (+11.07%)	215.67 ± 9.70* (+33.71%)	132.17 ± 7.87* **(+59.38%)**
Glibenclamide (10 mg/kg b.w)	338.5 ± 8.53	248.0 ± 10.81* (+26.74%)	161.5 ± 8.31* (+52.29%)	123.34 ± 5.32* **(+63.56%)**

#Values are mean ± SD (n = 6).
* p less than 0.05.

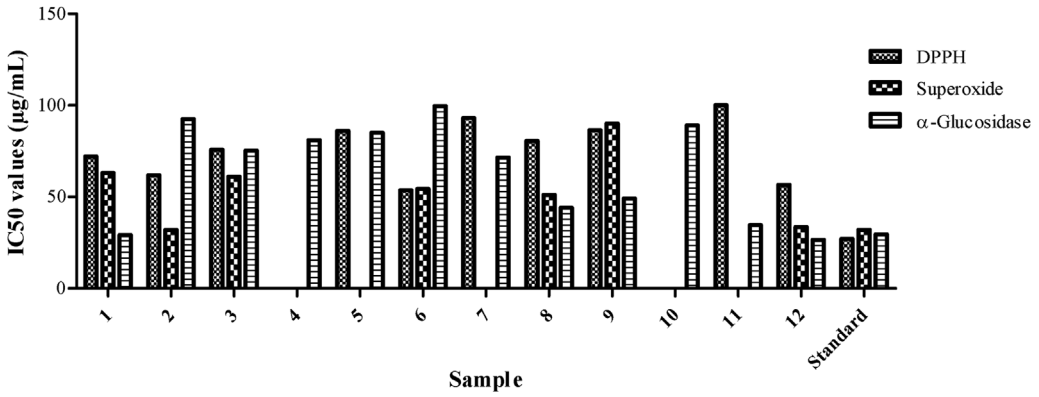

Fig. 2. IC_{50} values of indolizine derivatives against DPPH, superoxide free radicals and α-glucosidase enzyme.

hypoglycemic and anti-hyperglycemic activity for active indolizine derivatives (*i.e.* compounds possess IC_{50} value of below 50 μg/mL) by using a standard protocol of OGT test and STZ-induced diabetes in rats, respectively.

In OGT test, the control group animals (dosed with 0.5% CMC) showed linear levels of blood glucose levels in normal rats till 60 min. Subsequently, the normal rats showed sharp rise in plasma sugar levels due to the administration of 20% glucose solution using polyethylene gastric tube till 240 min (Table S4). In normal rats, the compound **8, 11** and **12** showed better reduction of plasma blood glucose levels at high dosage (Fig. 3). Moreover, the compound **12** (10 mg/kg b.w.) depicted prominent reduction of plasma sugar levels in normal animals with 35.0% at 60 min, which was more than that of the standard (glibenclamide, 10 mg/kg b.w.) with 33.48% at 60 min (Fig. 3).

Similarly, in glucose loaded rats, the compound **8, 11** and **12** showed augment reduction of the blood glucose levels till 240 min with respect to standard, thereafter the animals are relieved from hypoglycemic activity due to the biological degradation and excretion of the samples from the animal body (Fig. 3). Among all the animal groups, the compound **12** (10 mg/kg b.w.) group animals showed 46.03 and 48.62% reduction of plasma glucose levels in glucose loaded rats at 120 and 240 min, respectively, while the standard group animals revealed potent reduction and causes severe hypoglycemic conditions in glucose loaded rats at 240 min (Fig. 3).

In STZ-induced diabetic study, all tested compounds exhibited dose dependent reduction of plasma glucose levels. Among all tested samples, the compound **8, 9, 11** and **12** at 10 mg/kg b.w. depicted significant reduction of blood glucose levels in STZ-induced diabetic rats (Fig. 4). From the Fig. 4 it is interesting to noticed that the compound **12** (10 mg/kg b.w.) showed more pronounced hypoglycemic conditions in STZ-induced diabetic rats than that of standard drug and other compounds **(1, 8, 9** and **11)**. Hence, based on the *in vitro* and *in vivo* studies, it can be concluded that the highly active indolizine derivative *i.e.* compound **12** was selected for 21 days antidiabetic study.

