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Research Article
Antidiabetic Agent in Combination with Simvastatin Reduces Blood Glucose and Elevated Liver Enzymes Level in Diabetic Rats for Extended Period of Time

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Abstract

Background and Objective: Combination of dosages regimen of an antidiabetic agent (Glibenclamide) with a lipid lowering drug can be an effective medication for the patient with high blood glucose level and liver enzyme disfunctionality. The present study was undertaken to investigate the effect of a fixed dose combination of glibenclamide (1.2 mg/70 kg b.wt.) and simvastatin (10 mg/70 kg b.wt.) on blood glucose and liver enzymes dysfuntionality in alloxan-induced diabetic rats for an extended time period. **Materials and Methods:** Two protocols were developed to carry out the experiment. The first is designated as 4 weeks short-term and second one is termed as 12 weeks long-term treatment protocols, respectively. Diabetes Mellitus (DM) was induced by single intraperitoneal (i.p.) injection of freshly prepared alloxan solution in 0.9% saline. Diabetic rats received treatment with i.p., injection of glibenclamide (1.2 mg/70 kg b.wt.) and simvastatin (10 mg/70 kg b.wt.) for 4 weeks as monotherapy and combination therapy (glibenclamide 0.6 mg/70 kg b.wt., simvastatin 5 mg/70 kg b.wt.) for 12 weeks. Graph pad was used and the results were expressed as Mean±SEM. A one-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test or students paired or unpaired t-test was used in the study where appropriate. **Results:** Results were considered to be significant when p-values were less than 0.05 ($p<0.05$). Combination therapy demonstrated a significant ($p<0.05$) decrease in blood glucose and liver enzymes elevation compared with diabetic control group. The study also demonstrated that the short term treatment has satisfactory effect on lowering SGPT by 41% and SGOT by 50%. Long term administration of combination therapy showed more significant ($p<0.05$) potentiality on lowering SGPT (46%) and SGOT (53%), respectively and this level remain steady during total treatment period. **Conclusion:** The present study demonstrates that combination of glibenclamide with simvastatin at the dose level tested exhibits significant glucose and liver enzymes lowering activity in alloxan induced diabetic rats. When monotherapy with oral antidiabetic agents fails, combination therapy with glibenclamide plus simvastatin seems to be stable and effective for the treatment of diabetes mellitus.

Key words: Elevated blood glucose level, liver enzyme elevation, diabetic rats, dose combination, beneficial effects

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder and its incidence is continuously increasing which has subsequently caused a major public health concern. This disease is associated with multifactor disorders including hyperglycemia and problematic disruptions in carbohydrate, fat and protein metabolisms due to insufficient, deficient or disruption in insulin secretion (WHO., 2007). This has resulted in defects in reactive oxygen species scavenging enzymes (Wild *et al*., 2004) and high oxidative stress impairing pancreatic β-cells (Lamb and Goldstein, 2008). Hyperglycemia has been associated with tissue damage and several complications such as liver and kidney dysfunctions (Nathan *et al*., 2005; Tuomilehto, 2003). The phenomenon of DM is increasing worldwide at alarming rates which brought other serious diseases. Recent advancements has led to the development of several therapeutic strategies for the treatment of the disease that includes stimulation of endogenous insulin secretion, enhancement of insulin sensitivity at the target tissues, reduction of dietary starch and lipid degradation and treatment with oral hypoglycemic agents (Carmena, 2005). Millions of people are affected by diabetes mellitus worldwide and thus it has a large negative impact on parameters like the patient's health and quality of life (Davidson, 2006). This situation is rapidly getting worse and has the greatest burden on working age adult population of developing countries (Solano and Goldberg, 2006). Two potent liver enzymes namely Serum Glutamic Oxaloacetate Transaminase (SGOT) and Serum Glutamic Pyruvate Transaminase (SGPT) are commonly measured as indirect markers of liver inflammation or injury. Liver fat accumulation is closely related to the level of SGPT and has also been used as a surrogate marker for nonalcoholic fatty liver disease (NAFLD) in some epidemiologic studies. The NAFLD is frequently associated with obese subjects and is strongly involved in the pathogenesis of type 2 diabetes. The elevated level of SGPT and SGOT are indicated to the damage or inflammation of hepatocyte due to NAFLD, however, plasma SGPT and SGOT elevation level may also be a consequence of high systemic SGPT2 isoform levels derived from adipose tissue in obesity and insulin resistance as has been observed in rats (Jadaho *et al*., 2004). Pro-inflammatory cytokine production, oxidative stress and mitochondrial dysfunction were increased by insulin resistance reported to the hepatocyte inflammation/destruction have all been posed as important pathophysiological mechanisms (Day, 2002). Recent studies have shown a linkup between SGPT and SGOT with markers of inflammation and oxidative stress (Rikitake and Liao, 2005).

