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Antihyperglycemic and antidiabetic effects of Ethyl (S)-2-(1-cyclohexylsulfamide carbamoyloxy) propanoate in streptozotocin-induced diabetic Wistar rats

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ABSTRACT

In this study, we examined the antihyperglycemic and antidiabetic effects of a novel synthesized molecule, the Ethyl (S)-2-(1-cyclohexylsulfamide carbamoyloxy) propanoate (ESP1b), in streptozotocin (STZ)-induced diabetic Wistar rats. Experimental diabetes mellitus was produced by a single intraperitoneal injection of STZ (55 mg/kg b.w.). Seven day post-injection, animals have received ESP1b orally at the doses of 5, 10 and 20 mg/kg b.w. daily for 28 days. This resulted in a clear decline, in a dose dependent manner, of blood glucose levels during the oral glucose tolerance test (OGTT) and the four weeks of treatment period. ESP1b at 20 mg/kg b.w. has alleviated body weight loss, improved plasma insulin concentration and at the same time markedly decreased the values of glycosylated hemoglobin, lipoproteins and atherogenic ratios. Additionally, ESP1b notably restored renal as well as hepatic functions tests. Histopathological examinations of pancreatic tissue also confirmed the previous biochemical findings. Considering the obtained results, it may be concluded that ESP1b possess a potent antihyperglycemic activity in STZ-diabetic rats possibly related to an insulin-secretagogue effect, which may be responsible for the moderate decrease in blood glucose concentration observed in normal rats administrated with this tested compound.

1. Introduction

Diabetes mellitus (DM) at present is a major health problem and one of the most common endocrine diseases in the world. According to the recent global estimates of diabetes prevalence by the International Diabetes Federation (IDF), approximately 382 million people were suffering from DM in 2013; and is expected that this figure will rise to over 592 million by the year 2035 (Guariguata et al., 2014).

DM is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolisms, resulting from defective insulin secretion, resistance to insulin action, or both (American Diabetes Association, 2014). The persistent hyperglycemia in uncontrolled DM is associated with various micro- and macrovascular complications such as retinopathy, neuropathy, nephropathy, cardiomyopathy and foot amputation, which have substantial clinical impact, in terms of mortality, morbidity and quality of life (Holstein and Beil, 2009).

Several pharmacological approaches are employed in managing DM, such as the use of insulin therapy and different classes of oral antidiabetic drugs, which include agents that stimulate insulin secretion (Sulfonylureas and Short-acting insulin secretagogues), reduce hepatic glucose production (Biguanides), delay digestion and absorption of intestinal carbohydrates (Alpha-glucosidase inhibitors) or improve insulin action (Glitazones) (Cavallo Perin and Fornengo, 2011). All these therapeutic agents are often associated with certain adverse effects like causing hypoglycemia at higher doses, liver toxicity, lactic acidosis and diarrhea (Williams, 1994), or a decrease in response after prolonged use. Therefore, there is a need for novel drugs that have effective antidiabetic potential at low dose without undesirable side effects (Nathan et al., 2009). In this regard, the present study has been undertaken to investigate the antihyperglycemic and antidiabetic properties of, a tested compound, the Ethyl (S)-2-(1-cyclohexylsulfamide carbamoyloxy)

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propanoate (ESP1b) in experimental animal model of diabetes induced by a chemical toxin, the Streptozotocin (STZ).

STZ or 2-deoxy-2-({[methyl(nitroso)amino]carbonyl}amino)-β-D-glucopyranose is a broad spectrum antibiotic that was first isolated from *Streptomyces achromogenes* fermentations (Herr et al., 1960; Vavra et al., 1960). During the studies of preclinical pharmacology, it was soon noticed that STZ possess a diabetogenic action in rats and dogs, which is mediated by pancreatic β-cells destruction through necrosis (Rakieten et al., 1963). Although the exact mechanism of the cytotoxic effect of STZ has not been fully elucidated, it has generally been assumed that the selective uptake of this glucose toxic analog via the glucose transporter 2 (GLUT2) into the β-cell and subsequent cellular destruction through DNA fragmentation, as a result of the DNA alkylating ability of its methylnitrosourea moiety, are the main reason of the STZ diabetogenicity (Elsner et al., 2000). However, STZ was found to generate reactive oxygen species (Takasu et al., 1991) and to act as an intracellular nitric oxide donor (Turk et al., 1993). As a consequence, it was suggested that synergistic action of both reactive oxygen species and nitric oxide may also contribute to DNA damage and other deleterious changes caused by this toxin (Szkudelski, 2001).

2. Materials and methods

2.1. Chemicals

STZ was purchased from Sigma-Aldrich, USA. Glibenclamide (GLB) was obtained from Sanofi, Algeria. The all other chemicals and reagents used were of analytical grade procured from Sigma-Aldrich or Merck-Millipore, France.

2.2. Synthesis of the novel compound ESP1b

ESP1b (Fig. 1) was synthesized by our Laboratory of Applied Organic Chemistry, as part of a research to develop a general and mild two steps approach (carbamoylation and sulfamoylation) for the synthesis of alkyl or aryl sulfonamides. Synthetic route for the preparation of this compound was already described in detail in a prior paper (Cheloufi et al., 2014). Briefly, ESP1b and a series of other novel sulfonylureas and N-acylsulfonamides derivatives were prepared firstly using a reaction of chlorosulfonyl isocyanates (CSI) with ethyl lactate in the presence of an excess of triethylamine (TEA), which results in the formation of a corresponding N-chlorosulfonyl-carbamate (CSC), then, in a second step, carbamate reacted with primary or secondary amines in presence of TEA. Mixtures of obtained products were purified by column chromatography on silica gel using anhydrous dichloromethane (CH_2Cl_2) as eluent to give 70% of N-acylsulfonamides and 20% of sulfonylureas. Structures of all obtained compounds were unambiguously confirmed by Nuclear magnetic resonance (^1H and ^{13}C NMR) and High-resolution mass spectrometry (HRMS).

2.3. Animals

Male *Wistar* strain rats weighing about 180–220 g procured from the Pasteur Institute of Algeria, were used for the present investigations. These animals were fed with a standard pellet rat's diet (ONAB, Bejaia, Algeria), supplied with normal drinking water *ad libitum* and acclimatized to laboratory conditions for 2 weeks, prior to experiments, at 23 ± 2 °C and 45 ± 10% relative humidity with a natural light/dark cycle. The experimental protocol was conducted in strict accordance with the current animal ethical norms approved by the Badji Mokhtar-Annaba University.

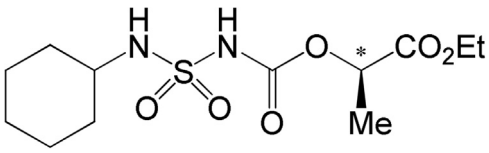

Fig. 1. Chemical structure of Ethyl (S)-2-(1-cyclohexylsulfamide carbamoyloxy) propanoate.

2.4. Induction of experimental diabetes

After an overnight fast, the animals were rendered diabetic by a single intraperitoneal injection of 55 mg/kg body weight of freshly dissolved STZ in 0.1 M citrate buffer (pH 4.5) (Altan et al., 1997). Since STZ administration is capable to produce fatal hypoglycemia as a result of massive release of pancreatic insulin, the rats were supplied, 6 h after the STZ injection, with 5% glucose solution for the next 48 h to prevent this reactive hypoglycemia (Ramachandran et al., 2004). Seven day post-injection, animals having glycosuria with hyperglycemia (i.e. blood glucose levels greater than 300 mg/dl) were considered diabetic (Ahmed et al., 1998) and selected for the experiment.

2.5. Experimental protocol

In present study, we have used 12 normal rats (Non-treated with the STZ) and 30 STZ-induced diabetic rats. These animals were divided into seven groups (n=6/each group) and treated orally once a day for 28 days as detailed follows: (1) NC**:** Normal control rats received a 0.5 ml of physiological saline (0.9% NaCl solution), (2) N+ESP1b 20 mg: Normal rats treated with ESP1b at 20 mg/kg b.w. dissolved in 0.5 ml of saline, (3) DC: STZ-induced diabetic control rats received a 0.5 ml of saline, (4) D+ESP1b 5 mg: STZ-induced diabetic rats treated with ESP1b at 5 mg/kg b.w. dissolved in 0.5 ml of saline, (5) D+ESP1b 10 mg: STZ-induced diabetic rats treated with ESP1b at 10 mg/kg b.w. dissolved in 0.5 ml of saline, (6) D+ESP1b 20 mg: STZ-induced diabetic rats treated with ESP1b at 20 mg/kg b.w. dissolved in 0.5 ml of saline, (7) D+GLB: STZ-induced diabetic rats treated with the well known antidiabetic sulfonylurea drug, glibenclamide, at the dose of 600 μg/kg b.w. (Subash-Babu et al., 2008) dissolved in 0.5 ml saline.

During the treatment period, the levels of fasting blood glucose (FBG) in the normal and streptozotocin-induced diabetic rats were estimated at the 1st, 3rd, 7th, 14th, 21th and 28th day by the method of Trinder (1969) using glucose oxidase-peroxidase reactive strips and ACCU-CHEK® Active glucometer (Roche diagnostics, France) on blood samples collected via tail vein by excision. The initial and final body weights of all animal groups were also recorded. At the end of the experiment, the rats were deprived of food overnight and then killed by cervical decapitation. Whole blood from each animal was collected into ethylene diamine tetra acetic acid (EDTA) containing tubes for the determination of total and glycosylated hemoglobin, whereas, fasting plasma samples were separated for the estimation of insulin, triglycerides, total cholesterol, high density lipoprotein cholesterol, total protein, albumin, hepatic and renal dysfunction parameters.

2.6. Oral glucose tolerance test

On the 12th day of treatment period, animals were subjected to an oral glucose tolerance test (OGTT). After overnight fasting (16 h), a baseline (t=0 min) blood sample was taken from rats in normal and diabetic groups. Without delay, a load of glucose (2 g/kg b.w.) was administered by gavage. Four more samples were also withdrawn from the tip of the tail at 30, 60, 90 and 120 min interval for the estimation of blood glucose levels by the method mentioned above.

2.7. Estimation of plasma insulin, total and glycosylated hemoglobin

Plasma insulin was assayed with an electrochemiluminescence immunoassay (ECLIA) (Sapin et al., 2001) using a Cobas e 411 Analyzer (Roche Diagnostics, Mannheim, Germany). Total hemoglobin (Hb) was estimated with a COULTER® HmX Hematology Analyzer (Beckman Coulter, Inc. Villepinte, France). Glycosylated hemoglobin (HbA1c) was determined by an ion-exchange high-performance liquid chromatography (HPLC) technique using a Bio-Rad D-10™ Analyzer (Bio-Rad, Marnes-la Coquette, France).

2.8. Determination of lipid profile

Triglycerides (TG) concentration was determined by GPO-POD enzymatic-colorimetric method (Fossati and Prencipe, 1982). Total cholesterol (TC) level was measured by the end point, CHOD-POD colorimetric-enzymatic method (Allain et al., 1974). High density lipoprotein cholesterol (HDL-C) was assayed by the method of precipitation with sodium phosphotungstate-magnesium (Grove, 1979). Very low density lipoprotein cholesterol (VLDL-C) and low density lipoprotein cholesterol (LDL-C) in plasma were calculated by the **Friedewald formula** (Friedewald et al., 1972); **VLDL-C=TG/5** and **LDL-C=total cholesterol−(HDL-C+VLDL-C)**, respectively. The atherogenic ratios such as TC/HDL-C and LDL-C/HDL-C were also calculated.