In 21 days STZ-induced diabetes model, it was observed that the normal group animals showed slight variation of blood sugar levels in respective intervals of time *i.e.* 1, 7, 14 and 21 day (Table 1). Besides, the diabetic control animals have radically intensifies their plasma glucose levels from 354.5 ± 4.23 to 403.34 ± 5.19 mg/dL with 13.78% raise at 21 day (Table 1). Further, animals treated with compound **12** at 5 and 10 mg/kg b.w. dosage for three weeks resulted in gradual reduction of plasma sugar levels from 301.5 ± 3.29 to 185.67 ± 7.87 mg/dL and 325.34 ± 6.83 to 132.17 ± 7.87 mg/dL, respectively, with 38.42 and 59.38% reduction of plasma glucose levels, respectively, at 21 day (Table 1). While standard (glibenclamide, 10 mg/kg b.w.) group animals exhibited 63.56% reduction in their blood glucose levels at 21 day (Table 1). From the complete antidiabetic activity analysis, it can be interesting to note that compound **12** at 10 mg/kg b.w. revealed good antidiabetic potentiality with respect to standard and control.

Body weight

During the antidiabetic experimental protocol, all the animal groups were noted for their body weight at 1, 7, 14 and 21 day (Table 2). For these observations, it can be observed that there was a chronological increase in the body weight in the normal control animals (10.43% at 21 day), whereas the diabetic control depicted a drastic decline in their body weight of about 22.26% reduction at 21 day (Table 2). The diabetic animals administered with compound **12** (10 mg/kg b.w.) and standard (glibenclamide, 10 mg/kg b.w.) showed slight reduction in their body weight with 5.36 and 4.54% reduction, respectively, at 21 day, whereas the group treated with compound **12** at 5 mg/kg b.w. exhibited 19.05% reduction in their body weight (Table 2). From these observations it can be concluded that the compound **12** and standard were very active in recovering from diabetic effects in albino rats.

Plasma analysis and TBARS content in tissues

The variations in biochemical parameters (total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL), thiobarbituric acid reactive substances (TBARS) in liver and kidneys) of diabetes in normal, sample treated (compound **12**, 5 and 10 mg/kg b.w.) and standard treated (glibenclamide, 10 mg/kg b.w) rats were estimated and presented in Table 3. The analysis of blood samples of normal, sample treated and standard treated rats' depicted large variation in their levels of TC, TG, LDL and HDL (Table 3). When compared to lower dosage (5 mg/kg b.w.), the compound **12** at higher dosage (10 mg/kg b.w.) exhibited better recovery from TC, TG, LDL and HDL levels with respect to the normal control and diabetic control, whereas the standard treated animals depicted to be almost became normal in all calibrated parameters (Table 3).

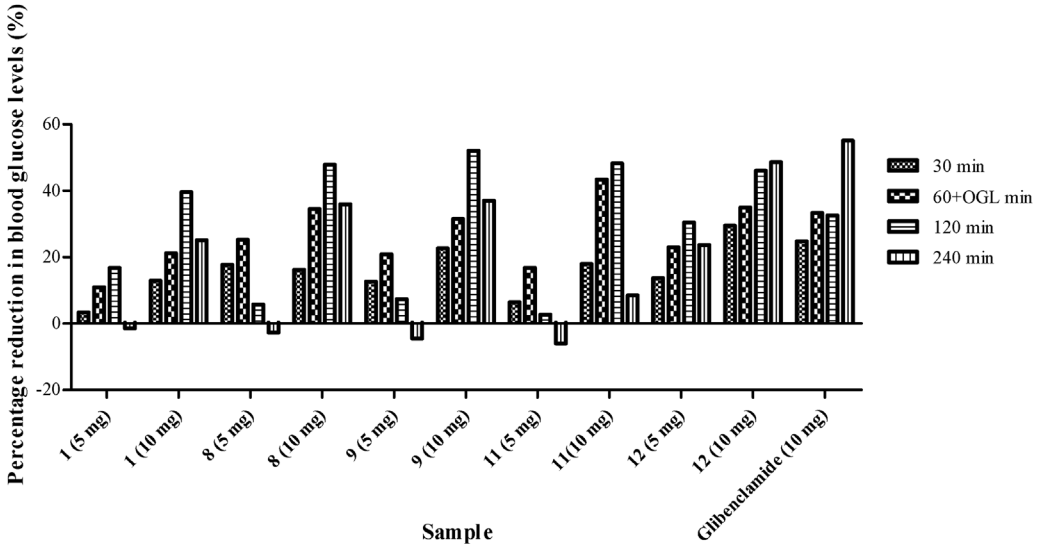

Fig. 3. Dose response effects of indolizine derivatives in normal and glucose loaded albino rats (n = 6).

