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ORIGINAL ARTICLE

Additive Effect of Lipid Lowering Drug (Simvastatin) in Combination with Antidiabetic Drug (Glibenclamide) on Alloxan Induced Diabetic Rats with Long Term Dyslipidemia

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Abstract High blood glucose level, elevated level of liver enzyme, necrosis and shrinkage of islets of Langerhans has been implicated in the pathogenesis of type 2 diabetes. High blood glucose cause oxidative stress, production of free radical as well as elevated SGPT and SGOT level. Both glibenclamide and simvastatin in fixed dose used as antihyperglycemic antidyslipidemic and antioxidative agents for type 2 diabetes treatment. This study therefore aimed to evaluate the antihyperglycemic, antidyslipidemic and antioxidative effect of fixed dose combination of glibenclamide (0.6 mg/70 kg body weight) and simvastatin (5 mg/70 kg body weight) on long term alloxan induced diabetic rats with cardiovascular disease using various diagnostic kits as a parameter of phamacotherapeutic and pharmacological effect. The study was carried out using 96 Swiss Albino male rats weighing about 200–220 g. Combination therapy induced a significant decrease in blood glucose level in alloxan induced diabetic rats, from 33.75 ± 1.65 to 5.80 ± 0.07 mmol/l 2 h after last dose administration, after 4 weeks treatment. In case of dyslipidemic effect, combination therapy reduced total cholesterol (45 %), triglyceride (36 %) and low density lipoprotein-cholesterol (32 %) levels significantly and increased high density lipoprotein-cholesterol level (57 %) in comparison with their respective diabetic control groups. Results of this study showed that combination therapy effectively decreased SGPT (ALAT) (55 %) and SGOT (ASAT) (51 %) in comparison with diabetic control group. It was also observed that catalase and superoxide dismutase enzyme activity was increased by 58 and 91 % respectively in comparison with diabetic control group after 4 weeks treatment with combination of both drugs. In conclusion, these findings of combination therapy (glibenclamide and simvastatin) on alloxan induced diabetes in rats are significantly better than monotherapy using single drug. The results of the present study suggest that, combination of the fixed dose of glibenclamide and simvastatin might be efficacious in patients with diabetic dyslipidemia and increased oxidative stress. Furthermore, this combination therapy offer dosage convenience to the patients and by virtue of its dual mode of action might be a useful addition to the therapeutic armamentarium for patients with diabetic dyslipidemia and oxidative stress.

Keywords Diabetes with CVD · Glibenclamide · Simvastatin · Combination drugs · Beneficial effects

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Introduction

Diabetes is a major threat to global public health that is rapidly getting worse, and the biggest impact is on adults of

working age in developing countries. In most developing countries at least one in ten deaths in adults aged 35–64 is attributable to diabetes, and in some the figure is as high as one in five [1]. Diabetes is a common condition and its frequency is dramatically rising all over the world. In 2010, there were 285 million people with diabetes worldwide, and by 2030 this figure is expected to nearly double to reach a total of 439 million [2]. Diabetes has become one of the major causes of premature illness and death in most countries, mainly through the increased risk of cardiovascular disease (CVD). Diabetes is usually accompanied by consistent hyperglycemia and increased production of free radicals [3–5] or impaired antioxidant defenses [6], and this oxidative stress ultimately leads to apoptosis or myocardial cell injury and dysfunction by alteration of gene expression and modification of cellular responses [7]. Mechanisms by which increased oxidative stress is involved in the diabetic complications are partly known, including activation of transcription factors, advanced glycated end products and protein kinase C [8]. High glucose level also promotes intracellular lipid accumulation in vascular smooth muscle cells by impairing cholesterol influx and efflux balance [9]. Hypercholesterolemia and hypertriglyceridemia are independent risk factors that alone or together can accelerate the development of atherosclerosis and progression of atherosclerotic lesions [10, 11]. At present, the treatment of diabetes mainly involves a sustained reduction in hyperglycemia by the use of biguanides, thiazolidinediones, sulfonylureas D-phenylalanine and α-glucosidase inhibitors in addition to insulin. However, due to unwanted side effects, the efficacies of these compounds are debatable and there is a demand as well as continuation of these drugs for the treatment of diabetes with CVD [12]. Moreover, long-term induction of diabetes without any lipid lowering drug leads to the risk of CVD. Hence, combination therapy has been suggested as a rich source of potentially useful antidiabetic and hypolipidemic drugs. Many traditionally combined treatments (only antidiabetic drugs) for diabetes are used throughout the world. So there is no alternative way to treat long term diabetes without using combination therapy containing antidiabetic and hypolipidemic drugs. The attributed antihyperglycemic and antidyslipidemic effects of this combination therapy is due to their ability to restore the function of pancreatic tissues by causing an increase insulin output, or to inhibit the intestinal absorption of glucose, or to facilitate the metabolites in insulin dependent processes [13], and their ability to inhibit the cholesterol biosynthetic pathway by inhibiting the enzyme HMG-CoA reductase [14]. Hence, treatment with combination therapy has an effect on protecting β-cells and smoothing out fluctuation in glucose level and cholesterol biosynthetic pathway. Therefore, the present study was aimed to provide a strong view on experimental studies in animals and to find out the most effective and commonly used hypoglycemic and lipid lowering drugs combination on long term alloxan induced diabetes with CVD in rats.