Adiponectin released by adipose tissue has also been implied in the pathogenesis of NAFLD. Lower level of adiponectin is present in obese subjects and patients with type 2 diabetes mellitus as compared to healthy controls. The SGPT, SGOT and GGT are associated inversely with adiponectin levels and these associations are independent of age, body mass index, insulin resistance, serum triglycerides and total cholesterol (Yokoyama *et al*., 2004). However, combination of an anti-diabetic drugs with lipid lowering agent has been reported for the correction of liver enzyme (SGPT and SGOT) level significantly (Begum *et al*., 2013). Aygun *et al*. (2006) have shown that adiponectin levels were lower in patients with biopsy-proven NAFLD and elevated liver enzymes as compared to patients with NAFLD and normal liver enzymes and to those without NAFLD. The exact mechanisms by which adiponectin is related to NAFLD is not fully elucidated and needs further clarification.

MATERIALS AND METHODS

Chemicals: Aloxan was purchased from Sigma chemical company, Kolkata. All other chemicals used in the experiments were purchased locally (Merck or SD fine Chemicals) and were of analytical grade.

Experimental animals: The test rats were collected from International Centre for Diarrhoeal Disease Research, Bangladesh (icddr, b). The experiments were performed by using Long-Evans male rats weighing about 200-220 g, aged 2-2.5 months. Polypropylene cages were used to house the test animals individually in well-ventilated rooms under hygienic conditions. Feeding of animals was done with normal pellet, along with drinking water and maintained at natural day night cycle. All protocols for the animal experiments were reviewed and approved by the Animal Care and Use Committee of Department of Clinical Pharmacy and Pharmacology, University of Dhaka.

Experimental design: After 1 week acclimation the test animals were divided into 5 major groups. Long Evan male rats (200) were divided into two groups to perform monotherapy and combination therapy. For 4 weeks protocol, A, B, C, D and E groups had been assigned with total 100 test rats and for 12 weeks protocol, A, B, C D and E groups had been assigned with total 100 test rats, respectively. Group A and B were considered as normal and diabetic control group, respectively. Group C, D and E were considered as glibenclamide, simvastatin and combination therapy test rats.

Induction of experimental diabetes: Diabetes was induced in rats by administering alloxan monohydrate at a dose of 120 mg kg^{-1} b. wt., intraperitoneally (Davidson, 2006). Alloxan was dissolved in citrate buffer at pH 4.5 and injected immediately to avoid degradation. After 72 h, blood was collected from the retro-orbital plexus and blood glucose level was determined by using auto analyzer (Microlab 2000) from all surviving rats. The experimental rats having a blood sugar level between 200-350 mg dL^{-1} along with polydipsia, polyuria and polyphagia were considered as diabetic and were employed in the study.

Preparation and administration of drugs: Diabetic rats received treatment with intra peritoneal (i.p.) injection of glibenclamide (1.2 mg/70 kg b.wt.) and simvastatin (10 mg/70 kg b.wt.) for 4 and 12 weeks as monotherapy and combination therapy (glibenclamide 0.6 mg/70 kg b.wt., simvastatin 5 mg/70 kg b.wt.), respectively.

Estimation of plasma glucose: Blood samples were collected from 18 h fasted rats 1 h after the last dose administration. Glucometer was used to test baseline glucose level of all the experimental rats. In addition, changes in body weight were recorded in both the control and treatment groups.

Blood serum, heart, liver, kidney and collection: Blood sample was collected from rats to obtain serum sample. After completion of the desired treatment protocol the rats were anesthetized with phenobarbital sodium (Emer, Opsonin Pharma Ltd., Barisal, Bangladesh). The thoracic artery was opened by cutting the abdominal skin and 3-5 mL blood was collected directly from thoracic artery by using heparinized syringe. Finally the blood was centrifuged at 4000 rpm for 15 min and the serum was collected carefully and stored at 4°C to conduct further experiments.