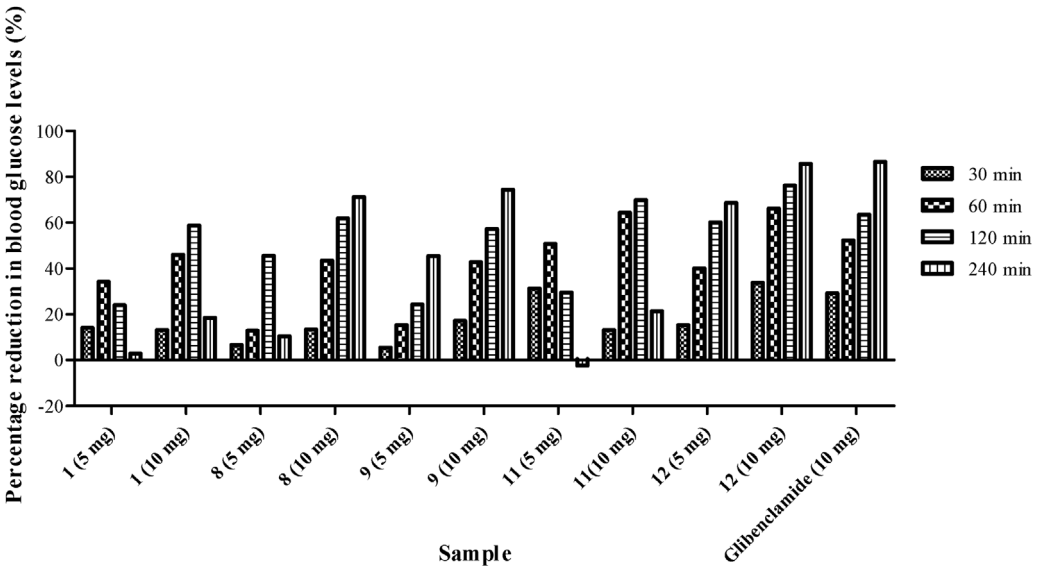

Fig. 4. Dose response effects of indolizine derivatives in STZ-induced diabetes in albino rats (n = 6).

Table 2
Effect of compound **12** on body weight in STZ-induced diabetic rats.

Sample	Body weight[#] (g) (% change)			
	Day 1	Day 7	Day 14	Day 21
Normal control	186.4 ± 7.62	191.6 ± 4.89 (+2.71%)	203.7 ± 5.32 (+8.49%)	208.1 ± 11.67 **(+10.43%)**
Diabetes control	194.4 ± 8.57	189.1 ± 4.89 (−2.80%)	171.3 ± 8.11 (−13.49%)	159.0 ± 7.62 (−22.26%)
12 (5 mg)	191.9 ± 7.26	184.9 ± 5.30 (−3.79%)	173.5 ± 4.20* (−10.61%)	161.2 ± 8.3* (−19.05%)
12 (10 mg)	190.6 ± 4.54	185.1 ± 9.58* (−.97%)	179.2 ± 4.48* (−6.36%)	180.9 ± 4.92* **(−5.36%)**
Glibenclamide (10 mg/kg b.w.)	195.7 ± 7.31	191.1 ± 5.19* (−2.41%)	185.7 ± 10.01* (−5.38%)	187.2 ± 11.8* **(−4.54%)**

[#] Values are mean ± SD (n = 6), data were analyzed using one way ANOVA analysis.
* *p* less than 0.05.

In addition, the higher doses of compound **12** (10 mg/kg b.w.) revealed 30.46 and 5.62% reduction of lipid peroxidation in kidney and liver tissues, respectively, than compound **12** with 5 mg/kg b. w. On the other hand, the standard exhibited 43.05 and 8.11% depletion in TBARS content in kidney and liver, respectively (Table 3).

Histopathological studies

The pancreas and testes of the all treated animals were subjected to histopathological observations. The pancreatic and testes sections were stained with Hematoxylin and Eosin and illustrated in Figs. 5 and 6, respectively.

