International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 11 (3): 226-235, 2015
ISSN 1811-7775

ansinet
Asian Network for Scientific Information

RESEARCH ARTICLE OPEN ACCESS

DOI: 10.3923/ijp.2015.226.235

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

[1]H. Berredjem, [1,2]Y. Réggami, [3]M. Benlaifa, [4]M. Berredjem and [1]N. Bouzerna
[1]Laboratory of Biochemistry and Applied Microbiology, Department of Biochemistry, Faculty of Sciences, University of Badji Mokhtar-Annaba, Algeria
[2]Department of Microbiology and Biochemistry, Faculty of Sciences, University of M'sila, Algeria
[3]Laboratory of Cellular Toxicology, Department of Biology, Faculty of Sciences, University of Badji Mokhtar-Annaba, Algeria
[4]Laboratory of Applied Organic Chemistry, Synthesis of Biomolecules and Molecular Modelling Group, Department of Chemistry, University of Badji Mokhtar-Annaba, Algeria

ARTICLE INFO
Article History:
Received: November 17, 2014
Accepted: February 12, 2015

Corresponding Author:
H. Berredjem,
Laboratory of Biochemistry and Applied Microbiology,
Department of Biochemistry,
Faculty of Sciences, University of Badji Mokhtar, P.O. Box 12, 23000 Annaba, Algeria
Tel: +21338871061
Fax: +21338871061

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.

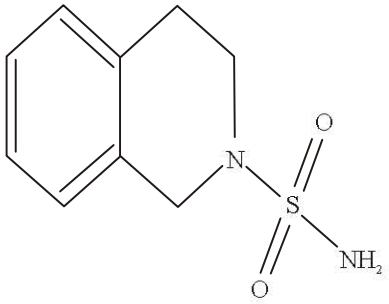

Fig. 1: Chemical structure of the 3, 4-dihydroisoquinolin-2(1H)-sulfonamide (3, 4 DHIQS)

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).