Materials and Methods

Animal Studies

All protocols for the animal experiments were reviewed and approved by the Animal Care and Use Committee of Institute of Biological Science, University of Rajshahi. Swiss Albino male rats weighing about 200–220 g, aged 2 months, were purchased from animal's house of International Centre for Diarrheal Disease Research, Bangladesh (ICDDRB). Within 96 Swiss Albino male rats, 12 rats were randomly assigned into group A, C and F, 4 rats in each group for glucose tolerance test. Another 12 rats were randomly assigned into 3 groups A, B, and C (4 rats in each group), for repeated dose treatment for 1 week for blood glucose test. For 2 weeks protocol, a total number of 16 rats were assigned into 4 groups A, B, C and D (4 rats in each group) for the determination of blood glucose and lipid profile. For 4 weeks protocol, a total number of 20 rats were assigned into 5 groups A, B, C, D and E (4 rats in each group) for the determination of blood glucose, lipid profile, glycogen content, antioxidant activity and histological studies. Prior to commencement of the experiments, all the rats were acclimatized to the new environmental condition for a period of 1 week. In both protocols (2 weeks for short term and 4 weeks for long term), alloxan [120 mg/kg body weight (BW)] was injected intraperitonially (i.p.) in rats. Alloxan induced rats were treated with i.p. injection of glibenclamide (1.2 mg/70 kg BW) and simvastatin (10 mg/70 kg BW) for 2 weeks in the monotherapy and for 4 weeks in combination therapy.

Time Limit Specification

Time limit has been designed into three categories for the treatment of alloxan induced diabetic rats named as 1 week treatment protocol, 2 weeks treatment protocol and 4 weeks treatment protocol. One and two weeks treatment protocol is termed as short term treatment protocol and specified for the determination of oral glucose tolerance test, blood glucose level, lipid profile level and liver glycogen level in monotherapy. On the other hand 4 weeks treatment protocol is termed as long term treatment protocol and specified for the determination of blood glucose level, lipid profile level; liver glycogen level, liver dysfunction indices, antioxidant activity test and islets of Langerhans cell morphology in combination therapy. Short term treatment protocol need not any combination therapy

because it takes minimum 3–4 weeks to develop CVD like atherosclerotic plaque, left ventricular hypertrophy etc. if alloxan induced rats were left untreated more than 3 weeks there is evidence to increase oxidative which lead to increase ROS production. ROS reduce bioavailability of nitric oxide (NO), leading to endothelial dysfunction. ROS influence the activity of variety of cellular signaling pathways ultimately leading the changes in the expression of redox-sensitive genes, which regulate cellular process involved in cellular apoptosis/death that may involved in the pathogenesis of CVD. The aim of 4 weeks treatment of combination therapy is to reduce the risk of CVD as well as dyslipidemia. In both short and long term treatment protocol alloxan induced rats were treated with i.p. injection of glibenclamide and simvastatin for 2 weeks in the monotherapy and for 4 weeks in combination therapy.

Collection of Blood Serum, Liver and Pancreases

After completing the 2 and 4 weeks treatment the rats were at first anesthetized with Phenobarbital sodium (Emer, Opsonin Pharma Ltd, Barisal, Bangladesh). Then after cutting the abdominal skin, thoracic artery was opened. 3–5 ml of blood was collected directly from thoracic artery by heparinized syringe. Pancreases were collected. At last the blood was centrifuged at 4,000 rpm for 15 min and the serum was obtained.

Measurement of Glucose Tolerance

The oral glucose tolerance test was determined according to the method specified for glucose tolerance test. After overnight fasting, all animals ($n = 4$) in control group received glucose (1.5 g/kg per oral) and blood glucose was estimated at 0, 0.5, 1, 2 h interval using glucometer. Glibenclamide (1.2 mg/70 kg BW per oral) was administered 2 h prior to glucose administration.