The histopathological examination of pancreas of control animals showed the well alignment of islets of Langerhans (Fig. 5(A)) and the diabetic animal depicted the degeneration and accumulation of fat in interlobular duct of the pancreas and decline in size and number of acini and islets of Langerhans (Fig. 5(B)). Besides, the standard treated animals revealed great recovery of damaged acini and islet cells (Fig. 5(C)), whereas compound **12** administrated diabetic animals displayed the regeneration of Langerhans islets and acini. Moreover, the decadence of fat from interlobular duct and also a clear reformation of intralobular duct were also observed (Fig. 5(D)). Further, the number of islet cells and their diameter significantly augmented in compound **12** and standard treated animals compared to diabetic group (Fig. 5).

The histopathological slides of control testes revealed the well pattern of presence of spermatogonia and Sertoli cells (Fig. 6(A)), while the diseased testes showed the disorganization of spermatogonia and necrosis of Sertoli cells (Fig. 6(B)). On the other hand, the standard testes displayed clear regeneration and

Table 3
The mean ± SD values (n = 6 per group) for the serum glucose concentrations in diabetic rats due to the effect of compound 2.

Sample	TC (mg/dL)	TG (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	TBARS in μmol/g ± SEM (% change)	
					Kidney	Liver
Normal Control	61.1 ± 1.22	40.5 ± 0.58	28.9 ± 0.34	66.3 ± 0.32	181.5 ± 1.35	341.17 ± 1.40
Diabetic Control	153.3 ± 2.10	176.7 ± 1.24	130.1 ± 0.80	34.9 ± 0.27	350.17 ± 1.05 (+87.80)	391.67 ± 2.62 (−13.98)
12 (5 mg)	105.6 ± 1.09	88.5 ± 0.94	57.4 ± 0.58	49.7 ± 0.18	275.17 ± 2.35 (−18.02)	389.5 ± 2.11 (−2.48)
12 (10 mg)	90.2 ± 0.88	56.8 ± 0.86	41.4 ± 0.28	54.6 ± 0.85	238.17 ± 2.89 **(−30.46)**	353.67 ± 1.45 **(−5.62)**
Glibenclamide (10 mg/kg b.w.)	72.9 ± 1.54	45.2 ± 0.92	34.04 ± 0.78	64.7 ± 0.66	200.67 ± 1.09 **(−43.05)**	349.84 ± 1.09 **(−8.11)**

TC: total cholesterol; TG: triglycerides; HDL: High density lipoprotein; LDL: low density lipoprotein; TBARS: Thiobarbituric acid reactive substances.