2.9. Measurement of total protein, albumin and renal dysfunction parameters

Total protein (TP) content was measured by the method of Bradford (1976) using bovine serum albumin (BSA) as a standard. Albumin concentration was estimated by the colorimetric method with Bromocresol green (BCG) (Doumas et al., 1971). Urea level was assayed by the UV kinetic method with Urease-GLDH (Talke and Schubert, 1965). Creatinine level was measured by the colorimetric-kinetic method of Jaffé (1886). Uric acid concentration was determined by the colorimetric-enzymatic method with Uricase-POD (Fossati et al., 1980).

2.10. Assessments of plasma enzymes activities and hepatic dysfunction parameters

Aspartate aminotransferase (AST; EC 2.6.1.1) and Alanine aminotransferase (ALT; EC 2.6.1.2) activities were estimated by the UV enzymatic-Kinetic methods (Bergmeyer et al., 1978). Alkaline phosphatase (ALP; EC 3.1.3.1) and Lactate dehydrogenase (LDH; EC 1.1.1.27) activities were determined by the Kinetic methods recommended by the German society of clinical chemistry (Deutschen Gesellschaft für Klinische Chemie, 1972). Gamma-glutamyl transpeptidase (GGT; EC 2.3.2.2) was determined according to the kinetic photometric method of Szasz (1969). Total and direct bilirubin (TB, DB) levels were estimated by the colorimetric method with dimethylsulfoxide (DMSO) (Malloy and Evelyn, 1937).

2.11. Histopathological study

Pancreatic tissue was dissected out, washed in an ice-cold physiological saline, fixed in a 10% buffered neutral formalin solution, dehydrated in graded ethanol solutions (70–100%) and embedded into paraffin blocks. Then the tissue was sliced into sections of 5 μm thickness by a rotator microtome and stained with hematoxylin-eosin (H&E). Obtained sections were examined under a light microscope, and photomicrographs were taken.

2.12. Statistical analysis

Results were expressed as mean ± S.E.M. (standard error of the mean) for six rats in each group. Statistical analysis was performed using MINITAB software package Version 13.4. The means of experimental groups were compared vs controls means by one-way analysis of variance (ANOVA) followed by Dennett's multiple comparison test. Differences were considered statistically significant at the level of $P < 0.05$.

3. Results

3.1. Blood glucose levels during the oral glucose tolerance test

Fig. 2 illustrates the results of the treatment with ESP1b on blood glucose levels during the OGTT test in normal and streptozotocin-induced diabetic rats. A clear difference in baseline fasting blood glucose between the normal and STZ induced-diabetic groups was noted. After glucose loading, the level of blood glucose in both normal control rats and normal rats treated with ESP1b at 20 mg/kg b.w. showed a high peak value at 30 min and then decreased to near normal values at 120 min. The decline rate of blood glucose was faster in normal animals treated with ESP1b than the normal control animals. In STZ induced-diabetic control rats, the blood glucose levels reached the peak value at 60 min and then started to decrease but remained higher, even after 120 min. A high peak value at 30 min was also observed in diabetic rats treated with the different doses of ESP1b and then a decline in a dose-dependent manner was remarked at 60 and 90 min. At the end of oral glucose tolerance test, the ESP1b at a dose of 20 mg/kg b.w. was as effective as the GLB (600 μg/kg b.w.) in reducing blood glucose levels.

3.2. Fasting blood glucose levels during the treatment period

During the 4 weeks of treatment period with ESP1b and GLB, Fasting blood glucose levels were estimated in normal and experimental rats on 1st, 3rd, 7th, 14th, 21th and 28th day. Our

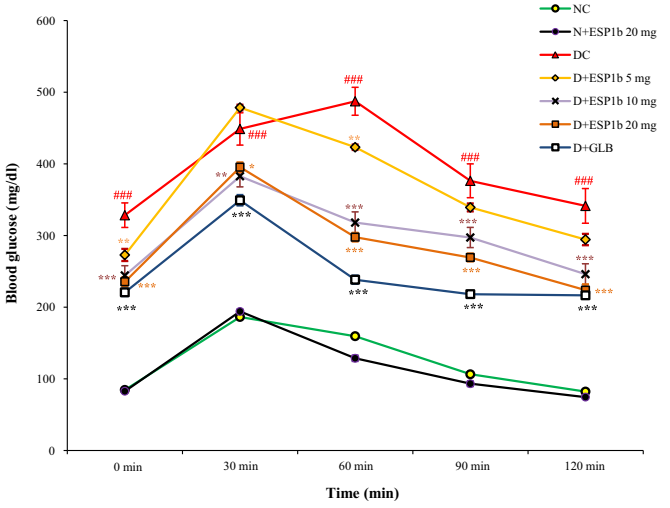

Fig. 2. Effect of ESP1b on blood glucose levels during the oral glucose tolerance test in normal and streptozotocin-induced diabetic Wistar rats. NC, Normal control rats; N+ESP1b 20 mg, Normal rats treated with high dose of ESP1b (20 mg/kg b.w./day); DC, STZ-induced diabetic control rats; D+ESP1b 5 mg, STZ-induced diabetic rats treated with low dose of ESP1b (5 mg/kg b.w./day); D+ESP1b 10 mg, STZ-induced diabetic rats treated with medium dose of ESP1b (10 mg/kg b.w./day); D+ESP1b 20 mg, STZ-induced diabetic rats treated with high dose of ESP1b (20 mg/kg b.w./day); and D+GLB, STZ-induced diabetic rats treated with glibenclamide (600 μg/kg b.w./day). Values are mean ± S.E.M. (n=6). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ compared to NC group. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to DC group.

Table 1
Effect of ESP1b on fasting blood glucose levels during the 4 weeks of treatment period in normal and streptozotocin-induced diabetic Wistar rats.

Groups	Fasting blood glucose levels (mg/dl)					
	1st day	3rd day	7th day	14th day	21th day	28th day
NC	89.33 ± 1.73	87.67 ± 1.15	84.50 ± 1.52	86.00 ± 2.03	89.67 ± 1.28	88.17 ± 1.49
N+ESP1b 20 mg	81.67 ± 2.43	80.33 ± 2.67	82.67 ± 1.15	80.50 ± 1.43	78.00 ± 1.57	73.83 ± 1.19
DC	332.17 ± 8.81^{c}	345.00 ± 13.5^{c}	360.50 ± 15.7^{c}	399.20 ± 21.2^{c}	384.80 ± 25.6^{c}	439.70 ± 17.0^{c}
D+ESP1b 5 mg	329.33 ± 7.26^{c}	341.67 ± 7.86^{c}	322.83 ± 8.45^{c}	254.00 ± 8.26^{c,f}	216,17 ± 4.27^{c,f}	174.50 ± 5.29^{c,f}
D+ESP1b 10 mg	340.83 ± 5.52^{c}	334.50 ± 9.83^{c}	264.20 ± 13.6^{c,f}	225.80 ± 15.3^{c,f}	177.30 ± 13.3^{c,f}	156.17 ± 6.69^{c,f}
D+ESP1b 20 mg	338.00 ± 7.77^{c}	295.67 ± 5.11^{c,e}	242.33 ± 6.38^{c,f}	181.67 ± 8.11^{c,f}	149.00 ± 4.81^{b,f}	130.67 ± 2.96^{b,f}
D+GLB	336.50 ± 4.06^{c}	268.00 ± 7.89^{c,f}	225.67 ± 5.20^{c,f}	156.17 ± 7.23^{c,f}	134.50 ± 5.16^{b,f}	119.33 ± 3.21^{a,f}

NC, Normal control rats; N+ESP1b 20 mg, Normal rats treated with high dose of ESP1b (20 mg/kg b.w./day); DC, STZ-induced diabetic control rats; D+ESP1b 5 mg, STZ-induced diabetic rats treated with low dose of ESP1b (5 mg/kg b.w./day); D+ESP1b 10 mg, STZ-induced diabetic rats treated with medium dose of ESP1b (10 mg/kg b.w./day); D+ESP1b 20 mg, STZ-induced diabetic rats treated with high dose of ESP1b (20 mg/kg b.w./day); and D+GLB, STZ-induced diabetic rats treated with glibenclamide (600 μg/kg b.w./day). Values are mean ± S.E.M. (n=6). $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$ compared to NC group. $^{d}P<0.05$, $^{e}P<0.01$, $^{f}P<0.001$ compared to DC group.

results (Table 1) showed that STZ injection in rats leads to a significant increase ($P<0.001$) in FBG level when compared to normal controls. A clear reduction in FBG levels was observed from the 3rd day after the treatment with the ESP1b ($P<0.01$) at 20 mg/kg b.w. and GLB ($P<0.001$) when compared to STZ induced-diabetic control values. Highest percentage decreases of FBG levels by 64.53%, 61.34%, 54.17% and 47.01% have been noted at the end of experiment for the treatments, with GLB, ESP1b at 20, 10 and 5 mg/kg b.w., respectively. The more efficient glucose lowering effect of ESP1b was remarked at the dose of 20 mg/kg b.w. than the other two doses.

3.3. Body weight, plasma insulin, total and glycosylated hemoglobin

Table 2 illustrates the effect of ESP1b on body weight, plasma insulin, total and glycosylated hemoglobin in normal and experimental rats. Oral gavage of normal animals with ESP1b had not produced any significant changes in body weight and total hemoglobin level, whereas a significant decrease ($P<0.001$) in glycosylated hemoglobin by 18.81% and a significant rise ($P<0.05$) in plasma insulin by 11.32% were observed in comparison with normal control group. STZ-induced DM significantly ($P<0.001$) decreased the body weight (28.39%), plasma insulin (44.27%), and total hemoglobin (26.38%) and increased glycosylated hemoglobin (66.84%) when compared to normal control animals. Oral administration of ESP1b at a dose of 20 mg/kg b.w. to STZ-induced diabetic animals significantly ($P<0.001$) increased the body weight (13.99%), plasma insulin (54.37%), and total hemoglobin level (15.26%) and decreased glycosylated hemoglobin (31.47%) when compared to STZ-induced diabetic control rats.

3.4. Plasma lipid profile and atherogenic ratios

Our results showed that treatment of normal rats with ESP1b had not involved any significant changes in the levels of plasma lipids and atherogenic ratios in comparison with normal control levels (Table 3). Diabetic control rats showed significantly ($P<0.001$) higher levels of TG, total, LDL and VLDL cholesterol associated with lower levels of HDL cholesterol, moreover, a significant ($P<0.001$) elevation in the atherogenic ratios was noted in this group, compared to normal control ratios. Treating diabetic animals with ESP1b returned back the levels of TG, HDL and VLDL cholesterol to near normal values and significantly ($P<0.001$) reduced the concentration of total and LDL cholesterol when compared to diabetic control values. Treatment with ESP1b also significantly ($P<0.001$) decreased the atherogenic ratios in diabetic rats (Table 3).

3.5. Total protein, albumin, creatinine, urea and uric acid

Changes in total protein, albumin, creatinine, urea and uric acid concentrations in normal and STZ-induced diabetic rats are listed in Table 4. Untreated diabetic rats exhibited significant ($P<0.001$) decreases of total protein and albumin concentrations by 24.56% and 23.87%. However, this group show significant ($P<0.001$) increases in creatinine, urea and uric acid levels by 92.85%, 76.42% and 66.95% respectively when compared to the values of normal control animals. Treating diabetic rats with ESP1b significantly ($P<0.001$) raised the levels of total protein and albumin when compared to diabetic control group, whereas, it reduced the concentration of creatinine, urea and uric acid to near normal control

Table 2
Effect of ESP1b on body weight, plasma insulin, total and glycosylated hemoglobin in normal and streptozotocin-induced diabetic Wistar rats.