Measurement of SGPT and SGOT level: Serum SGPT and SGOT levels were tested using blood serum samples collected from experimental rats. The concentrations were analyzed by taking absorbance from UV spectrophotometer using diagnostic kits (Human, Germany). Kinetic method was used for the determination of SGPT and SGOT activity according to the recommendations of the expert panel of the IFCC without pyridoxal phosphate activation.

Statistical analysis: Graph pad prism (version 4.0) computer program (Graph pad software san diego, CA, USA) was used and the results were expressed as Mean±SEM. A one-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test or students paired or unpaired t-test was used in the study where appropriate. The statistical method applied in each analysis was described in each figure. Results were considered to be significant when $p<0.05$.

RESULTS

Observation of blood glucose level before and after for short-term and long-term treatment protocols in alloxan-induced diabetic rats with mono and combination therapy: Short-term i.p., injection of alloxan in rats significantly increased blood glucose level (16 mmol L^{-1}) when compared with normal rats (5-6.5 mmol L^{-1}) (Fig. 1a). To clarify the individual effects of glibenclamide and simvastatin on blood glucose level we estimated blood glucose level after 4 weeks treatment with glibenclamide and simvastatin on alloxan induced diabetic rats. Repeated treatment with glibenclamide for 4 weeks showed a regular decline of blood glucose level in diabetic rats which are shown in Fig. 1a. Glibenclamide alone effectively decreased blood glucose level from 16.36±0.14 to 8.37±0.66 mmol L^{-1}, whereas simvastatin alone failed to scale down blood glucose level in significant amount when compared with diabetic control rats (Fig. 1a). On the other hand combination therapy lowered blood glucose level from 16.36±0.14 to 6.31±0.094 mmol L^{-1} significantly ($p<0.05$) after 4 weeks treatment. In opposite point of view, long-term alloxan administration appreciably raised blood glucose level (33.50 mmol L^{-1}) when compared with normal rats (5-6.5 mmol L^{-1}) (Fig. 1b). Glibenclamide alone and in combination showed potent ($p<0.05$) blood glucose lowering effect from 33.50±0.31 to 7.00±0.07 mmol L^{-1} and 33.26±0.34 to 5.88±0.09 mmol L^{-1}, respectively whereas simvastatin alone failed to lessen blood glucose level as shown in Fig. 1b.

Effects of mono and combination therapy on elevated liver enzymes (SGPT and SGOT) levels in short term and long term treatment protocols in alloxan-induced diabetic rats: Glibenclamide mono and combination therapy showed reduction effects on elevated liver enzyme level in alloxan-induced diabetic rats. After 4 weeks treatment with glibenclamide, simvastatin and combination, liver enzyme SGPT level declined by 32.25, 3.65 and 40.88%, respectively (Fig. 1c) and SGOT 17, 2.84 and 49.23%, respectively (Fig. 1d) as compared with normal control group.

Following 12 weeks treatment with the glibenclamide, simvastatin and combination therapy, it was observed that liver enzyme SGPT level scale down by 42.36, 3.75 and 46% (Fig. 1e) and SGOT 31, 3.01 and 53.33% (Fig. 1f) remarkably ($p<0.05$) in comparison with normal control group.