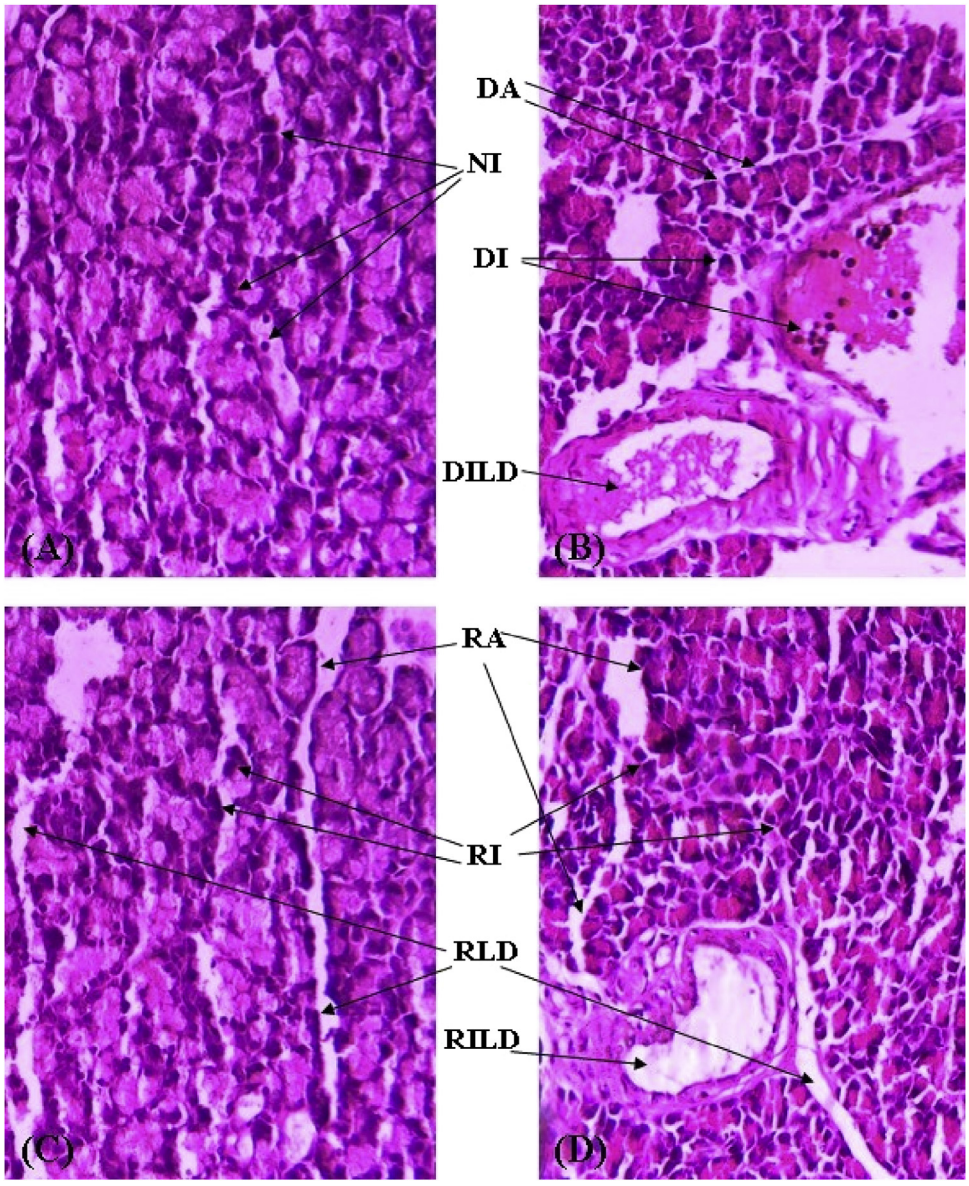

Fig. 5. Histopathological study of pancreatic tissues in rats **(A)** Control pancreas; **(B)** Diseased pancreas; **(C)** Glibenclamide treated pancreas; **(D)** Compound **12** treated pancreas at 10 mg/kg body weight. [DA: damaged acini; DI: damaged islets; DILD: damaged interlobular duct; NI: normal islets; RA: recovered acini; RI: recovered islets; RILD: recovered interlobular duct; RLD: recovered intralobular duct].

disposition of spermatogonia followed with good organization of Sertoli cells (Fig. 6(C)). Similarly, the compound **12** treated animals showed the partial regeneration of spermatogonia and Sertoli cells, furthermore, their arrangement were not properly arrayed (Fig. 6(D)). In addition, it is interesting to notice that the basement membrane of the seminiferous tubule is not effected in all the animal groups (Fig. 6).

Hence, from the observation of biological parameters and histopathological study of pancreas and testes it can be concluded that the pyrrolo[1,2-*a*]pyrazine incorporated indolizine derivatives has a great aptitude to act against type 2 diabetes mellitus.

Discussion

In the present study, the indolizine derivatives showed prominent inhibitory profile against free radicals as well as α-glucosidase enzyme (key intestinal enzyme involved in the digestion of carbohydrates) and most of the derivatives exhibited remarkable protective hypoglycemic effects in normal, glucose loaded and STZ-induced diabetic rats by reducing the plasma glucose levels. Particularly, compound **12** revealed significant increase in the body weight and diminished plasma glucose, metabolic parameters and TBARS content variations in STZ-induced diabetic rats. The histopathological slides showed a clear and well envisions of regeneration of islets of Langerhans and exocrine acini in compound **12** treated pancreas and good arrangement of spermatogonia and sertoli cells in compound **12** treated testes.