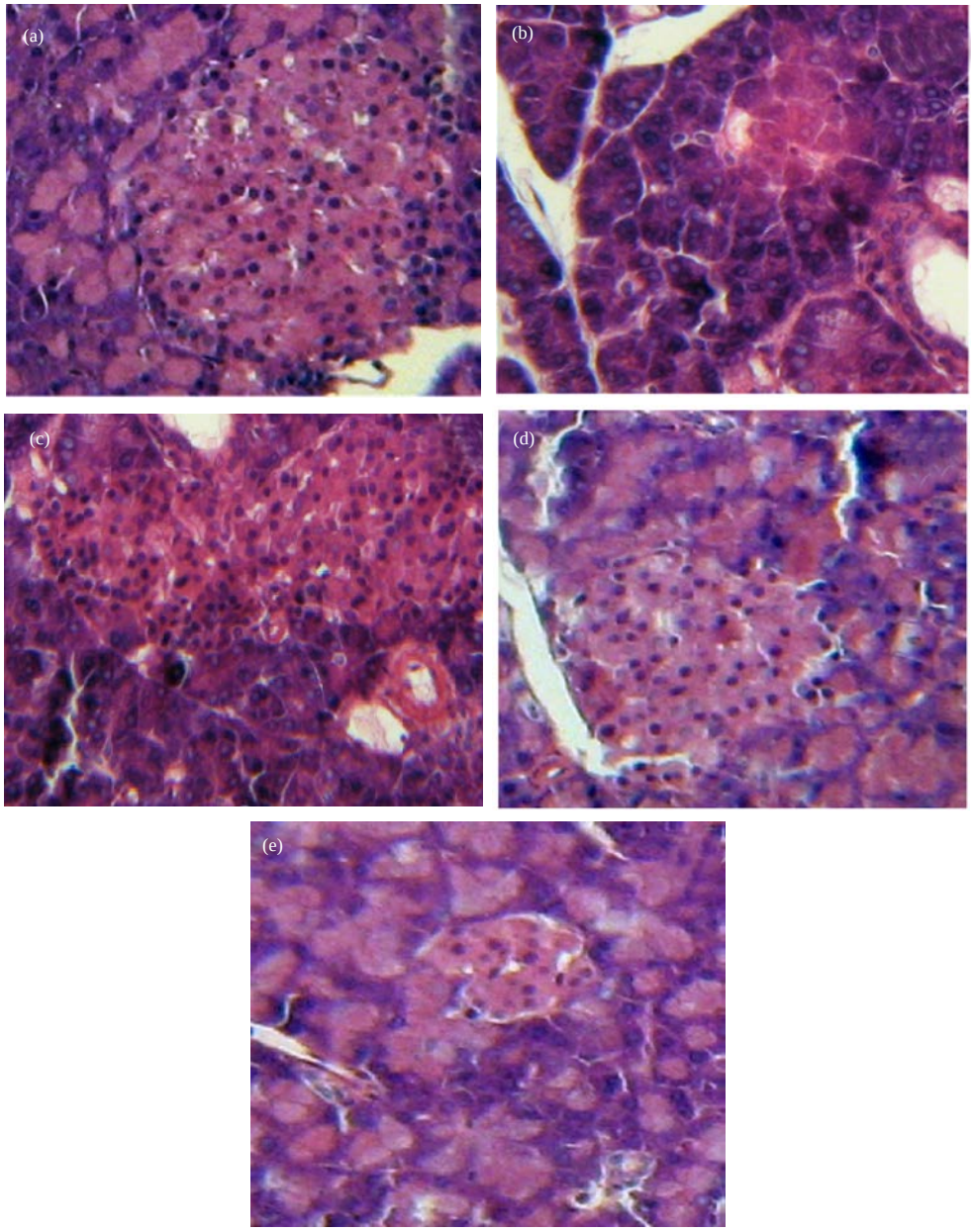

Fig. 2(a-e): Histological comparison of the pancreatic islet tissues in normal and ALX-induced diabetic rats. Photomicrograph showing one single pancreatic islet of the normal group (a) Diabetic control, (b) Diabetic +GLB at 2.5 mg kg^{-1} BW group, (c) Diabetic + 3, 4 DHIQS at 2.5 mg kg^{-1} BW group, (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.

ACKNOWLEDGMENTS

We thank Dr. Omar Laouar, from the Anatomopathology Department of CHU IBEN ROCHED (Annaba, Algeria), for helping us to carry out histological studies.

REFERENCES

Akhtar, A., M. Rashid, M.I. Ibne Wahed, M.R. Islam and S.M. Shaheen *et al*., 2007. Comparison of long-term antihyperglycemic and hypolipidemic effects between *Coccinia cordifolia* (Linn.) and *Catharanthus roseus* (Linn.) in Alloxan-induced diabetic rats. Res. J. Med. Med. Sci., 2: 29-34.

Al-Shamaony, L., S.M. Al-khazraji and H.A.A. Twaij, 1994. Hypoglycaemic effect of *Artemisia herba alba*. II. Effect of a valuable extract on some blood parameters in diabetic animals. J. Ethnopharmacol., 43: 167-171.

Almdal, T.P. and H. Vilstrup, 1988. Strict insulin therapy normalises organ nitrogen contents and the capacity of urea nitrogen synthesis in experimental diabetes in rats. Diabetlogia, 31: 114-118.

American Diabetes Association, 2009. Diagnosis and classification of diabetes mellitus. Diabetes Care, 32: S62-S67.

Annamala, P.T. and K.T. Augusti, 1980. Studies on the biochemical effects of glibenclamide on alloxan diabetic rabbits. Experientia, 36: 383-384.

Anwar, M.M. and A.R.M.A. Meki, 2003. Oxidative stress in streptozotocin-induced diabetic rats: Effects of garlic oil and melatonin. Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol., 135: 539-547.

Balakumar, P., V.A. Chakkarwar, V. Kumar, A. Jain, J. Reddy and M. Singh, 2008. Experimental models for nephropathy. J. Renin Angiotensin Aldosterone Syst., 9: 189-195.

Bopanna, K.N., J. Kannan, G. Sushma, R. Balaraman and S.P. Rathod, 1997. Antidiabetic and antihyperlipaemic effects of neem seed kernel powder on alloxan diabetic rabbits. Indian J. Pharmacol., 29: 162-172.

Bouasla, R., M. Berredjem, S. Hessainia, Z. Chereait, H. Berredjem and N.E. Aouf, 2011. Synthesis, acylation, antibacterial and hypoglycemic activity of novel sulfonamide. J. Chem. Chem. Eng., 5: 1153-1159.

Brownlee, M., 2001. Biochemistry and molecular cell biology of diabetic complications. Nature, 414: 813-820.

Chen, L., D.J. Magliano and P.Z. Zimmet, 2012. The worldwide epidemiology of type 2 diabetes mellitus-present and future perspectives. Nat. Rev. Endocrinol., 8: 228-236.