Measurement of Glucose Level

Glibenclamide, simvastatin and combination of both were administered daily in alloxan induced diabetic rats for different and distinct time limit protocols defined as 1, 2 and 4 weeks treatment protocol. After 1, 2 and 4 week's treatment with drugs, blood glucose level was determined 2 h after last dose using glucometer (One Touch Horizon, USA).

Measurement of Lipid Profile

After completing the treatment of drugs, the rats were at first anesthetized with sodium phenobarbital. Then abdominal skin was cut and thoracic artery was opened. Finally 3–5 ml of blood was collected directly from thoracic artery by syringes. The blood was centrifuged at 4,000 rpm for 10 min and the serum was obtained. Serum lipid profile, such as total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C), was assessed using diagnostic kits (Human, Germany).

Evaluation of Superoxide Dismutase (SOD) and Catalase Enzyme Activity

After the experimental period, the animals were sacrificed; liver was isolated, homogenized in chilled Tris buffer at a concentration of 10 % (w/v). The homogenate was centrifuge at 4,000 rpm for 15 min in cold centrifuge and supernatant was assayed for SOD and catalase activity. The catalase activity was assayed by the method of [15] and SOD activity was determined by the method of [16].

Measurement of SGPT (ALAT) and SGOT (ASAT) Level

The blood serum was used for testing the serum SGPT (ALAT) and SGOT (ASAT) levels. The concentrations were analyzed by taking absorbance by UV spectrophotometer, using diagnostic kits (Human, Germany). Kinetic method is used for the determination of SGPT (ALAT) and SGOT (ALAT) activity according to the recommendations of the Expert Panel of the IFCC without pyridoxalphosphate activation. This test is carried out as the parameter of liver dysfunction indices.

Histopathological Analysis

Histological studies were performed for the investigation of pancreatic beta cell recovery. In brief one section was obtained from each pancreas, and mounted on slides and stained with hematoxylin and eosin. To evaluate the extent of pancreacyte recovery cross-sectional images of pancreacyte were scanned at 400× magnifications. Approximately 20 cross-sections of pancreacyte were analyzed in each pancreas. Average values for each pancreas were used for analysis. All images were taken using a KRUSS (A Kruss Optronic, Humburg, Germany), and all measurements were determined using Scion Image software (Scion Corporation, Frederick, MD, USA).

Statistical Analysis

The results were expressed as mean ± SEM using Graph Pad Prism (version 4.0) computer program (Graph pad Software San Diego, CA, USA). We used a one-way analysis of variance (ANOVA), followed by Dunnett's post hoc test or students paired or unpaired t test where

Fig. 1 Effect of glibenclamide on oral glucose tolerance test. Data were presented as mean ± SEM; n = 4 in each group, $*p < 0.05$ compared to glucose control group (student's unpaired t test)

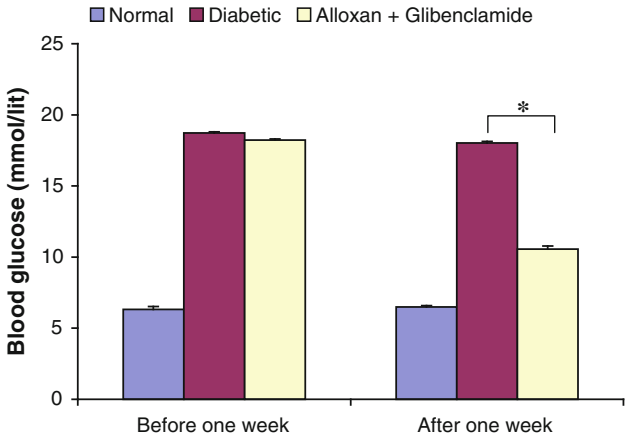

Fig. 2 Effect of repeated dose treatment of glibenclamide for 1 week on blood glucose level in alloxan induced diabetic rats (data were presented as mean ± SEM; n = 4 each group, $*p < 0.05$ compared to diabetic control group)

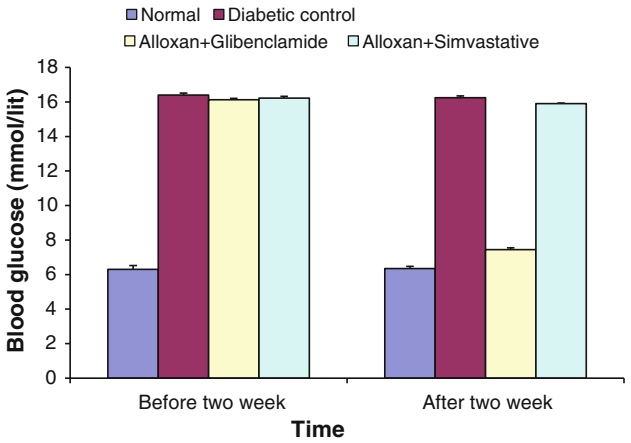

Fig. 3 Effect of repeated dose treatment of glibenclamide and simvastatin for 2 weeks on blood glucose level in alloxan induced diabetic rats. Data were presented as mean ± SEM; n = 4 in each group, $*p < 0.05$ compared to diabetic control group)

appropriate. The statistical method applied in each analysis was described in each figure. Results were considered to be significant when p values were less than 0.05 ($p < 0.05$).