Groups	Body weight (g/day)			Hb (g/dl)	HbA1C (%Hb)	Insulin (U/l)
	Initial	Final	Difference between 1st and 28th day (%)			
NC	202.53 ± 4.31	226.05 ± 3.20^{h}	11.61	13.53 ± 0.18	5.58 ± 0.06	14.75 ± 0.10
N+ESP1b 20 mg	198.08 ± 3.03	232.48 ± 2.15^{i}	17.36	13.16 ± 0.15	4.53 ± 0.09^{c}	16.42 ± 0.07^{c}
DC	199.50 ± 2.74	161.87 ± 1.55^{c,i}	−18.86	9.96 ± 0.11^{c}	9.31 ± 0.22^{c}	8.22 ± 0.20^{c}
D+ESP1b 5 mg	196.47 ± 2.14	176.93 ± 3.74^{c,e,h}	−9.94	10.21 ± 0.15^{c}	8.40 ± 0.18^{c,e}	9.17 ± 0.27^{c,e}
D+ESP1b 10 mg	193.85 ± 3.98	177.22 ± 3.56^{c,e,g}	−8.57	10.85 ± 0.19^{c,e}	7.95 ± 0.25^{c,f}	11.18 ± 0.19^{c,f}
D+ESP1b 20 mg	195.23 ± 2.92	184.52 ± 2.45^{c,f,g}	−5.48	11.48 ± 0.19^{c,f}	6.38 ± 0.14^{b,f}	12.69 ± 0.13^{c,f}
D+GLB	198.43 ± 3.35	179.17 ± 1.73^{c,f,i}	−9.70	12.51 ± 0.14^{c,f}	5.46 ± 0.08^{f}	13.48 ± 0.15^{c,f}

Hb, total hemoglobin; HbA1C, glycosylated hemoglobin; NC, Normal control rats; N+ESP1b 20 mg, Normal rats treated with high dose of ESP1b (20 mg/kg b.w./day); DC, STZ-induced diabetic control rats; D+ESP1b 5 mg, STZ-induced diabetic rats treated with low dose of ESP1b (5 mg/kg b.w./day); D+ESP1b 10 mg, STZ-induced diabetic rats treated with medium dose of ESP1b (10 mg/kg b.w./day); D+ESP1b 20 mg, STZ-induced diabetic rats treated with high dose of ESP1b (20 mg/kg b.w./day); and D+GLB, STZ-induced diabetic rats treated with glibenclamide (600 μg/kg b.w./day). Values are mean ± S.E.M. (n=6). $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$ compared to NC group. $^{d}P<0.05$, $^{e}P<0.01$, $^{f}P<0.001$ compared to DC group. $^{g}P<0.05$, $^{h}P<0.01$, $^{i}P<0.001$ compared to Initial body weight

Table 3
Effect of ESP1b on lipid profile and atherogenic ratios in normal and streptozotocin-induced diabetic Wistar rats.

Groups	TG (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	TC/HDL-C ratio	LDL-C/HDL-C ratio
NC	87.89 ± 1.90	95.41 ± 0.86	27.84 ± 0.63	49.99 ± 0.25	17.57 ± 0.38	3.43 ± 0.05	1.80 ± 0.04
N+ESP1b 20 mg	81.20 ± 1.68	99.46 ± 1.07	29.56 ± 0.81	53.65 ± 0.52	16.24 ± 0.33	3.37 ± 0.06	1.82 ± 0.05
DC	144.21 ± 1.90^{c}	138.70 ± 1.48^{c}	14.70 ± 0.87^{c}	95.15 ± 1.94^{c}	28.84 ± 0.37^{c}	9.62 ± 0.65^{c}	6.62 ± 0.5^{c}
D+ESP1b 5 mg	116.70 ± 1.89^{c,f}	130.57 ± 1.60^{c,e}	22.26 ± 0.75^{c,f}	84.95 ± 0.59^{c,f}	23.34 ± 0.37^{c,f}	5.88 ± 0.13^{c,f}	3.83 ± 0.11^{c,f}
D+ESP1b 10 mg	98.61 ± 1.72^{b,f}	123.15 ± 1.75^{c,f}	24.47 ± 0.54^{b,f}	78.95 ± 1.49^{c,f}	19.72 ± 0.34^{b,f}	5.04 ± 0.12^{c,f}	3.23 ± 0.10^{c,f}
D+ESP1b 20 mg	91.45 ± 2.31^{f}	116.09 ± 1.38^{c,f}	25.71 ± 0.46 ^{f}	72.08 ± 0.63^{c,f}	18.28 ± 0.46^{f}	4.51 ± 0.04^{a,f}	2.80 ± 0.04^{b,f}
D+GLB	92.09 ± 2.28^{f}	100.90 ± 1.29^{a,f}	22.64 ± 0.65^{c,f}	59.83 ± 0.63^{c,f}	18.41 ± 0.45^{f}	4.46 ± 0.09^{a,f}	2.65 ± 0.08^{b,f}

TG, Triglycerides; TC, Total cholesterol; HDL-C, High density lipoprotein cholesterol; VLDL-C, Very low density lipoprotein cholesterol; LDL-C, Low density lipoprotein cholesterol; NC, Normal control rats; N+ESP1b 20 mg, Normal rats treated with high dose of ESP1b (20 mg/kg b.w./day); DC, STZ-induced diabetic control rats; D+ESP1b 5 mg, STZ-induced diabetic rats treated with low dose of ESP1b (5 mg/kg b.w./day); D+ESP1b 10 mg, STZ-induced diabetic rats treated with medium dose of ESP1b (10 mg/kg b.w./day); D+ESP1b 20 mg, STZ-induced diabetic rats treated with high dose of ESP1b (20 mg/kg b.w./day); and D+GLB, STZ-induced diabetic rats treated with glibenclamide (600 μg/kg b.w./day). Values are mean ± S.E.M. (n=6). $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$ compared to NC group. $^{d}P<0.05$, $^{e}P<0.01$, $^{f}P<0.001$ compared to DC group.

Table 4
Effect of ESP1b on total protein, albumin, creatinine, urea and uric acid in normal and streptozotocin-induced diabetic Wistar rats.

Groups	Total protein (g/dl)	Albumin (g/dl)	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
NC	7.45 ± 0.07	4.44 ± 0.04	0.70 ± 0.05	32.84 ± 0.59	1.15 ± 0.04
N+ESP1b 20 mg	7.49 ± 0.09	4.46 ± 0.06	0.65 ± 0.04	34.11 ± 0.40	1.38 ± 0.09
DC	5.62 ± 0.12^{c}	3.38 ± 0.07^{c}	1.35 ± 0.05^{c}	58.01 ± 1.71^{c}	1.92 ± 0.04^{c}
D+ESP1b 5 mg	6.30 ± 0.09^{c,f}	3.75 ± 0.05^{c,f}	0.78 ± 0.05^{f}	46.15 ± 1.02^{c,f}	1.57 ± 0.08^{c,e}
D+ESP1b 10 mg	6.52 ± 0.13^{c,f}	3.87 ± 0.08^{c,f}	0.71 ± 0.05 ^{f}	42.34 ± 0.58^{c,f}	1.43 ± 0.06^{a,f}
D+ESP1b 20 mg	6.97 ± 0.07^{b,f}	4.13 ± 0.05^{b,f}	0.69 ± 0.04^{f}	40.76 ± 0.45^{c,f}	1.24 ± 0.05 ^{f}
D+GLB	7.24 ± 0.08^{f}	4.32 ± 0.03 ^{f}	0.67 ± 0.05^{f}	38.17 ± 0.62^{c,f}	1.13 ± 0.04^{f}

NC, Normal control rats; N+ESP1b 20 mg, Normal rats treated with high dose of ESP1b (20 mg/kg b.w./day); DC, STZ-induced diabetic control rats; D+ESP1b 5 mg, STZ-induced diabetic rats treated with low dose of ESP1b (5 mg/kg b.w./day); D+ESP1b 10 mg, STZ-induced diabetic rats treated with medium dose of ESP1b (10 mg/kg b.w./day); D+ESP1b 20 mg, STZ-induced diabetic rats treated with high dose of ESP1b (20 mg/kg b.w./day); and D+GLB, STZ-induced diabetic rats treated with glibenclamide (600 μg/kg b.w./day). Values are mean ± S.E.M. (n=6). $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$ compared to NC group. $^{d}P<0.05$, $^{e}P<0.01$, $^{f}P<0.001$ compared to DC group.

values. The ESP1b treatment in normal rats had produced a moderate increase in the levels of total protein and albumin.

3.6. Plasma enzymes, total and direct bilirubin

Compared with normal control animals, diabetic untreated group exhibited significantly ($P<0.001$) increased activities of plasma enzymes ALT, AST, LDH, ALP and GGT respectively by 66.40%, 30.28%, 50.69%, 59.74% and 65.71% (Table 5). Administration of ESP1b to diabetic animals significantly restored the enzyme activities to near normal control values, whereas, in normal rats, we noted that the oral gavage of ESP1b resulted in significant ($P<0.05$) decrease of LDH activity and a slight reduction of other plasma enzymes activities.

There were a clear rise in the levels of TB ($P<0.001$) and DB ($P<0.01$) of untreated diabetic animals respectively, by 45.71% and 51.02% when compared to normal controls levels. Significant decreases in the levels of TB and DB respectively, by 30.25% and 31.08% were observed in diabetic group treated with ESP1b compared to diabetic controls; however, we noted a moderate elevation in the level of TB and DB respectively by 6.93% and 12.24% in normal rats treated with ESP1b when compared to normal controls.

3.7. Histological changes

Photomicrograph of untreated diabetic rats showed severe atrophy of pancreatic islets and degranulation of β-cells (Fig. 3C) when compared to normal control pancreatic histology (Fig. 3A). Treating diabetic rats with ESP1b resulted in a remarkable improvement in the volume of islets (Fig. 3D) in comparison with diabetic control animals (Fig. 3C). No degenerative changes of

Table 5
Effect of ESP1b on ALT, AST, LDH, ALP, GGT, Total and Direct Bilirubin in normal and streptozotocin-induced diabetic Wistar rats.

Groups	ALT (IU/l)	AST (IU/l)	LDH (IU/l)	ALP (IU/l)	GGT (IU/l)	TB (mg/l)	DB (mg/l)
NC	24.20 ± 0.60	39.53 ± 1.37	1164.5 ± 33.7	87.54 ± 1.88	16.39 ± 0.60	2.45 ± 0.08	0.49 ± 0.05
N+ESP1b 20 mg	22.74 ± 0.72	38.97 ± 0.75	1041.2 ± 42.5^{a}	86.63 ± 1.57	15.02 ± 0.42	2.62 ± 0.05	0.55 ± 0.03
DC	40.27 ± 0.90^{c}	51.50 ± 1.53^{c}	1754.8 ± 31.5^{c}	139.84 ± 2.95^{c}	27.16 ± 0.92^{c}	3.57 ± 0.23^{c}	0.74 ± 0.05^{b}
D+ESP1b 5 mg	34.48 ± 0.50^{c,f}	49.86 ± 0.94^{c}	1631.2 ± 23.6^{c,d}	123.57 ± 1.76^{c,f}	25.84 ± 0.69^{c}	3.43 ± 0.05^{c}	0.67 ± 0.04^{a}
D+ESP1b 10 mg	30.63 ± 0.71^{c,f}	46.39 ± 0.89^{c,e}	1534.2 ± 20.8^{c,f}	114.50 ± 1.31^{c,f}	23.35 ± 0.85^{c,e}	2.78 ± 0.10^{f}	0.58 ± 0.02^{d}
D+ESP1b 20 mg	27.95 ± 0.52^{b,f}	42.21 ± 0.84^{f}	1293.0 ± 32.0^{a,f}	98.06 ± 1.71^{b,f}	18.45 ± 0.51^{f}	2.49 ± 0.06^{f}	0.51 ± 0.02^{e}
D+GLB	31.22 ± 0.64^{c,f}	40.72 ± 1.04^{f}	1326.8 ± 26.8^{b,f}	91.76 ± 1.47^{f}	18.61 ± 0.60 ^{f}	2.64 ± 0.06^{f}	0.54 ± 0.04^{e}

ALT, Alanine aminotransferase; AST, Aspartate aminotransférase; LDH, Lactate dehydrogenase; ALP, Alkaline phosphatase; GGT, Gamma-glutamyl transpeptidase; TB, Total bilirubin; DB, Direct bilirubin; NC, Normal control rats; N+ESP1b 20 mg, Normal rats treated with high dose of ESP1b (20 mg/kg b.w./day); DC, STZ-induced diabetic control rats; D+ESP1b 5 mg, STZ-induced diabetic rats treated with low dose of ESP1b (5 mg/kg b.w./day); D+ESP1b 10 mg, STZ-induced diabetic rats treated with medium dose of ESP1b (10 mg/kg b.w./day); D+ESP1b 20 mg, STZ-induced diabetic rats treated with high dose of ESP1b (20 mg/kg b.w./day); and D+GLB, STZ-induced diabetic rats treated with glibenclamide (600 μg/kg b.w./day). Values are mean ± S.E.M. (n=6). $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$ compared to NC group. $^{d}P<0.05$, $^{e}P<0.01$, $^{f}P<0.001$ compared to DC group.