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia, which later develops to vascular complications [1,2]. In our study, induction of diabetes in albino rats by single intraperitoneal injection of STZ at 55 mg/kg b.w. and the hypoglycemic and anti-hyperglycemic activity of indolizine derivatives were determined. The plasma blood glucose level was determined in normal, glucose loaded and STZ-induced diabetes rats. After the administration with STZ, the fasting plasma glucose level was significantly increased in the range of 200–275 mg/dL and it was significantly (p less than 0.05) diminished by

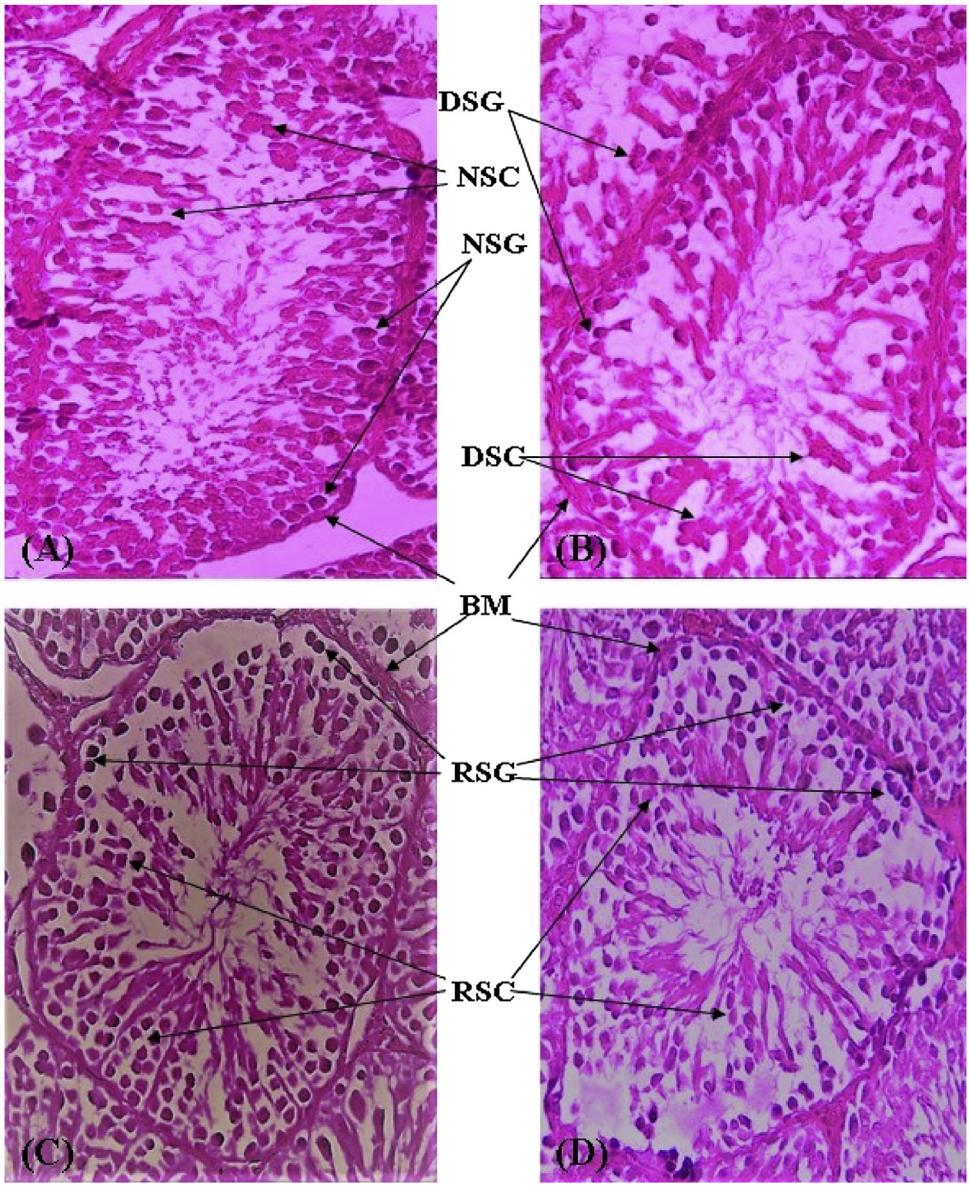

Fig. 6. Histopathological study of testes in albino rats **(A)** Control testes; **(B)** Diseased testes; **(C)** Glibenclamide treated testes; **(D)** Compound **12** treated testes at 10 mg/kg b.w. [BM: basement membrance; DSC: damaged; DSG: damaged spermatogonia; NSC: normal sertoli cells; NSG: normal spermatogonia; RSC: recovered sertoli cells; RSG: recovered spermatogonia].