Results

The effect of drug alone (glibenclamide/simvastatin) and combination (glibenclamide and simvastatin) on the parameters of oral glucose tolerance, blood glucose level, lipid profile (TC, TG, LDL-C, HDL-C), antioxidant property liver dysfunction indices and histopathology of pancreas of islets of langerhans were performed for both short and long term alloxan induced diabetic rats.

Effect of Glibenlcamide on Oral Glucose Tolerance Test

The effect of glibenclamide on FBG level in glucose induced hyperglycemic rats is shown in the Fig. 1. Most significant decrease in blood glucose level had been observed at the dose of 1.5 mg/70 kg BW from 9.575 ± 0.22 to 7.15 ± 0.10 mmol/l at half hour compared to control glucose fed rats.

Effect of Glibenclamide and Simvastatin on Blood Glucose Level in Short Term Alloxan Induced Diabetic Rats

Short term i.p. injection of alloxan in rats significantly increased blood glucose level when compared with control rats. We estimated blood glucose level and showed that in 1 week treatment protocol glibenclamide alone decreased blood glucose level from 18.72 ± 0.08 to 10.65 ± 0.22 mmol/l, and in 2 weeks treatment protocol glibenclamide alone significantly decreased blood glucose level from 16.40 ± 0.12 to 7.45 ± 0.10 mmol/l whereas simvastatin alone failed to reduce significant amount of blood glucose level when compared with alloxan induced diabetic rats (Figs. 2, 3).

Effect of Glibenclamide and Simvastatin on Lipid Profile in Short Term Alloxan Induced Diabetic Rats

Short term induction of diabetes by alloxan in rats significantly altered lipid profile when compared with normal rats. To make clear the individual effect of glibenclamide and simvastatin on lipid profile, we examined TC, TG, LDL-C and HDL-C level. After 2 weeks treatment by drugs individually, we found that both glibenclamide and simvastatin reduced TC level 4.3 and 42.58 %, TG level 8.39 and 32.17 %, LDL-C level 4.17 and 27.32 %, whereas they increased HDL-C level 9.35 and 51.07 % respectively when compared with diabetic rats. These results suggested

Table 1 Effect of glibenclamide and simvastatin on lipid profile after 2 weeks treatment in short term alloxan induced diabetic rats

Lipid Profile (mg/dl)	Treatment group			
	Normal	Alloxan	Alloxan + glibenclamide	Alloxan + simvastatin
TC	165.25 ± 4.90	263 ± 13.16*	251.75 ± 13.12	151 ± 0.91*
TG	120.5 ± 8.09	193.5 ± 2.39*	177.25 ± 0.85	131.25 ± 1.31*
LDL-C	124 ± 2.48	185.75 ± 5.57*	178 ± 3.54	135 ± 1.47*
HDL-C	55.25 ± 1.25	34.75 ± 0.85*	38 ± 1.08	52.5 ± 0.64*

Data were presented as mean ± SEM; $n = 4$ in each group, * $p < 0.05$ compared to diabetic control group

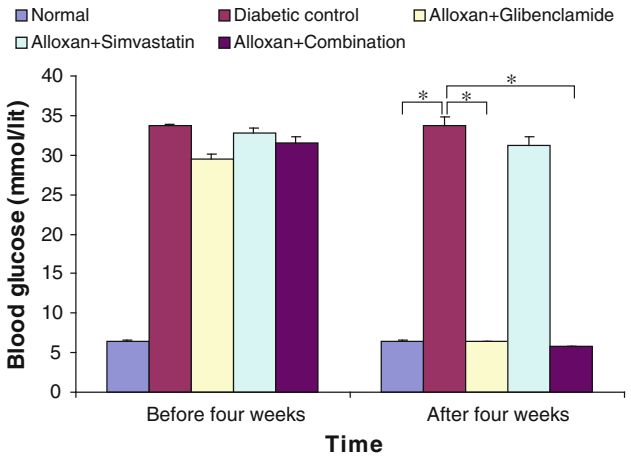

Fig. 4 Effect of repeated dose treatment of glibenlamide, simvastatin and combination for 4 weeks on blood glucose level in alloxan-induced diabetic rats (data were presented as mean ± SEM; n = 4 in each group, *$p < 0.05$ compared to diabetic control group)

that simvastatin but not glibenclamide acted as lipid lowering agents. We performed histological analysis in alloxan induced rats to check whether or not short term induction could recover Langerhans cell of islets of pancreas. Interestingly, we could not observe any change on pancreas islets of Langerhans morphology in diabetic rats (Table 1).