Chisolm, G.M., K.C. Irwin and M.S. Penn, 1992. Lipoprotein oxidation and lipoprotein-induced cell injury in diabetes. Diabetes, 41: 61-66.

Dehghan, A., M. Van Hoek, E.J.G. Sijbrands, A. Hofman and J.C.M. Witteman, 2008. High serum uric acid as a novel risk factor for type 2 diabetes. Diabetes Care, 31: 361-362.

Del Prato, S. and N. Pulizzi, 2006. The place of sulfonylureas in the therapy for type 2 diabetes mellitus. Metabolism, 55: 20-27.

Deng, Y.G., Z.S. Zhao, M. Wang, S.O. Su and X.X. Yao, 2006. Diabetes mellitus with hepatic infarction: A case report with literature review. World J. Gastroenterol., 12: 5091-5093.

El-Demerdash, F.M., M.I. Yousef and N.I.A. El-Naga, 2005. Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. Food Chem. Toxicol., 43: 57-63.

Evan, A.P., S.A. Mong, B.A. Connors, G.R. Aronoff and F.C. Luft, 1984. The effect of alloxan and alloxan-induced diabetes on the kidney. Anatom. Rec., 208: 33-47.

Fawcett, J.K. and J.E. Scott, 1960. A rapid and precise method for the determination of urea. J. Clin. Pathol., 13: 156-159.

Green, J.B. and M.N. Feinglos, 2006. Are sulfonylureas passe? Curr. Diab. Rep., 6: 373-377.

Gupta, M.P., N.G. Solis, M. Esposito and S. Sanchez, 1984. Hypoglycemic activity of *Neurolaena lobata* (L.) R. Br. J. Ethnopharmacol., 10: 323-327.

Hamden, K., S. Carreau, K. Jamoussi, S. Miladi, S. Lajmi, D. Aloulou, F. Ayadi and A. Elfeki, 2009. 1α, 25 dihydroxyvitamin D3: Therapeutic and preventive effects against oxidative stress, hepatic, pancreatic and renal injury in Alloxan-induced diabetes in rats. J. Nutr. Sci. Vitaminol., 55: 215-222.

Howard, B.V., P.J. Savage, L.J. Bennion and P.H. Bennett, 1978. Lipoprotein composition in diabetes mellitus. Atherosclerosis, 30: 153-162.

Jefferson, L.S., W.S.L. Liao, D.E. Peavy, T.B. Miller, M.C. Appel and J.M. Taylor, 1983. Diabetes-induced alterations in liver protein synthesis. Changes in the relative abundance of mRNAs for albumin and other plasma proteins. J. Biol. Chem., 258: 1369-1375.

Joy, K.L. and R. Kuttan, 1999. Anti-diabetic activity of *Picrorrhiza kurroa* extract. J. Ethropharmacol., 67: 143-148.

Kang, J., X.S. Dai, T.B. Yu, B. Wen and Z.W. Yang, 2005. Glycogen accumulation in renal tubules, a key morphological change in the diabetic rat kidney. Acta Diabetologica, 42: 110-116.

Kechrid, Z. and N. Bouzerna, 2004. Effect of zinc deficiency and experimental diabetes on glutamate oxaloacetate, glutamate pyruvate aminotransferases and alkaline phosphatase activities in rats. Int. J. Diabetes Metabol., 11: 14-18.

Kim, J.S., B.B. Ju, C.W. Choi and S.C. Kim, 2006. Hypoglycemic and antihyperlipidemic effect of four Korean medicinal plants in alloxan induced diabetic rats. Am. J. Biochem. Biotechn., 2: 154-160.

Lenzen, S., 2008. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia, 51: 216-226.

Madianov, I.V., M.I. Balabolkin, D.S. Markov and T.N. Markova, 2002. [Main causes of hyperuricemia in diabetes mellitus]. Ter. Arkh., 72: 55-58, (In Russian).

Mansour, H.A., A.S.A. Newairy, M.I. Yousef and S.A. Sheweita, 2002. Biochemical study on the effects of some Egyptian herbs in alloxan-induced diabetic rats. Toxicology, 170: 221-228.

Maren, T.H., 1976. Relations between structure and biological activity of sulfonamides. Annu. Rev. Pharmacol. Toxicol., 16: 309-327.

Mauer, S.M., M.W. Steffes and D.M. Brown, 1981. The kidney in diabetes. Am. J. Med., 70: 603-612.

Modan, M., H. Halkin, A. Karasik and A. Lusky, 1987. Elevated serum uric acid: A facet of hyperinsulinaemia. Diabetologia, 30: 713-718.