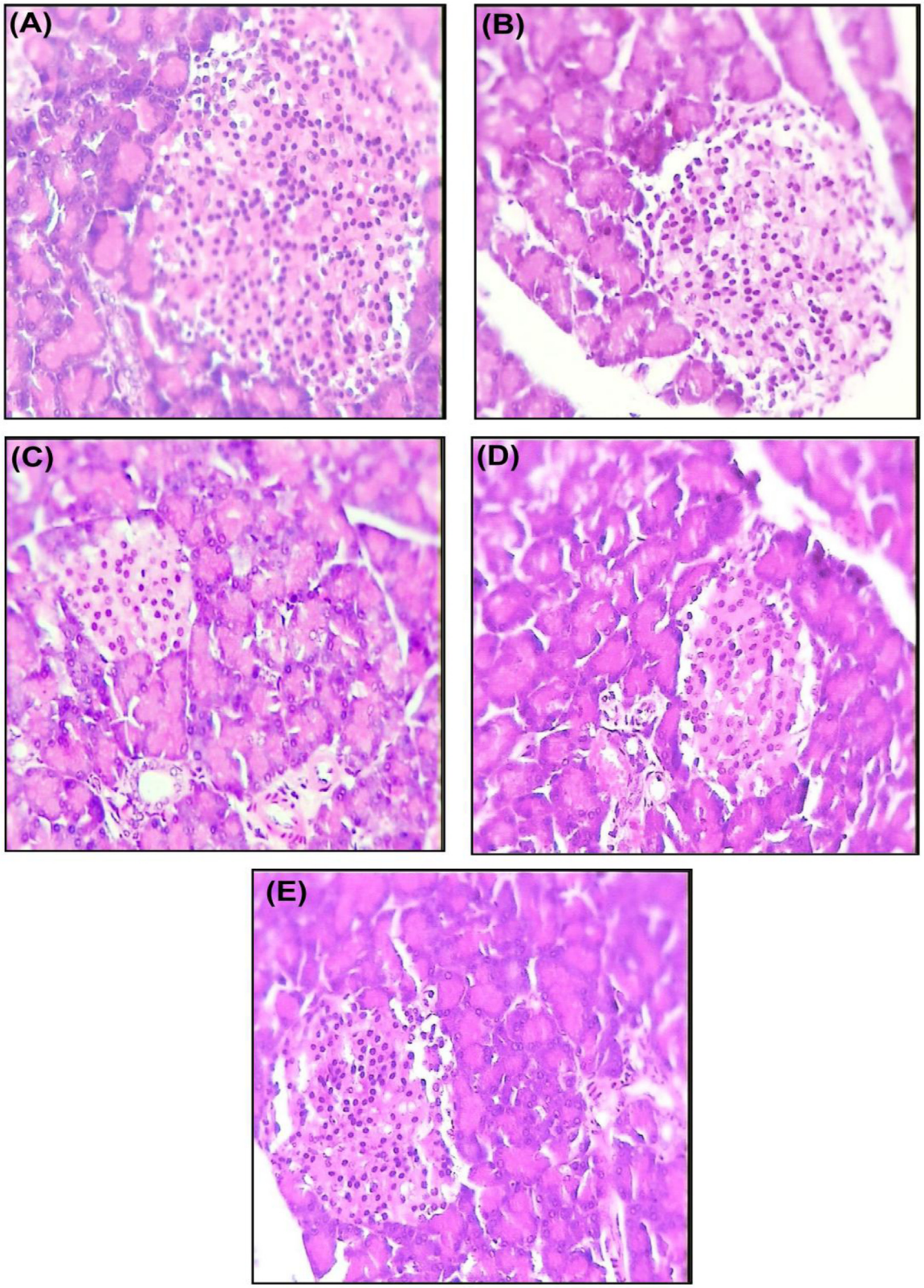

Fig. 3. Histopathological comparison of the pancreatic islet tissues in normal and streptozotocin-induced diabetic Wistar rats. Photomicrographs showing one single pancreatic islet of (A) Normal control rats; (B) Normal rats treated with high dose of ESP1b (20 mg/kg b.w./day); (C) STZ-induced diabetic control rats; (D) STZ-induced diabetic rats treated with high dose of ESP1b (20 mg/kg b.w./day); and (E) STZ-induced diabetic rats treated with glibenclamide (600 μg/kg b.w./day). In diabetic control rats, a severe atrophy of pancreatic islets was clearly observed. An improvement in the volume of islets was noted after ESP1b and GLB treatments. Examinations were carried out at × 400 magnifications with hematoxylin-eosin's stain.

pancreatic β-cells were observed in normal animals treated with ESP1b (Fig. 3B).

4. Discussion

In the present study, we investigated the effects of a novel synthesized molecule, ESP1b, in STZ-induced diabetic Wistar rats. The obtained results show that intraperitoneal injection of STZ to our experimental rats has provoked a severe atrophy of pancreatic islets (Fig. 3C), diminished plasma insulin concentration, decreased final body weight, augmented persistently the levels of fasting blood glucose, and increased HbA1c percentage to over 9% of total hemoglobin (Tables 1 and 2). These observed alterations are similar to those reported previously (Chandramohan et al., 2008; Harini et al., 2012). Gavage of diabetic rats with the novel synthesized molecule ESP1b resulted in a decline, in a dose dependent manner, of blood glucose levels during the OGTT test (Fig. 2) and the four weeks of treatment period (Table 1). Moreover, a rise in insulin concentration associated with a diminished HbA1c value ($< 6.5\%$ of total hemoglobin) (Table 2) were noted in these animals. This signifies a better glycemic control and a reduced glycation of proteins as a result of increased release of insulin from Langerhans islets, which may be with the same mode of action of GLB and all other hypoglycemic sulfonylurea drugs (Green and Feinglos, 2006).

Photomicrographs of pancreatic specimen have confirmed the preceding effects of treating diabetic rats with ESP1b, showing an improvement in the total volume of Langerhans islets (Fig. 3D), which can be explained by an increase in the β-cells number, cellular volume, or a combination of both. The expansion of the β-cells mass may be viewed as a result of the reduced injurious effect of the overproduction of reactive oxygen species on β-cells, which have a particularly low antioxidative defense capacity, or as a compensatory response of pancreatic islet for the increased insulin demands in order to control blood glucose homeostasis. Diabetic animals on treatment with ESPb1 have also showed an enhancement in their body weight (Table 2). This ability of ESPb1 to protect rats from weight loss seems to be related to increased insulin level, which lead to reduced lipids and proteins breakdown.

DM is usually associated with several abnormalities of fats and lipoproteins (Dunn, 1990). The most typical feature of these disorders is the abundance of TG-rich lipoproteins such as chylomicrons and VLDLs in the blood (Laakso, 2009), which was noticed in STZ-diabetic animals (Table 3). An elevation in plasma concentrations of TG, total and LDL cholesterol associated with reduced levels of HDL were also observed. This may be regarded as a result of profound decreases in catabolism of TG-rich lipoproteins secondary to reduced activity of lipoprotein lipase (LPL) (Taskinen, 1987), and/or may be due to hepatic overproduction of VLDL particles that are stimulated by increased circulating free fatty acids (FFA), the substrates of VLDL, as a result of the uninhibited action of the hormone-sensitive lipase (HSL) in adipose tissue (Vergès, 2009). Oral treatment of diabetic rats with ESPb1 has decreased the disturbance of lipid profile and atherogenic ratios (Table 3). This hypolipidemic effect may be attributed to the augmented circulating levels of the antilipolytic hormone, insulin, which is considered as a potent activator of LPL, the enzyme that catalyzes the hydrolysis of the plasma TG-rich lipoproteins. Moreover, the improvement in insulin secretion inhibits the HSL activity and increases the utilization of glucose, which in turn decreases the mobilization of FFA from the fat depots and the synthesis of VLDL by the liver.

Metabolic disorders induced by DM involve not only the metabolism of carbohydrates and lipids but also that of proteins. In this study, plasma proteins concentrations (Table 4) were found to be reduced in diabetic animals. This confirm the negative nitrogen balance as well as loss of nitrogen from most organs that are commonly suggested in STZ-induced DM (Almdal and Vilstrup, 1987). Furthermore, it could indicate a diminished synthetic function of the liver secondary to insulin deficiency (Biolo and Wolfe, 1993) or the onset of microalbuminuria and microproteinuria, which are regarded as important predictors of diabetic nephropathy (Mogensen, 1984). ESP1b treated group has exhibited increased levels of total protein, albumin (Table 4) and Hb (Table 2) when compared to diabetic controls. This may be mainly related to boosted insulin secretion. Indeed, in most circumstances this hormone is anabolic, meaning that it stimulates net protein synthesis (Biolo and Wolfe, 1993), which contributes to the restoration of plasma proteins concentration in this condition.

STZ-diabetic animals have also enhanced plasma levels of urea, creatinine and uric acid (Table 4). This could be attributed to reduced clearance of these substances, reflecting a decline in the glomerular filtration rate (GFR) (Almdal and Vilstrup, 1988) and/or an increased net tubular absorption (Johnson et al., 2003). However, during this metabolic disorder, the augmented levels of urea, the main end product of protein metabolism, and creatinine, the degradation derivative of creatine and phosphocreatine that are viewed as energy storage compounds in skeletal muscles, could also be a result of an extensive muscles breakdown associated with an increased catabolism of liver and plasma proteins (Jordá et al., 1982). Moreover, the increment in the level of uric acid, which is a product of purine metabolism and one of the major endogenous antioxidants in the body, could relate to oxidative stress, which increases the activity of xanthine oxidase, the only enzyme that is able to produce uric acid (Becker, 1993). In addition to oxidative stress, hyperuricemia may be a consequence of metabolic disturbance (Madianov et al., 2000) and more particularly, protein glycation, which lead to muscle wasting and increased release of purine, the main source of uric acid as well as in activity of xanthine oxidase (Anwar and Meki, 2003). Our data (Table 4) indicate that diabetic rats administrated with ESP1b have significantly ($P < 0.001$) diminished the levels of urea, creatinine and uric acid compared to the mean values of diabetic control group. This may be interpreted by an improved renal function associated with decreased protein degradation secondary to reduced glucose concentration and subsequent glycation.