Effect of Combination Therapy on Blood Glucose Level in Long Term Alloxan Induced Diabetic Rats

Long term induction of diabetes by alloxan in rats increased blood glucose level (33.75 mmol/l) twice when compared with short term induction of diabetes (16.4 mmol/l) (Figs. 2, 3). To investigate the effect of mono and combination therapy on blood glucose level, we examined blood glucose level after 4 weeks treatment of glibenclamide and simvastatin alone and combination of both in diabetic rats. After 4 weeks treatment, glibenclamide alone and combination of both significantly decreased blood glucose level from 33.75 ± 1.65 to 6.37 ± 0.08 and 33.75 ± 1.65 to 5.8 ± 0.07 mmol/l, respectively when compared with diabetic rats, but the effect of combination therapy on blood glucose level was higher than that of monotherapy alone. Simvastatin failed to reduce significant amount of blood glucose level when compared with diabetic rats (Fig. 4).

Effect of Combination Therapy on Lipid Profile in Long Term Alloxan Induced Diabetic Rats

To clarify the effect of mono and combination therapy on lipid profile, we examined TC, TG, LDL-C and HDL-C level, after 2 weeks treatment with glibenclamide and simvastatin alone and combination of both in long term alloxan induced rats. After treatment, it was found that glibenclamide, simvastatin and combination of both reduced TC level 2.24, 40.55 and 45.05 %, TG level 3.51, 30.68 and 35.76 %, and LDL-C level 4.80, 28.39 and 32.23 %, and they increased HDL-C level 8.9, 45.89 and 56.84 %, respectively when compared with diabetic rats. In contrast, the effect of combination therapy on lipid profile was higher than that of monotherapy alone. Glibenclamide did not show significant effect on lipid profile (Table 2). These results showed that combination therapy was more effective in lipid profile than the monotherapy in long term alloxan induced diabetic rats.

Effect of Combination Therapy on Catalase and Superoxide Dismutase Enzyme Activity on Long Term Alloxan Induced Diabetic Rats

The effect of glibenclamide, simvastatin and combination of both drugs on catalase enzyme activity in alloxan induced diabetic rats was shown. After 4 weeks treatment with glibenlamide, simvastatin and combination of both drugs it was observed that catalase enzyme activity was increased 6.72, 34.97 and 57.84 % and superoxide enzyme activity was increased 7.25, 86.10, and 90.67 % in comparison with diabetic control group respectively. This result revealed that the combination therapy showed better antioxidant activity than monotherapy in long term alloxan induced diabetic rats. We performed same test for short term alloxan induced diabetic rats for both enzyme activity but interestingly, we could not observe any change in enzyme activity on diabetic rats (Table 3).

Table 2 Effect of combination therapy (glibenclamide and simvastatin) on lipid profile after 4 weeks treatment in long term alloxan induced diabetic rats

Lipid profile (mg/dl)	Treatment group				
	Normal	Alloxan	Alloxan + glibenclamide	Alloxan + simvastatin	Alloxan + combination
TC	165 ± 2.19	278 ± 1.29*	271.75 ± 1.03*	165.25 ± 0.95	152.75 ± 0.95*
TG	125.5 ± 2.25	192.25 ± 2.62*	176.75 ± 3.12*	133.25 ± 0.85	123.5 ± 1.05*
LDL-C	125.7 ± 1.49	182.25 ± 3.64*	173.5 ± 1.04	130.5 ± 0.64*	123.5 ± 1.19*
HDL-C	55.25 ± 1.11	36.5 ± 1.19*	39.75 ± 0.45	53.25 ± 0.85*	57.25 ± 0.85*

Data were presented as mean ± SEM; $n = 4$ in each group, * $p < 0.05$ compared to diabetic control group

Table 3 Effect of glibenclamide and simvastatin (alone and combination of both drugs) on catalase enzyme activity after 4 weeks treatment in long term alloxan induced diabetic rats

Enzyme activity (U/mg protein)	Treatment group				
	Normal	Alloxan	Alloxan + glibenclamide	Alloxan + simvastatin	Alloxan + combination
Catalase activity	86.25 ± 1.25	55.75 ± 1.11*	59.5 ± 0.64	75.25 ± 0.85*	88 ± 0.91*
SOD activity	9.7 ± 0.11	4.825 ± 0.08*	5.175 ± 0.11	8.975 ± 0.09*	9.2 ± 0.09*