Nathan, D.M., J.B. Buse, M.B. Davidson, E. Ferrannini and R.R. Holman *et al*., 2009. Medical management of hyperglycemia in type 2 diabetes: A consensus algorithm for the initiation and adjustment of therapy: A consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care, 32: 193-203.

Navarro, C.M., P.M. Montilla, A. Martin, J. Jimenez and P.M. Utrilla, 1993. Free radical scavenger and antihepatotoxic activity of *Rosmarinus tomentosus*. Plant Med., 59: 312-314.

Odetola, A.A., O. Akinloye, C. Egunjobi, W.A. Adekunle and A.O. Ayoola, 2006. Possible antidiabetic and antihyperlipidaemic effect of fermented *Parkia biglobosa* (JACQ) extract in alloxan-induced diabetic rats. Clin. Exp. Pharmacol. Physiol., 33: 808-812.

Prentki, M. and C.J. Nolan, 2006. Islet β cell failure in type 2 diabetes. J. Clin. Invest., 116: 1802-1812.

Pupim, L.B., O. Heimburger, A.R. Qureshi, T.A. Ikizler and P. Stenvinkel, 2005. Accelerated lean body mass loss in incident chronic dialysis patients with diabetes mellitus. Kidney Int., 68: 2368-2374.

Rajasekaran, S., K. Ravi, K. Sivagnanam and S. Subramanian, 2006. Beneficial effects of *aloe vera* leaf gel extract on lipid profile status in rats with streptozotocin diabetes. Clin. Exp. Pharmacol. Physiol., 33: 232-237.

Rana, S.V., R. Singh and S. Verma, 1996. Protective effects of few antioxidants on liver function in rats treated with cadmium and mercury. Indian J. Exp. Biol., 34: 177-179.

Saravanan, G. and L. Pari, 2003. Effect of Cogent db, a herbal drug on serum and tissue lipid metabolism in experimental hyperglycaemic rats. Diabetes Obes. Metab., 5: 156-162.

Saravanan, G. and P. Ponmurugan, 2012. Ameliorative potential of S-allylcysteine: Effect on lipid profile and changes in tissue fatty acid composition in experimental diabetes. Exp. Toxicol. Pathol., 64: 639-644.

Schrijvers, B.F., A. De Vriese and A. Flyvbjerg, 2004. From hyperglycemia to diabetic kidney disease: The role of metabolic, hemodynamic, intracellular factors and growth factors/cytokines. Endocrine Rev., 25: 971-1010.

Scozzafava, A., T. Owa, A. Mastrolorenzo and C.T. Supuran, 2003. Anticancer and antiviral sulfonamides. Curr. Med. Chem., 10: 925-953.

Shaw, J.E., R.A. Sicree and P.Z. Zimmet, 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res. Clin. Pract., 87: 4-14.

Smith, D.A. and R.M. Jones, 2008. The sulfonamide group as a structural alert: A distorted story? Curr. Opin. Drug Discov. Develop., 11: 72-79.

Tragl, K.H. and G.M. Reaven, 1972. Effect of insulin deficiency on hepatic ribosomal aggregation. Diabetes, 21: 84-88.

Udayakumar, R., S. Kasthurirengan, T.S. Mariashibu, M. Rajesh and V.R. Anbazhagan *et al*., 2009. Hypoglycaemic and hypolipidaemic effects of *Withania somnifera* root and leaf extracts on alloxan-induced diabetic rats. Int. J. Mol. Sci., 10: 2367-2382.

Virdi, J., S. Sivakami, S. Shahani, A.C. Suthar, M.M. Banavalikar and M.K. Biyani, 2003. Antihyperglycemic effects of three extracts from *Momordica charantia*. J. Ethnopharmacol., 88: 107-111.

Wool, I.G., O.R. Rampersad and A.N. Moyer, 1966. Effect of insulin and diabetes on protein synthesis by ribosomes from heart muscle: Significance for theories of the Hormone's mechanism of action. Am. J. Med., 40: 716-720.

Wun, Y.T., C.S. Chan and C.S. Lui, 1999. Hyperuricaemia in type 2 diabetes mellitus. Diabetes Nutr. Metab., 12: 286-291.

Yassin, M.M., A.R.A. Ashour and N.R. Elyazji, 2004. Alterations in body weight, protein profile, non-protein nitrogen constituents and kidney structure in diabetic rats under glibenclamide treatment. J. Islamic Univ. Gaza, 12: P37-P54.