21 days treatment with compound **12**. On the progression of dosage with compound **12**, fasting plasma glucose reduced from 1 st week. Similarly, in STZ-induced diabetic rats, compound **12** treatment significantly improved the body weight and reduced blood glucose levels. The obtain results is far better than Du and Saha hypoglycemic studies [3]. The authors [3] used their synthesized indolizine derivatives at 250 mg/kg b.w. dose (*i.e.* 25 fold higher), instead of 5 and 10 mg/kg b.w. used in this present study. In addition, the maximum percentage lowering of blood sugar level in normal albino rats was 3 folds lesser than our study. Hence, our novel indolizine derivatives are very active oral hypoglycemic agents than earlier reports.

Generally, β-cells are the most abundant cell type in endocrine pancreas. Their number is the most essential factor that determines islet of Langerhans area. During diabetes, there is rupture of islet of Langerhans and decreased in β-cells of pancreas [36]. Similarly, the experimental diabetes induction in rats using diabetogenic agents like STZ, will selectively damages the pancreatic β-cells by reactive oxygen species. Hence, the administration of STZ to normal albino rats result in the destruction/necrosis of pancreatic β-cells of islets of Langerhans and also malfunctioning of the pancreas, eventually results in this diabetic condition leading to the elevation in the plasma glucose levels (due to lowering of plasma insulin) and depletion of body weight in the untreated diabetic albino rats. The concurrent supplementation with compound **12** and glibenclamide to the diabetic rats for 21 days significantly lowered blood glucose levels most probably due to improvement in the pancreatic secretion of insulin from β-cells of islets of Langerhans. The elevated levels of insulin and restoration of pancreatic damage may be improved control over the toxic activity of free radicals and α-glucosidase inhibitory profile by compound **12** treated diabetic rats. In addition, glycation reaction in diabetes occurs in several tissues including β-cells. The activity of antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase which is low in islets of Langerhans cells when compared to other tissues under diabetes [37]. Glycation mediated reactive oxygen species leads to necrosis of pancreatic β-cells and reduced insulin gene

transcription [37,38]. The administration of diabetic rats with compound **12** prevents the glycation process by its antidiabetic activity and this it protects the pancreatic β-cells from hyperglycemia induced injuries.

Similarly, the reproductive tract functions and sexual behavior are also affected by diabetes mellitus which eventually decrease fertility [39]. During diabetes there is disruption of glucose transport from the blood to testicular germ cells through blood-testes-barrier, which mainly composed of Sertoli cells [40]. The impairment of glucose transport is followed by accumulation of free radicals and oxidative stress, which result in higher percentage of spermatic cellular death [41]. In the present study, the STZ-induced diabetic rats caused distortion and reduction of diameter of seminiferous tubules and Steroli cells. In addition, there was also increases in the thickness of basal lamina in somniferous tubules which accompanies reduction of total size of somniferous tubules may be due to increased testicular oxidative stress markers and lipid peroxidation content. The concurrent supplementation with compound **12** and glibenclamide to the diabetic rats for 21 days succeeded in restoring of testicular structure to normal and regeneration of spermatogonia and Steroli cells. This was attained due to reduction of lipid peroxidation content as well as down regulation of the oxidative stress markers.

Hence, the indolizine derivatives have the tissue repairing potential from diabetic related complications and its action is much comparable with the glibenclamide (standard drug). The improved glycemic control, metabolic parameters and antioxidant potential of indolizine derivatives showed its tissue repair capability from diabetes.

To conclude, the experimental findings suggested that the administration of pyrrolo[1,2-*a*]pyrazine incorporated indolizine derivatives at a safe dose, suppresses free radicals and α-glucosidase enzyme along with STZ-induced diabetes in albino rats. Furthermore, the free alkyl indolizine derivatives (compound **8** and **12**) showed more pronounced antidiabetic activities than any other substituents. Thus, the observations of the histopathological study revealed improved effect on regeneration of islets of Langerhans, spermatogonia and Sertoli cells and from the outcomes, it elucidated the biological importance of pyrrolo[1,2-*a*] pyrazine incorporated indolizine derivatives in the production of novel agents which impetus in the treatment of oxidative stress conditions.

Conflict of interest

No financial support or relationship with any organization to carry out this research. The authors declare that they have no conflict of interest.

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