STZ is known to exert toxic effects not only on pancreatic β cells but also on other organs including liver (Lenzen, 2008), which is regarded as the central metabolic organ in the body that plays a pivotal role in the homeostasis of glucose; through regulation of its uptake, storage, release and *de novo* synthesis from other precursors such as amino acids (AAs) (Hagopian et al., 2003). In our study, experimental DM has increased activities of plasma enzymes; ALT, AST, LDH, ALP and GGT (Table 5). This might be primarily due to the leakage of these enzymes, and more specifically ALT, from liver cytosol into blood stream (Navarro et al., 1993), demonstrating a hepatocellular damage (El-Demerdash et al., 2005). However, the high activities of aminotransferases (ALT and AST) during DM, could also be due to excessive accumulation of gluconeogenic AAs (alanine and glutamate) as a result of AAs mobilization from peripheral protein stores (Felig et al., 1970). Furthermore, the increment in the activities of circulating AST and LDH that are predominantly presents in cardiac and skeletal muscles could attributed to myocardial injury (Awaji et al., 1990). In addition to these extra-hepatic sources of the raised enzymatic activities in circulation, serum isoenzymes determinations have revealed a solely intestinal origin of the observed hyperphosphatasemia in diabetic rats (Chua and Shrago, 1978), leading, to suggest that the elevated activity of circulating ALP may be secondary to the hyperphagia (Chua and Shrago, 1978) or to a direct toxic effects of STZ on the intestine (Hough et al., 1981).

Although, increased activity of GGT in patients sera was initially viewed as sensitive marker of alcohol consumption, liver inflammation, hepatic steatosis and hepatitis (Teschke et al., 1977), more recent studies (Lee et al., 2003; Mason et al., 2010) have reported that high activity of circulating GGT is also strongly associated with the development of metabolic syndrome, DM and cardiovascular diseases, which all characterized by increased oxidative stress. Thus, the elevated activity of GGT in plasma of diabetic rats (Table 5) may be explained by an enhanced need of these organisms to reproduce more glutathione (GSH), the most abundant nonprotein-thiol antioxidant in the body, in order to modify the existing oxidative stress during this pathologic state (Bidel et al., 2008).

Hyperbilirubinemia noticed in STZ-induced DM (Table 5) could reflect alterations of one or several hepatic processes responsible for the elimination of this heme catabolite from plasma, including bilirubin uptake and conjugation (Tuñon et al., 1991). Although bilirubin was long considered to be a toxic waste product, in particular for central nervous system; this yellow pigment has also exhibited a potent antioxidant activity (Stocker et al., 1987). This valuable property has supported an emerging hypothesis that the moderate rise in plasma bilirubin level may be also a physiological response to oxidative stress (Kapitulnik, 2004).

Treating diabetic rats with ESP1b has decreased the activities of plasma enzymes and levels of bilirubin to near normal mean values (Table 5). This may be mainly due to enhanced release of insulin, which consequently reduces oxidative stress, amino acids mobilization and hyperphagia of experimental animals.

5. Conclusion

The finding of this study evidently indicates that oral treatment of diabetic rats with ESP1b has lowered blood glucose levels, enhanced insulin secretion from surviving or regenerated β-cells and modulated the disturbances of lipids as well as proteins metabolism caused by STZ-induced DM. Furthermore, an improvement in renal and hepatic functions was also noticed in experimental animals. These therapeutic effects exhibited by the novel synthesized molecule were comparables with those of glibenclamide, a standard drug of type 2 DM.

Conflict of interest

The authors state that they do not have any conflict of interest.

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Antidiabetic and Hypolipidemic Potential of 3, 4-dihydroisoquinolin-2(1H)-Sulfonamide in Alloxan Induced Diabetic Rats

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ABSTRACT
The present study aimed to investigate the effects of the 3, 4-dihydroisoquinolin-2(1H)-sulfonamide on blood glucose, lipid profile, hepatic and renal functions and enzyme activities in alloxan-induced diabetic Wistar rats. Experimental diabetes was produced by a single intraperitoneal injection of alloxan (140 mg kg^{-1} b.wt.). Three groups of diabetic rats were administered orally with two doses of the novel sulfonamide (2.5 and 5 mg kg^{-1} b.wt.) and a standard drug, glibenclamide (2.5 mg kg^{-1} b.wt.), for 10 days. Changes in body weight, food consumption, water intake and blood glucose levels were recorded regularly. At the end of the treatment period, blood was collected to determine biochemical parameters and enzyme activities in serum samples. Pancreas, liver, kidneys, spleen and heart were weighed in order to evaluate the relative organ weight. Histological changes in pancreas were examined by microscopy according to procedure with hematoxylin-eosin. The treatment with 2.5 and 5 mg kg^{-1} of the tested compound decreased the pathophysiological disturbances of diabetic syndrome. A significant ($p<0.001$) anti-hyperglycemic activity was clearly observed from the 4th day. Significant decreases ($p<0.05$) in total lipid and total cholesterol levels and highly significant decreases ($p<0.001$) in the level of Triglyceride were also noted with the two doses of the tested compound. Moreover, renal and hepatic functions were improved and lesions of pancreas were reversed in treated animals. However, the tested compound at 5 mg kg^{-1} produced highly significant increase ($p<0.001$) in the relative spleen weight. Our findings suggest that the 3, 4-dihydroisoquinolin-2(1H)-sulfonamide is endowed with an interesting anti-diabetic activity comparable to glibenclamide; however, it could present an immunological risk, so further more studies must be undertaken.

Key words: Diabetes mellitus, sulfonamide, hypoglycemic, Biochemical parameters, histopathology

INTRODUCTION

Diabetes Mellitus (DM) is one of the most challenging health problems in the 21st century (Chen *et al*., 2012). Actually, more than 285 million individuals (aged 20-79 years) worldwide are living with the disease. By the year 2030 the DM incidence is projected to rise to over 439 million (Shaw *et al*., 2010). The majority of these individuals have

type 2 DM (Brownlee, 2001). This disease is characterized by a chronic hyperglycemia, resulting from insulin resistance and/or relative insulin deficiency, associated with disturbances in carbohydrate, lipid and protein metabolism (American Diabetes Association, 2009). Those metabolic changes lead to damages and functional impairments of various organs and tissues (Prentki and Nolan, 2006).

Sulfonamides constitute an important class of drugs, with several types of pharmacological agents possessing antibacterial, antitumor, anti-carbonic anhydrase, diuretic, hypoglycemic, antithyroid, or protease inhibitor activity among others (Scozzafava *et al*., 2003). These compounds differ in structure, molecular weight and lipophilicity and act at different receptors via differing modes; they have no common link except the presence of a sulfonamide group (SO_2NH_2) (Smith and Jones, 2008).

Stimulation of glucose-mediated insulin secretion was the first pharmacologic approach for the treatment of type 2 DM as heralded by the introduction of sulfonylureas (SUs) into the antidiabetic pharmacopoeia more than 50 years ago (Del Prato and Pulizzi, 2006). The standard SUs drug for many years was Tolbutamide, a sulfonylurea with no antibacterial activity (no free aryl-NH_2) and no activity against carbonic anhydrase (no free aryl-SO_2-NH_2). Further work, however, showed that the urea structure (-NH-CO-NH-) was not essential and that C = O or C = S could give way to C-N, as in Glycodiazine. Then, the fundamental hypoglycemic structure is simply (R_1-SO_2-NH-). Of course, not all compounds with this group are active but it is worth noting that compounds in the other classes of sulfonamides have the possibility of lowering blood glucose (Maren, 1976).

Nowadays, several synthetic classes of hypoglycemic agents are used for the treatment of DM, such as Sulfonylureas, Biguanides, α-Glycosidase inhibitors and Thiazolidinediones. Many of these oral drugs are often associated with undesirable side effects or a decrease in response after prolonged use. Hence, the search continues for new therapies with effective antidiabetic activity at low dose without adverse effects (Nathan *et al*., 2009).

The objective of this study is to determine the anti-hyperglycemic activity of the 3, 4-dihydroisoquinolin-2(1H)-sulfonamide and its effects on biochemical and histopathological parameters in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Experimental animals: Adult male albino Wistar rats 7-8 weeks old, weighing about 200-220 g, were purchased from the Pasteur Institute of Algeria. They were acclimatized to animal house conditions for 2 weeks in air-conditioned room at 21±2°C and 50±10% relative humidity on a 12 h light/dark cycle. The animals were fed with a standard pellet rat's diet (ONAB, Bejaia, Algeria) and water was supplied *ad libitum*. The experiments were conducted in strict accordance with the current animal ethical norms approved by the University.

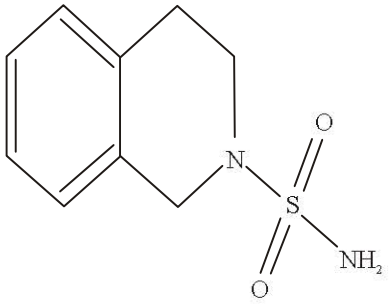

Fig. 1: Chemical structure of the 3, 4-dihydroisoquinolin-2(1H)-sulfonamide (3, 4 DHIQS)

Drugs and chemicals: Both Alloxan monohydrate (ALX) (Sigma-Aldrich Co, USA) and Glibenclamide (GLB) (Sanofi, Algeria) were purchased from reputable pharmaceutical companies.

The biochemical parameters estimation was performed using commercially available kits. Urea, Uric acid, Total protein, Total lipid, Triglycerides, Alanine transaminase, Aspartate transaminase, Alkaline phosphatase, Total and Direct Bilirubin kits were purchased from SPINREACT SAU (Girona, Spain), Creatinine and Total Cholesterol kits were purchased from ELITechGroup (Paris, France). The all other chemicals and reagents used were of analytical grade.

The 3, 4-dihydroisoquinolin-2 (1H)-sulfonamide (3, 4 DHIQS) (Fig. 1), was synthesized by the laboratory of Applied Bio-Organic Chemistry, University of Annaba. The synthesis routes for the preparation of the test compound, were already described in detail in a prior report (Bouasla *et al*., 2011). Briefly, as part as the research for new derivatives of sulfonamide, the 3, 4 DHIQS was prepared in four steps (carbamoylation, sulfamoylation, deprotection and acylation) by the reaction of chlorosulfonyl isocyanate and tertiobutanol in anhydrous CH_2Cl_2. The obtained residue was purified by chromatography on silica gel (eluted with CH_2Cl_2) to give 85% of N-Boc sulfonamide as white solid. The structure of this compound was unambiguously confirmed by usual spectroscopic methods: The ^{1}H NMR, mass spectrometry, IR and X-ray.

Induction of diabetes mellitus: Animals were fasted for 12 h prior to the induction of diabetes as described by Joy and Kuttan (1999) with slight modification. A freshly prepared solution of ALX in normal saline was administered intraperitoneally at single dose of 140 mg kg^{-1} (b.wt.). Since the injection of ALX is capable to produce fatal hypoglycemia as a result of a reactive massive release of pancreatic insulin, rats were also orally given 5-10 mL of a 20% glucose solution after 6 h. Animals were then kept for the next 24 h on 5% glucose solution to prevent severe hypoglycemia (Gupta *et al*., 1984). After 5 days, rats with moderate diabetes having glycosuria and hyperglycemia (i.e., with Fasting Blood Glucose (FBG) levels greater than 200 mg dL^{-1}) were chosen for the experiment.

Experimental design:

Group 1: Normal healthy control: Given only vehicle (9% NaCl)
Group 2: Diabetic control rats
Group 3: Diabetic rats treated with 3, 4 DHIQS (2.5 mg kg^{-1} b.wt.)
Group 4: Diabetic rats treated with 3, 4 DHIQS (5 mg kg^{-1} b.wt.)
Group 5: Diabetic positive control rats treated with GLB (2.5 mg kg^{-1} b.wt.)

The 3, 4 DHIQS and the GLB were given in aqueous solution using an intragastric tube.

On the 1, 2, 4, 7 and 10th day of the treatment period, blood was collected via tail vein by excision, applied to a test strip and analyzed immediately *via* a blood glucose monitoring system with a blood glucose monitoring device (ACCU-CHEK® Active, Roche diagnostics, France). The FBG results are expressed as mg dL^{-1}.