Data were presented as mean ± SEM; $n = 4$ in each group, * $p < 0.05$ compared to diabetic control group

Table 4 Effect of glibenclamide and simvastatin (alone and combination of both drugs) on liver dysfunction indices activity after 4 weeks treatment in long term alloxan induced diabetic rats

Liver dysfunction indices activity (U/L)	Treatment group				
	Normal	Alloxan	Alloxan + glibenclamide	Alloxan + simvastatin	Alloxan + combination
SGPT	24.75 ± 0.63	73 ± 1.29*	42.75 ± 0.85*	69.5 ± 0.64	33 ± 1.47*
SGOT	32 ± 0.41	75.25 ± 0.85*	53.25 ± 0.48*	72.25 ± 0.85	72.25 ± 0.85*

Data were presented as mean ± SEM; $n = 4$ in each group, * $p < 0.05$ compared to diabetic control group

Effect of Drugs and Combination Therapy of Glibenclamide and Simvastatin on Liver Dysfunction Indices Activity on Long Term Alloxan Induced Diabetic Rats

The effect of glibenclamide, simvastatin and combination of both drugs on liver dysfunction indices activity in alloxan induced diabetic rats was shown. After 4 weeks treatment with the glibenclamide, simvastatin and combination of both drugs it was observed that liver dysfunction indices activity was decreased 41.43, 4.79 and 54.79 % in case of SGPT (ALAT) and 29.23, 3.98 and 50.49 % in case of SGOT (ASAT) in comparison with normal control group respectively. We performed same test for short term alloxan induced diabetic rats on liver dysfunction indices activity but interestingly, we could not observe any change in enzyme activity on alloxan induced diabetic rats (Table 4).

Effect of Drugs and Combination Therapy on Pancreas Islets of Langerhans Morphology in Long Term Alloxan Induced Diabetic Rats

Injection of alloxan in rats for 4 weeks significantly shrunk islets of Langerhans in comparison with normal rats. Treatment with simvastatin significantly recovered islets of Langerhans from shrinkage in AIDRs. Glibenclamide exhibited no effects on Langerhans cell recovery. But in case of combination therapy, noticeable recovery of Langerhans cell was reported (Fig. 5). The images shows tiny incompatibility though images were taken at same fixed magnification. Incompatibly indicates abnormal pancreacyte, there is also variation in staining due to changed morphology of pancreacyte of various groups like normal, alloxan induced, glibenclamide treated simvastatin treated and combination treated.

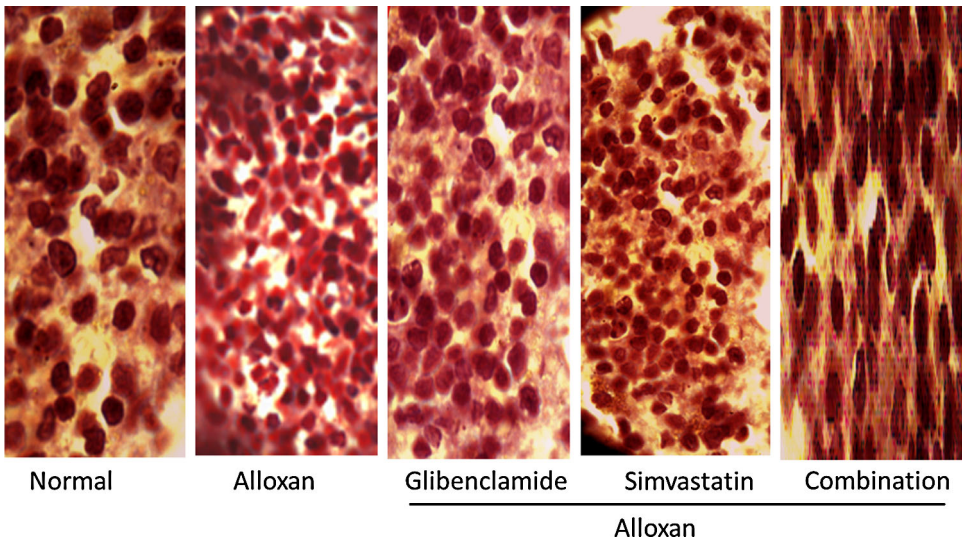

Fig. 5 Effect of repeated dose treatment of glibenlamide, simvastatin alone and combination of both drugs for 4 weeks on pancreas islets of Langerhans morphology in alloxan induced diabetic rats