During the experimental period, daily fluid intake, feed consumption and body weight changes were also recorded periodically.

Evaluation of vital organs relative weight: On the 10th day of the treatment period, the normal and experimental animals were deprived of food overnight before being sacrificed by decapitation. After taking the blood, the abdominal cavity of each animal was opened and organs (pancreas, liver, spleen, heart and kidneys) were quickly removed, cleaned with ice-cold saline solution, patted dry and weighed. The Relative Organ Weight (ROW) of each animal was then calculated as follows:

$$ROW = \frac{AOW}{FBW} \times 100$$

AOW is the Absolute Organ Weight and FBW is Final Body Weight (the b.wt. of rat on day of sacrifice).

Biochemical analysis: After decapitation, blood samples were collected into dry no heparinized centrifuge tubes, immediately centrifuged at 4000 rpm for 10 min and the obtained serum was submitted to biochemical tests. Total Lipid (TL) content was estimated by the sulfophosphovanillin method. Triglycerides (TG) concentration was determined by the glycero-3-phosphate: The O_2 2-oxidoreductase (GPO) method. Total Cholesterol (TC) level was estimated by the end point, colorimetric-enzymatic method of Trinder. Total Protein (TP) content was measured by the method of Biuret. Urea level was estimated by the method of Berthelot according to Fawcett and Scott (1960) Creatinine level was measured by the method of Jaffe. Uric acid concentration was determined by the colorimetric-enzymatic method with Uricase-POD. Total and direct bilirubin (TB, DB) levels were estimated by the colorimetric method with dimethylsulfoxide (DMSO). Aspartate transaminase (AST) and Alanine transaminase (ALT) activities were estimated by the UV enzymatic-Kinetic method. Alkaline phosphatase (ALP) activity was determined by the Kinetic method with p-Nitrophenylphosphate. The All assessment assays and kits were performed in accordance with the manufacturers' instructions and protocols.

Histopathological analysis: The pancreas were harvested from the sacrificed rats after dissection, washed with saline, cleared of fat and lymph nodes and fixed in a Bouin solution for 24 h. The fixed specimens were embedded into paraffin blocks. The tissues were cut into 5 μm paraffin sections by a rotator microtome, stained with hematoxylin-eosin and examined under a light microscope; photomicrographs were taken.

Statistical analysis: Statistical analysis was performed using MINITAB software package Version 13.4. The values were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. All the results were expressed as Mean±SEM (standard error of the mean) for five rats in each group. Values of $p<0.05$ were considered significant.

RESULTS

Effect on body weight, food consumption and water intake: Table 1 illustrates the variations in b.wt., food consumption and water intake of normal, diabetic control and diabetic treated groups. ALX-induced DM significantly ($p<0.001$) reduced the b.wt. of rats in comparison with normal animals which gained weight; the weight loss was significantly ($p<0.01$) improved in diabetic rats treated with 3, 4 DHIQS at 5 mg kg^{-1}. However, the standard drug GLB demonstrated more beneficial effect.Diabetic control rats showed also higher intake of food and water when compared with normal group. These disturbances were significantly decreased ($p<0.001$) in diabetic rats treated with 3, 4 DHIQS and GLB for 10 days.

Table 1: Effects of 3, 4 DHIQS and GLB on body weight, food consumption and water intake in ALX-induced diabetic rats

Groups/treatments	b.wt. before treatment (g)	Change in b.wt. (g)	Food consumption (g day^{-1})	Water intake (mL day^{-1})
Normal	248.06±10.6	19.82±6.25	30.09±0.94	60.82±2.58
Diabetic control	240.83±7.19	-48.48±2.47^{c}	44.33±1.30^{c}	222.96±9.23^{c}
D+3,4DHIQS/2.5 mg kg^{-1} b.wt.	244.85±6.43	-41.60±3.91	42.60±1.45^{B}	208.89±4.48^{A}
D+3,4DHIQS/5 mg kg^{-1} b.wt.	245.95±7.12	-35.84±1.84^{B}	38.93±0.95^{C}	207.38±6.97^{C}
D+GLB/2.5 mg kg^{-1} b.wt.	243.67±6.69	-24.53±3.63^{C}	38.09±0.74^{C}	205.91±8.45^{C}

Data represent Mean±SEM (n = 5 for each group), D: Diabetic, b.wt., ^{a,b,c}Indicate statistical significance in comparison with normal group at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, ^{A,B,C}Denote statistical significance in comparison with diabetic control group at $p<0.05$, $p<0.01$ and $p<0.001$, respectively

Table 2: Effects of 3, 4 DHIQS and GLB on FBG levels in ALX-induced diabetic rats

	Day (mg dL^{-1})					
Groups/treatments	1	2	4	7	10	Changes in FBG (%)
Normal	107.89±6.86	109.83±6.68	108.89±4.93	109.37±5.84	107.68±5.28	(-) 0.19
Diabetic control	298.51±18.04^{c}	307.08±19.14^{c}	341.60±24.30^{c}	378.48±14.57^{c}	396.20±27.40^{c}	(+) 32.72
D+3,4DHIQS/2.5 mg kg^{-1} b.wt.	299.06±13.33	290.60±31.90	263.02±12.16^{C}	188.11±8.73^{C}	152.27±18.47^{C}	(-) 49.08
D+3,4DHIQS/5 mg kg^{-1} b.wt.	303.99±18.76	299.18±13.20	234.33±11.66^{C}	177.19±21.73^{C}	138.63±16.06^{C}	(-) 54.39
D+GLB/2.5 mg kg^{-1} b.wt.	298.49±18.22	290.70±28.40	230.83±18.95^{C}	142.63±7.13^{C}	125.29±12.44^{C}	(-) 58.02

Data represent Mean±SEM (n = 5 for each group), D: Diabetic, b.wt., ^{a,b,c}Indicate statistical significance in comparison with normal group at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, ^{A,B,C}Denote statistical significance in comparison with diabetic control group at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, change in FBG (%): [(final FBG-initial FBG)/initial FBG)]×100, (+): Indicates a increase in FBG, (-): Indicates a decrease in FBG

Table 3: Effects of 3, 4 DHIQS and GLB on vital organs relative weight in ALX-induced diabetic rats

		Relative organ weight (%)				
Groups/treatments	Final b.wt. (g)	Pancreas	Liver	Kidneys	Spleen	Heart
Normal	267.87±11.64	0.32±0.01	3.62±0.15	0.68±0.02	0.24±0.01	0.34±0.03
Diabetic control	192.35±9.64^{c}	0.27±0.02^{b}	4.58±0.37^{c}	0.88±0.08^{c}	0.29±0.01^{c}	0.42±0.03^{b}
D+3,4DHIQS/2.5 mg kg^{-1} b.wt.	203.26±4.28	0.32±0.02^{C}	4.34±0.23	0.85±0.05	0.32±0.01^{A}	0.39±0.01
D+3,4DHIQS/5 mg kg^{-1} b.wt.	210.09±6.80^{B}	0.34±0.02^{C}	4.10±0.15^{A}	0.84±0.05^{A}	0.34±0.01^{C}	0.36±0.02^{B}
D+GLB/2.5 mg kg^{-1} b.wt.	219.14±5.49^{C}	0.35±0.01^{C}	3.94±0.32^{B}	0.78±0.05^{C}	0.28±0.02	0.41±0.02

Data represent Mean±SEM (n = 5 for each group). D: Diabetic, b.wt., ^{a,b,c}Indicate statistical significance in comparison with normal group at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, ^{A,B,C}Denote statistical significance in comparison with diabetic control group at $p<0.05$, $p<0.01$ and $p<0.001$, respectively

Table 4: Effects of 3, 4 DHIQS and GLB on Lipid profile in ALX-induced diabetic rats

	Total lipid	Triglyceride	Total cholesterol
Groups/treatments		(mg dL^{-1})	
Normal	315.37±5.57	74.95±6.08	74.30±2.29
Diabetic control	479.60±25.40^{c}	281.40±57.20^{c}	113.30±10.05^{c}
D+3,4DHIQS/2.5 mg kg^{-1} b.wt.	423.39±9.39^{B}	133.54±10.77^{C}	100.71±5.70^{A}
D+3,4DHIQS/5 mg kg^{-1} b.wt.	424.20±22.60^{A}	114.97±9.94^{C}	99.06±6.20^{A}
D+GLB/2.5 mg kg^{-1} b.wt.	425.30±38.10^{A}	90.49±4.86^{C}	98.84±5.22^{A}

Data represent Mean±SEM (n = 5 for each group). D: Diabetic, b.wt., ^{a,b,c}Indicate statistical significance in comparison with normal group at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, ^{A,B,C}Denote statistical significance in comparison with diabetic control group at $p<0.05$, $p< 0.01$ and $p<0.001$, respectively

Fasting Blood Glucose (FBG): FBG levels were estimated in normal and experimental rats on 1, 2, 4, 7 and 10th day of treatment period with 3, 4 DHIQS and GLB. Our results showed that in group 2, FBG levels are 3-4 times higher than of group 1 (Table 2). A clear decline in FBG levels was observed from the 4th day after the treatment with two doses of 3, 4 DHIQS and GLB. Highest percentage decreases of FBG levels by 58.02, 49.08 and 54.39% have been observed at the end of experiment for the treatments, with GLB, 3, 4 DHIQS at 2.5 and 5 mg kg^{-1}, respectively.

Vital organs relative weight: Effects of oral administration of 3, 4 DHIQS and GLB on ROW are presented in Table 3. The relative pancreas weight in diabetic control group decreased significantly ($p<0.01$) by 15.62%, whereas, the ROW of liver, kidneys, spleen and heart increased by 26.51, 29.41, 20.83 and 23.52%, respectively, when compared to normal animals. Treating the diabetic rats with GLB, 3, 4 DHIQS at 2.5 and at 5 mg kg^{-1} improved significantly ($p<0.001$) the ROW of pancreas by 29.62, 18.51 and 25.92%, respectively, in comparison with the diabetic controls. In addition, there was a significant ($p<0.01$; $p<0.05$) decrease by 13.97 and 10.48% of the relative liver weight in diabetic rats treated with GLB and 3, 4 DHIQS at 5 mg kg^{-1} respectively. However, diabetic rats orally administered with 2.5 mg kg^{-1} of 3, 4 DHIQS showed moderate decreases by 5.23% of this ROW when compared to diabetic untreated rats. Moreover, in ALX-diabetic groups treated with 3, 4 DHIQS, there were moderate decreases by 3-5% of the ROW of kidneys, whereas GLB decreased this ROW by 11.36% in comparison with the diabetic control group. Also we note a reduction by 3.44% of the ROW of spleen in diabetic rats treated with GLB. However, 3, 4 DHIQS at 2.5 and 5 mg kg^{-1} produced significant ($p<0.05$, $p<0.001$) increases of the relative spleen weight by 10.34 and 17.24%, respectively, in comparison with diabetic untreated rats. Finally, there was no significant change in the ROW of heart in diabetic animals treated with GLB and 3, 4 DHIQS at 2.5 mg kg^{-1} (2.38 and 7.13%, respectively), whereas, the diabetic group treated with 3, 4 DHIQS at 5 mg kg^{-1} presents a very significant ($p<0.01$) reduction by 14.28%, in comparison with the diabetic controls.

Lipid profile: Our results showed that experimental DM increased significantly ($p<0.001$) TL and TC levels, respectively by 20.36 and 52.48% and involved a particularly important rise in the concentration of TG by 275.45% in comparison with normal levels (Table 4). Significant decreases

Table 5: Effects of the treatment with 3, 4 DHIQS and GLB on total protein, creatinine, urea and uric acid in ALX-induced rats

Groups/treatments	Total protein (g dL^{-1})	Creatinine (mg dL^{-1})	Urea (mg dL^{-1})	Uric acid (mg dL^{-1})
Normal	5.56±0.19	54.85±1.02	33.89±1.72	3.47±0.31
Diabetic control	4.66±0.34^{c}	76.57±1.54^{c}	57.14±1.45^{c}	3.93±0.24^{a}
D+3,4DHIQS/2.5 mg kg^{-1} b.wt.	5.01±0.19	70.06±5.36^{A}	50.18±3.68^{A}	3.13±0.08^{B}
D+3,4DHIQS/5 m kg^{-1} b.wt.	5.11±0.28^{A}	73.70±2.34	55.96±2.92	3.38±0.41^{A}
D+GLB/2.5 mg kg^{-1} b.wt.	5.19±0.25^{A}	67.47±3.90^{B}	50.23±6.14^{A}	3.30±0.36^{A}

Data represent Mean±SEM (n = 5 for each group). D: Diabetic, b.wt., ^{a,b,c}Indicate statistical significance in comparison with normal group at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, ^{A,B,C}Denote statistical significance in comparison with diabetic control group at $p<0.05$, $p<0.01$ and $p<0.001$, respectively

Table 6: Effects of the treatment with 3, 4 DHIQS and GLB on PAL, AST, ALT, total and direct bilirubin in ALX-induced diabetic rats

Groups/treatments	ALP (g dL^{-1})	AST (mg dL^{-1})	ALT (mg dL^{-1})	DB (mg dL^{-1})	TB (mg dL^{-1})
Normal	26.50±1.32	160.26±4.48	100.88±3.61	0.22±0.01	7.30±0.20
Diabetic control	43.74±9.77^{b}	376.60±36.6^{c}	308.60±45.4^{c}	0.42±0.02^{c}	12.55±1.42^{c}
D+3,4DHIQS/2.5 mg kg^{-1} b.wt.	27.40±2.09^{C}	329.98±5.84^{B}	254.13±9.44^{A}	0.23±0.02^{C}	7.37±0.20^{C}
D+3,4DHIQS/5 mg kg^{-1} b.wt.	38.00±1.98	332.95±16.55^{A}	259.64±10.57^{A}	0.37±0.04	10.50±1.27^{A}
D+GLB/2.5 mg kg^{-1} b.wt.	41.95±2.02	328.70±15.69^{B}	262.10±27.60^{A}	0.36±0.04^{A}	10.65±0.98^{A}

Data represent Mean±SEM (n = 5 for each group). D: Diabetic, b.wt., ^{a,b,c}Indicate statistical significance in comparison with normal group at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, ^{A,B,C}Denote statistical significance in comparison with diabetic control group at $p<0.05$, $p<0.01$ and $p<0.001$, respectively

($p<0.05$; $p<0.01$) by 11-13% in TL and TC levels and highly significant ($p<0.001$) decreases in the level of TG were observed in all treated diabetic rats compared to diabetic control group.

Total protein, kidney toxicity indices in serum (creatinine, urea) and uric acid: Table 5 lists changes in TP, creatinine, urea and uric acid levels in normal and ALX-induced diabetic rats. As can be seen, diabetic controls showed a highly significant ($p<0.001$) decrease of 16.18% of TP level; However, they show highly significant ($p<0.001$) increases in serum urea and creatinine levels by 68.60 and 39.59%, respectively when compared to the normal concentrations. Moreover, the level of uric acid in this group was significantly ($p<0.05$) increased by 13.25%. There was no significant increase (7.51%) in TP levels in diabetic rats treated with 3, 4 DHIQS at 2.5 mg kg^{-1}. However, significant ($p<0.05$) increases by 11.37 and 9.65% were noted in diabetic groups treated with GLB and 3, 4 DHIQS at 5 mg kg^{-1}, respectively, when compared to untreated diabetic rats. There were also significant ($p<0.05$) increases in uric acid levels by 20.35, 13.99 and 15.26%, in diabetic rats treated with, 3, 4 DHIQS at 2.5 and 5 mg kg^{-1}, respectively, compared to untreated diabetic rats. In addition, we observed significant ($p<0.05$) decreases in serum urea levels by 12.10 and 12.09%, respectively, in diabetic groups treated with GLB and 3, 4 DHIQS at 2.5 mg kg^{-1}. We also noticed a no significant decline by 2.06% with 3, 4 DHIQS at 5 mg kg^{-1}, compared to diabetic control. Moreover, creatinine levels were significantly ($p<0.05$) decreased by 8.50 and 11.88%, respectively, in diabetic rats treated with GLB and 3, 4 DHIQS at 2.5 mg kg^{-1}, when compared to diabetic control. The 3, 4 DHIQS at 5 mg kg^{-1} show moderate decrease with 3.74% compared to untreated diabetic rats.

Hepatotoxicity indices (PAL, AST, ALT, Total and Direct Bilirubin): Changes in enzyme activities of alkaline phosphatase (ALP) and transaminases (AST, ALT) in normal and diabetic rats are illustrated in Table 6. ALP activity was very significantly ($p<0.01$) increased by 65% in diabetic controls; however, the increase was more pronounced ($p<0.001$) in AST and ALT activities by 135 and 206%, respectively, compared to normal values. Treating the diabetic rats with 3, 4 DHIQS and GLB involved significant decreases by 12-13 and 15-17%, respectively in AST and ALT activities. Moreover, ALP enzymatic activity has decreased to a statistically highly significant ($p<0.001$) value, reaching 37.35% in the diabetic group treated with 3, 4 DHIQS at 2.5 mg kg^{-1}, whereas, 3, 4 DHIQS at 5 mg kg^{-1} and GLB resulted in only a slight reduction about 13.12 and 4.09% respectively, compared to diabetic control.

Changes in total and direct bilirubin are shown in Table 6. There was a clear rise ($p<0.001$) in the levels of TB and DB (71.91 and 90.90%, respectively) in untreated diabetic animals. Significant ($p<0.05$) decreases by 41.27 and 45.23% were observed, respectively, in the concentrations of TB and DB in diabetic group treated with GLB; however, we noted a moderate reduction in the level of DB by 11.90% and a significant ($p<0.05$) decrease by 16.33% in the level of TB in rats treated with 3, 4 DHIQS at 5 mg kg^{-1}, respectively, compared to diabetic controls. Moreover, Significant ($p<0.05$) decreases by 15.13 and 14.28% were observed in the levels of TB and DB, respectively, in diabetic group treated with 3, 4 DHIQS at 2.5 mg kg^{-1} compared to diabetic controls.

Histological changes: Histopathological examinations showed that ALX administration elicited severe injury of pancreatic β-cells, such as decreasing the islets cell numbers, cell damage and death (Fig. 2a) compared with normal rats (Fig. 2b). Treatment of diabetic rats with GLB and 3, 4 DHIQS at 5 mg kg^{-1} (Fig. 2c-e) resulted in a remarkable improvement in the volume of pancreatic islets compared to untreated diabetic rats (Fig. 2b). However, the 3, 4 DHIQS at 2.5 mg kg^{-1} initiated only a moderate improvement in the atrophy of pancreatic islets (Fig. 2d).

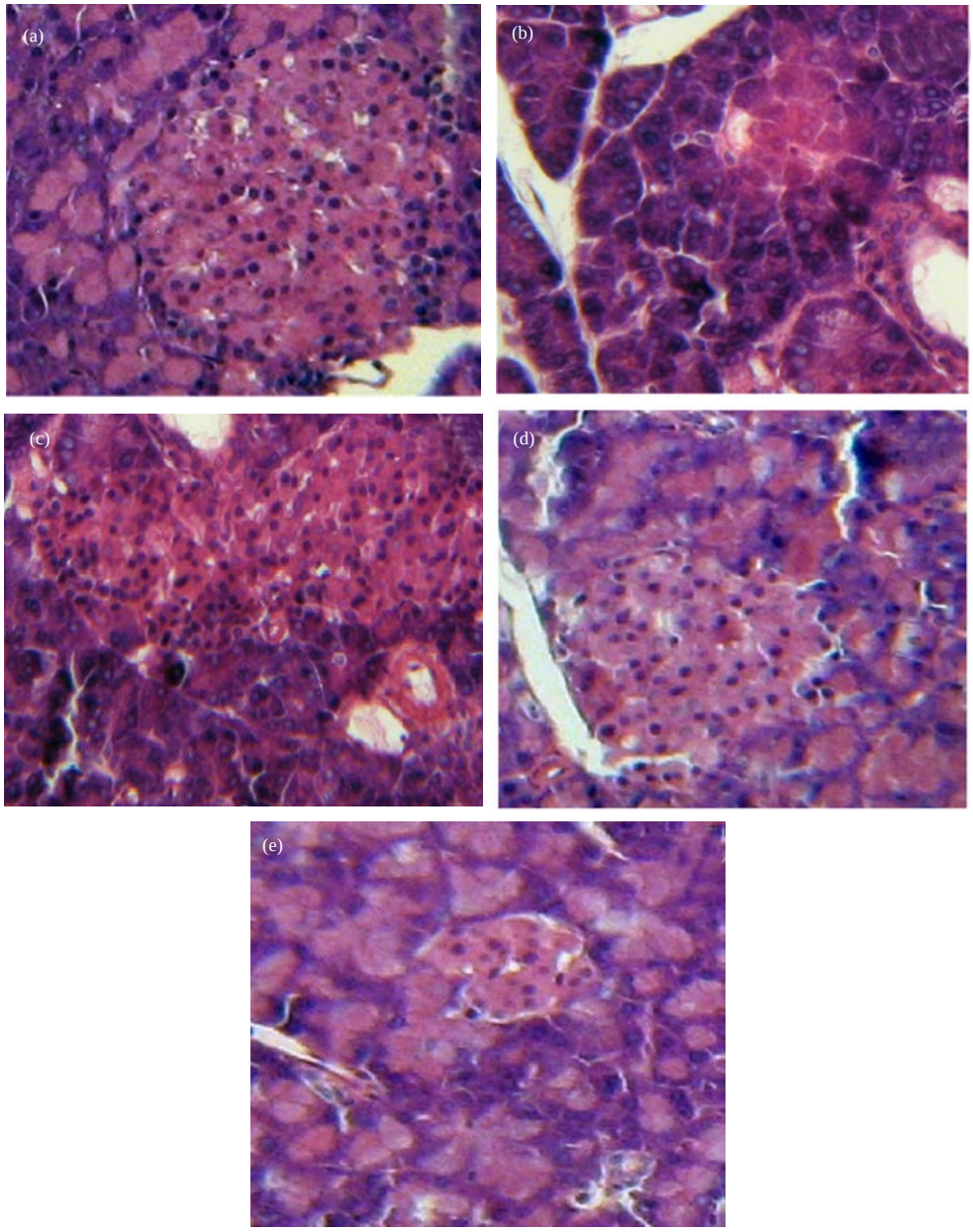

Fig. 2(a-e): Histological comparison of the pancreatic islet tissues in normal and ALX-induced diabetic rats. Photomicrograph showing one single pancreatic islet of the normal group (a) Diabetic control, (b) Diabetic +GLB at 2.5 mg kg^{-1} BW group, (c) Diabetic + 3, 4 DHIQS at 2.5 mg kg^{-1} BW group, (d) Diabetic + 3, 4 DHIQS at 5 mg kg^{-1} BW group and (e) with hematoxylin-eosin's stain. In diabetic rats, a decrease in pancreatic β-cells was clearly observed. An improvement in the volume of pancreatic islets was observed after GLB and 3, 4 DHIQS treatments. Examinations were carried out at ×200

DISCUSSION

Currently, in the field of diabetes research, several techniques are used to produce, in animals, a condition similar to DM, to better understand human diabetes or to find new therapies. In this study, we have chosen ALX as a diabetogenic agent. This molecule causes selective destruction of pancreatic β cells. Moreover, as a thiol reagent, ALX also selectively inhibits glucose-induced insulin secretion through its ability to inhibit the β cell glucose sensor glucokinase (Lenzen, 2008). β-cell destruction may be total or partial, depending on the injected doses of ALX, specie, strain, age,

weight and physiological state of experimental animals. This chemical can causes, respectively, mild or intense diabetic condition (Akhtar *et al*., 2007), similar to type 1 or type 2 DM. It is well established that GLB and all SUs drugs exert their glucose-lowering effect via increased release of insulin from the β cells of the pancreas (Green and Feinglos, 2006). These compounds are active in mild ALX-induced DM whereas they are inactive in intense ALX diabetes (nearly all β-cells have been destroyed) (Akhtar *et al*., 2007). However, since our results showed that GLB reduced FBG levels in diabetic animals, the state of DM in our experimental model is not severe. This conclusion was confirmed by histological findings of pancreatic tissues (Fig. 1). Just like GLB, the 3, 4 DHIQS at 2.5 and 5 mg kg^{-1} b.wt. has caused significant decreases in FBG levels reaching, respectively, the minimum values of 152.27±18.47 and 138.63±16.06 mg dL^{-1}. Hypoglycemic activity of 3, 4 DHIQS may be attributed to the stimulation of insulin secretion from pancreatic β-cells principally and this by inhibiting K_{ATP} channels.