Discussion

The risk of developing complications reduced substantially if diabetes can be effectively managed [17]. Diabetes, hyperlipidemia and CVD are a common debilitating illness among peoples in both developed and developing countries. Community surveys in industrialized countries have shown a prevalence of diabetes of 15–33 % in people aged 30 years. The disease continues to be a leading cause of morbidity and mortality from the coronary artery disease and stroke. Simple life style adjustments, such as a healthy diet and physical activity, often combined with medication have been shown to be effective in promoting a full and healthy life with heart diseases. In many cases, type 2 diabetes mellitus accounting for over 90 % of all cases of diabetes can be prevented through life style interventions alone. Though it may be true, but the maximum diabetic patient cannot take care to prevent the diabetes through life style innervations. In recent era, scientists and researchers all over the world are always engaged in research to find out new drug combination therapy and natural plant products to combat diabetes with CVD. The combination of glibenclamide (antidiabetic drug) and simvastatin (lipid lowering agent) may be used as an effective medicine in the treatment of diabetes and diabetes related risk factors especially CVD.

In the present study, diabetes was induced in rats by injecting alloxan (120 mg/kg BW) i.p. [18]. Alloxan is cytotoxic agent induced diabetes in a wide variety of animal species by damaging insulin secreting β-cell, resulting in decrease of endogenous insulin release, which paved the way for the decrease utilization of glucose by the tissues. Hypercholesterolemia and hypertriglyceridemia are common complications of diabetes mellitus in addition to hyperglycemia [19]. This work has evaluated the effect of combination therapy of glibenclamide and simvastatin on diabetes in experimental animal of rats, and also on other important biochemical parameters, such as oxidative properties, liver dysfunction indices, morphology of pancreas islets of Langerhans cells which caused CVD after long-term induction of diabetes.

Effect of Glibenclamide on Oral Glucose Tolerance Test

Glucose administration to normal rats caused significant ($p < 0.05$) rise in mean blood glucose level, half hour after administration. Two hours prior administration of glibenclamide significantly controlled the rise in blood glucose level after half hour (Fig. 1).

Thus, the oral administration of glibenclamide suppressed the increase in glucose level induced by glucose loading. Such an effect might be due to decrease in the rate of intestinal glucose absorption or by potentiation of pancreatic secretions or increasing the glucose uptake.

Effect of Monotherapy and Combination Therapy on Blood Glucose Level

The present study showed that glibenclamide produced significant decrease in blood glucose level in alloxan induced diabetic rats. On the other hand, simvastatin did not produce any significant change of this parameter. Interestingly, we observed that the combination therapy was more effective for controlling diabetes than the glibenclamide alone in long term alloxan induced diabetic rats. However, further studies are needed to clarify the exact mechanism of this effect of combination therapy.

Effect of Monotherapy and Combination Therapy on Lipid Profile

It was known that the factors which influence the glucose metabolism, under various physiological conditions, can influence lipid metabolism as well [20]. It had also been revealed that TG accumulation increased considerably in diabetes mellitus [21]. Hypercholesterolemia and hypertriglyceridemia had been reported to occur in diabetic [22]. A significant increase in TC, TG, and LDL-C level; and decrease in HDL-C level observed in our experiment as compared to normal rats in both protocol of diabetic rats. The serum TC, TG, LDL-C levels were significantly decreased and HDL-C level was significantly increased, after 2 weeks treatment with simvastatin compared to alloxan-induced diabetic rats. We also found similar results on lipid profile after 4 weeks treatment with simvastatin alone and combination therapy (glibenclamide and simvastatin) compared to alloxan induced diabetic rats. Interestingly the combination therapy was more significant than simvastatin alone. Glibenclamide alone was lack of significance effect on lipid profile in both 2 and 4 week's studies.

Recent evidences suggested that the serum lipids were usually increased in diabetes mellitus. For the distinct lipid lowering capacity of this combination therapy, it might be proposed that the drugs of the combination therapy may act as inhibitors for enzymes, such as hydroxyl-methyl-glutaryl-CoA reductase, which participates in de novo cholesterol biosynthesis.

Effect of Drugs and Combination Therapy on Liver Dysfunction Indices Activity

To date, an elevated ALAT and ASAT are considered a consequence of hepatocyte damage due to NAFLD. However, the measured plasma elevations of ALAT and ASAT may also be a consequence of high systemic ALAT2 isoform levels that is largely derived from adipose tissue in obesity and insulin resistance, as has been observed in rat [23]. Insulin resistance, increased pro-inflammatory cytokine production, oxidative stress and mitochondrial dysfunction leading to hepatocyte damage/destruction, have all been posed as important pathophysiological mechanisms [24]. Indeed a recent study reported on the association of ALAT and ASAT with markers of inflammation and oxidative stress [25].