Dehydration and loss of body weight have been associated with human DM (Pupim *et al*., 2005). In the diabetic rats, following experimentally DM, increased water intake, food consumption and decreased b.wt. were consistent with previous studies (Hamden *et al*., 2009; Udayakumar *et al*., 2009). Body weight loss in diabetic rats could be due to dehydration and catabolism of fats and proteins, as a result of unavailability of carbohydrates for utilization as an energy source (Virdi *et al*., 2003). Oral administration of 3.4DHIQS and GLB for 10 consecutive days to diabetic rats decreased their food consumption, water intake and improved b.wt. This could be due to a better control of the hyperglycemic state in the diabetic rats.

DM is also associated with hyperlipidemia with profound alteration in the concentration and composition of lipids (Odetola *et al*., 2006). The abnormally high concentration of serum lipids in DM is mainly due to an increase in the mobilization of free fatty acids (FAs) from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots (Al-Shamaony *et al*., 1994). Many studies have revealed that there is a significant alteration in the FAs composition of serum and a variety of tissues in both experimental and human DM (Saravanan and Ponmurugan, 2012). FAs, an important component of cell membranes, are eicosanoid precursors and are therefore required for both the structure and function of every cell in the body (Rajasekaran *et al*., 2006). Excess production of serum FAs by ALX-induced DM promotes the conversion of excess FAs into phospholipids and cholesterol in liver. These two substances along with excess of TG formed in the liver may be discharged into the blood in the form of lipoproteins (Bopanna *et al*., 1997). Changes in the concentrations of the lipid with DM contribute to the development of vascular disease (Howard *et al*., 1978). In our experiment, heart's ROW levels for TC, TG and TL were increased in diabetic controls. A decrease in relative heart weight was noted after treatment with GLB and especially with 3, 4 DHIQS at a dose of 5 mg kg^{-1}, this may be secondary to the improvement of b.wt. Significant reductions in TC, TG and TL concentrations were also observed after treatment with 3, 4 DHIQS and GLB. This hypolipidemic effect may be due to an increase in insulin secretion that ultimately led to a decrease in the synthesis of cholesterol and FAs.

Serum TP levels were found to be decreased in all diabetic rats. This is in agreement with the results obtained by Mansour *et al*. (2002). The decrease in TP might be due to microproteinuria which is an important clinical marker of Diabetic Nephropathy (DN) (Mauer *et al*., 1981) and/or might be due to increased protein catabolism (Almdal and Vilstrup, 1988). This decline also may be due to the inhibited oxidative phosphorylation processes which lead to decrease of protein synthesis, to the increase in the catabolic processes and to the reduction of protein absorption. Two mechanisms may account for the alterations in protein synthesis in diabetic rats. The defect in hepatic protein synthesis resulting from insulin deficiency is most likely due to a decrease in the amount of mRNA bound to ribosomes, leading to a decrease in the hepatic polysome population (Tragl and Reaven, 1972; Jefferson *et al*., 1983) and to the reduction in the number of ribosomal protein synthesis (Wool *et al*., 1966); thus, the capacity of the tissue for protein synthesis is decreased. Transport and uptake of amino acids (AAs) in peripheral tissues are depressed, causing an elevated circulating level of AAs particularly alanine which further enhance gluconeogenesis in the liver. Decline in ATP production and direct requirement for insulin-protein synthesis is decreased in all tissues (Yassin *et al*., 2004). The efficiency of GLB to restore TP concentrations is presumably due to its ability to increase insulin secretion (Annamala and Augusti, 1980). The proposed sites of action include hepatic uptake of glucogenic AAs, stimulation of AAs incorporation into protein and decreased proteolysis by activation and synthesis of transaminases and other enzymes catalyzing AAs transamination; the 3, 4 DHIQS could have the same mechanisms and sites of action.

Kidneys maintain optimum chemical composition of body fluids by acidification of urine and removal of metabolite wastes such as urea, uric acid, creatinine and ions (Virdi *et al*., 2003). The elevated levels of serum creatinine and blood urea nitrogen are significant markers of renal dysfunction reflecting a decline in the glomerular filtration rate (Mauer *et al*., 1981) and are considered to be an index of DN, one of the most common microvascular complications of DM where an uncontrolled increase in cellular glucose in kidney is seen (Balakumar *et al*., 2008). Metabolic factors such as advanced glycation end products (AGEs), sorbitol, beyond blood glucose level are also implicated in the pathogenesis of DN (Schrijvers *et al*., 2004). Moreover, increased lipid oxidation is also thought to trigger DN (Chisolm *et al*., 1992). This complication is associated with albuminuria and proteinuria

(Balakumar *et al*., 2008). Our results showed significant increases in the levels of urea and creatinine in diabetic rats when compared with normal rats. These results indicated that diabetes might lead to renal dysfunction. After treatment of ALX-diabetic rats with 3, 4 DHIQS and GLB, the levels of these parameters were significantly decreased when compared to the mean value of diabetic control group. This reduction may be a result of improved renal function due to reduced glucose concentration and subsequent glycosylation.

Increased levels of serum uric acid have been associated with insulin resistance (Modan *et al*., 1987) and with established type 2 DM (Wun *et al*., 1999). Previous studies have also demonstrated that uric acid is an independent predictor of incident type 2 DM in general populations (Dehghan *et al*., 2008), Our data showed that uric acid levels were increased in diabetic rats. This may be due to metabolic disturbance in DM reflected in high activities of xanthine oxidase, lipid peroxidation and increased TG and cholesterol (Madianov *et al*., 2002). Moreover, protein glycation in diabetes may lead to muscle wasting and increased release of purine, the main source of uric acid as well as in activity of xanthine oxidase (Anwar and Meki, 2003). ROW of kidney in diabetic controls was also found to be increased when compared with normal animals. This may be due to enlargement of lining cells of tubules, fatty infiltration, large area of hemorrhage and lymphocyte infiltration in ALX-induced diabetic rats (Evan *et al*., 1984). Moreover, a key morphological change associated with sustained hyperglycemia was the accumulation of glycogen granules in distal tubules which leads to the renal hypertrophy (Kang *et al*., 2005). In our present study, oral administration of 3, 4 DHIQS and GLB significantly decreased ROW of kidney and uric acid levels. The capability of GLB and 3, 4 DHIQS to protect renal weight and dysfunction seems to be as a result of its ability to protect from diabetes.

Liver is regarded as the central metabolic organ in the body, with an important role in glucose and lipid homeostasis (Saravanan and Pari, 2003). In the present study the serum enzyme activities of AST, ALT and ALP were increased in diabetic untreated rats. In diabetic animals, the changes in the levels of AST, ALT and ALP are directly related to changes in metabolism in which the enzymes are involved (Udayakumar *et al*., 2009). The increased activities of transaminases, during DM, could relate to excessive accumulation of AAs (glutamate and alanine) in the serum of diabetic animals as a result of AAs mobilization from protein stores. These excessive AAs are then converted to ketone bodies (α keto-glutaric and pyruvate) for which the enzyme GOT and GPT (AST and ALT) are needed, leading to increased enzyme activity. The higher levels of GOT and GPT in the diabetic animals, may give rise to a high concentration of glucose. In other words, the gluconeogenetic action of GOT and GPT plays the role of providing new supplies of glucose from other sources such as AAs (Kechrid and Bouzerna, 2004). Therefore, the transaminases are also responsible for the increased gluconeogenesis and ketogenesis (Udayakumar *et al*., 2009). On the other hand, the activities of transaminases are cytosolic marker enzymes reflecting hepatocellular necrosis as they are released into the blood after cell membrane damage (Kim *et al*., 2006). Therefore, we used the activities of AST, ALT and ALP in the circulation as indicators of hepatic damage. Moreover, AST and ALT levels act as an indicator of liver function hence restoration of normal level of these enzymes indicates the normal functioning of liver (Udayakumar *et al*., 2009). The increase in the activities of serum AST, ALT and ALP indicated that diabetes and/or alloxan may induced hepatic dysfunction and damage. Supporting our results it has been found that liver was necrotized in diabetic rats (El-Demerdash *et al*., 2005) and in diabetic patients (Deng *et al*., 2006). Therefore, the increment of the activities of AST, ALT and ALP in serum may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro *et al*., 1993) which gives an indication on the hepatotoxic effect of alloxan and chronic hyperglycemia. Lesion and disturbance of hepatic enzymes was demonstrated indirectly by inflammation and increased relative liver weight in the diabetic control group. The treatment of the diabetic rats with either GLB or 3.4DHIQS caused reduction in the activity of these enzymes in serum compared to the mean values of diabetic group. A possible explanation for the hepatic protective effects of GLB or 3.4DHIQS is that these treatments may inhibit the liver damage provoked by experimental diabetes.

Furthermore, the improvement of the liver damage by oral administration of GLB or 3, 4 DHIQS could be confirmed through studying their effect on the level of serum bilirubin. The results in Table 6 showed that the experimentally induced diabetes increased the level of direct and total bilirubin. However, GLB and 3, 4 DHIQS intake produced decrease in serum bilirubin of ALX-diabetic rats when compared to the diabetic rats. The increase in serum bilirubin (hyper-bilirubenimia) may be resulted from the decrease of liver uptake, conjugation or increase bilirubin production from hemolysis (Rana *et al*., 1996). Also, the elevation in plasma bilirubin indicates liver damage (El-Demerdash *et al*., 2005) as confirmed by the changes in the activities of serum enzymes. Our data showed a significant increase of relative spleen weight during experimental diabetes, this can be due to toxicity or immunogenicity of alloxan. We observed a moderate decrease of 3.44% of the ROW in diabetic rats treated with GLB compared to the diabetic control group. This decrease may be attributed to improved body weight after treatment as a result of better glycemic control. In spite of the remarkable antihyperglycemic activity of novel synthesized molecule, the diabetic rats treated with 3.4DHIQS at 2.5 and 5 mg kg^{-1} showed significant increases of the relative spleen weight by 10.34 and 17.24%, respectively, in comparison with diabetic untreated rats. This counterintuitive result can be explained by an immunological reaction manifested by splenic proliferative response against the new sulfonamide.

CONCLUSION

The results of the present investigation clearly indicate a significant dose dependant anti-diabetic and hypolipidemic effect of the 3, 4-dihydroisoquinolin-2(1H)-sulfonamide on ALX-induced diabetic rats. These activities were comparable with the glibenclamide wich is used for therapeutic as conventional drug. However, the increases of the relative spleen weight could indicate an immunological risk, so further more studies must be undertaken to elucidate the exact mechanism of action and to evaluate its immunogenicity.

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