Adiponectin, released by adipose tissue has been implied in the pathogenesis of NAFLD. Obese subjects and patients with type 2 diabetes mellitus have lower levels of adiponectin compared to healthy controls. There is an inverse association of ALAT, ASAT and GGT with adiponectin levels. These associations were independent of age, body mass index, insulin resistance, serum triglycerides and total cholesterol [26]. Aygun et al. [27] found that adiponectin levels were lower in patients with biopsy-proven NAFLD and elevated liver enzymes, compared to patients with NAFLD and normal liver enzymes and to those without NAFLD. The exact mechanisms by which adiponectin is related to NAFLD are not fully elucidated and need further clarification.

In our study it was observed that treatment with combination therapy (glibenclamide and simvastatin) significantly ($p < 0.01$) decreased SGPT (ALAT) and SGOT (ASAT) as a parameter of liver dysfunction indices activity in long term alloxan induced diabetic rats. The effect of combination therapy (glibenclamide and simvastatin) showed enhancing effect than simvastatin alone in long term alloxan induced rats compared with normal rats. On the contrary, glibenclamide alone had no significant liver dysfunction indices activity activity in comparison with normal rats.

Effect of Drugs and Combination Therapy on Superoxide Dismutase and Catalase Enzyme Activity

Antioxidant defense mechanism involves both enzymatic and nonenzymatic strategies. Common antioxidants include the enzymes like SOD, catalase, glutathione peroxidase, and vitamins like A, vitamin C and vitamine E. SOD convert the oxide anion to hydrogen peroxide, then catalase, located in peroxisomes, decomposes hydrogen peroxide to water and oxygen. Discrepancies in observed biomarkers for oxidative stress have to be seen, especially in the activities of SOD, catalase and glutathione peroxidase in experimentally diabetic animals [28]. So during diabetes hyperglycemia engenders free radicals, on the other hand it also impairs the endogenous antioxidant defense system in many ways.

It has been reported that in diabetic rat super oxide dismutase and catalase enzyme activity usually decreased. To investigate the beneficial effects of combination therapy (glibenclamide and simvastatin), we had further examined enzyme activity in alloxan induced rats after 4 weeks induction of diabetes.

The present study revealed that induction of alloxan in rats for long term significantly decreased antioxidant enzyme (SOD and catalase) activity in comparison with normal rats. Treatment with combination therapy significantly increased these enzyme activities in alloxan induced diabetic rats. Simvastatin alone also significantly increased antioxidant enzyme (SOD and catalase) activity whereas combination therapy showed more significant effect than simvastatin alone. On the contrary, glibenclamide had lack of significance in increasing antioxidant enzyme (SOD and catalase) activity in long term alloxan induced diabetic rats compared with alloxan induced diabetic rats.

Effect of Drugs and Combination Therapy on Pancreas Islets of Langerhans Morphology

Finally, histological studies of the pancreas demonstrated marked shrinkage of islets of Langerhans cells of pancreas in comparison with normal rats. Administration of glibenclamide recovered Langerhans cells from shrinkage which was demonstrated earlier where as simvastatin also displayed recovery of the cells. Treatment with combination therapy almost turns back shrinkage.

In this study treatment of normal and alloxan induced diabetic rats with patent medicine glibenclamide has profound effect to lower blood glucose level. Alloxan induced diabetic rats receiving combination therapy (glibenclamide and simvastatin) produced significant reduction compared to the diabetic control group. This effect is observed in 4 weeks experimental protocol model. In glucose induced hyperglycemic rats, normalization of blood glucose level was observed. This could be due to exert their insulin releasing effect by combination therapy (glibenclamide and simvastatin). Histological studies were carried out to prove this but further studies should be carried out to specify the mechanism of action precisely.

Conclusion

Alloxan-induced diabetes in rats represents well-established animal model for both types of diabetes mellitus. Increased production of high levels of oxygen free radicals had been linked to glucose oxidation and non-enzymatic glycation of proteins. Furthermore, characteristic diabetes raised LDL-C level, lowered HDL-C level and elevated TG level which contributed the development of diabetic complications (such as CVD). Our study also revealed that long term induction of diabetes by alloxan produced CVD. The present study clearly indicated that the combination therapy possessed antihyperglycemic, antidyslipidemic and antioxidative effects and had the beneficial effects on the islets of Langerhans cells recovery. Therefore the present study suggested that simvastatin together with glibenclamide might be effective combination drug in the treatment of diabetes with CVD. However, further study is necessary to clarify the exact mechanism of inhibitory action of combination drugs on diabetes with CVD.

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