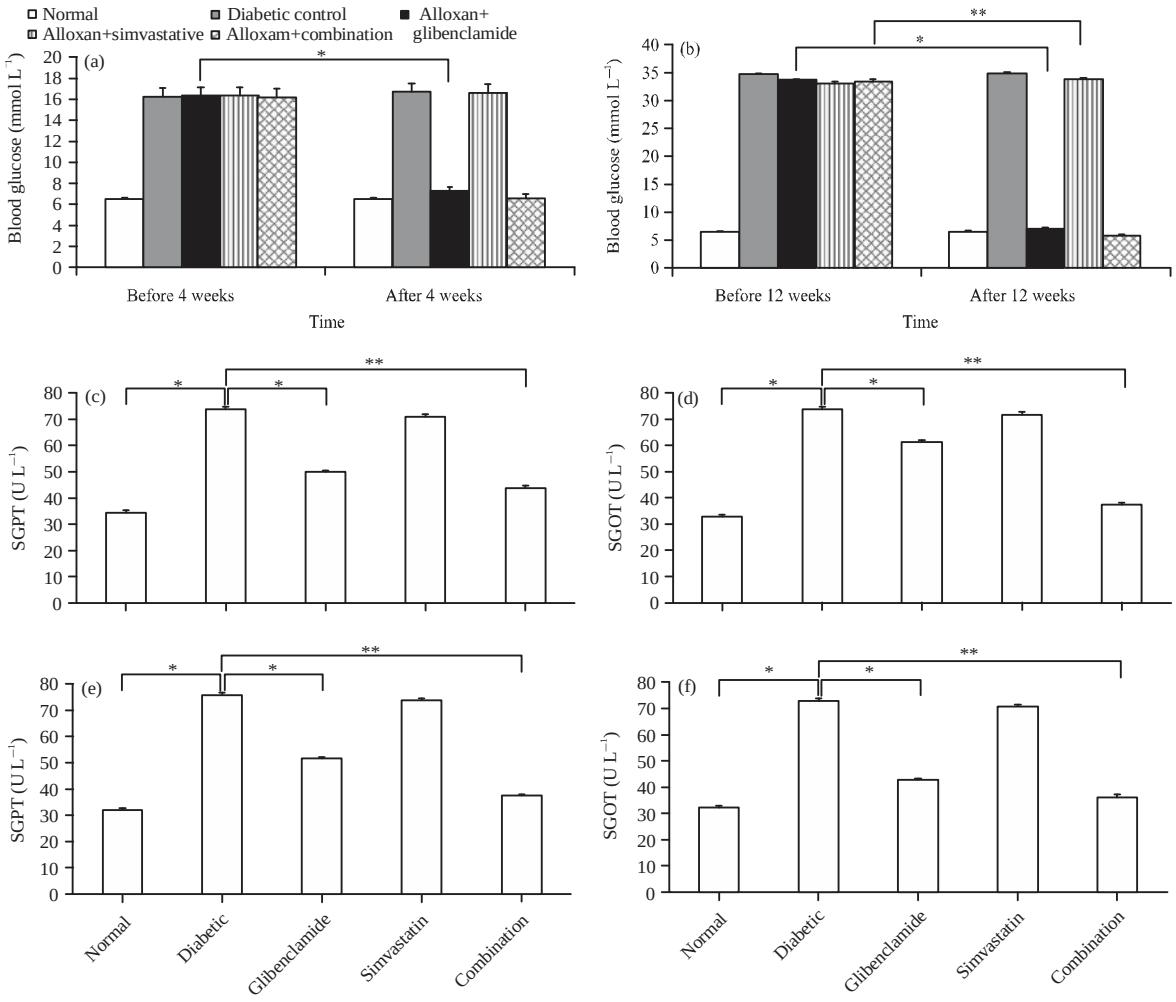

Fig. 1(a-f): Effect of repeated dose treatment of (a) Mono and combination therapy for 4 weeks on blood glucose level, (b) Glibenclamide, simvastatin and combination for 12 weeks on blood glucose level, (c) Glibenclamide, simvastatin and combination for 4 weeks on elevated liver enzyme (SGPT/ALAT) level, (d) Glibenclamide, simvastatin and combination for 4 weeks on elevated liver enzyme level (SGOT/ASAT), (e) Glibenclamide, simvastatin and combination for 12 weeks on elevated liver enzyme (SGPT/ALAT) activity and (f) Glibenclamide, simvastatin and combination for 12 weeks on elevated liver enzyme (SGOT/ASAT) level in alloxan-induced diabetic rats. Data were presented as Mean±SEM, n = 10 in each group, *p<0.05 compared to diabetic control group (ANOVA followed by Dunnett's test), *p<0.05 vs normal, **p<0.05 vs alloxan

Effects of repeated dose treatment of glibenclamide, simvastatin and combination therapy for 12 weeks on different physiologic parameters in alloxan-induced diabetic rats: Table 1 demonstrates the effects of repeated dose treatment of glibenclamide, simvastatin and combination therapy for 12 weeks on different parameters in alloxan-induced diabetic rats. These results indicate that the body weight fall in diabetic control and then increased notably (p<0.05) during treatment with drugs. Heart weight was also increased during the treatment. The heart weight was 0.645±0.0033 for normal control, 0.608±0.0023 for diabetic control, 0.617±0.0011 for glibenclamide, 0.631±0.0011 for simvastatin and 0.639±0.0021 for combination therapy. Liver weight increased in the glibenclamide treated group (4.90±0.24 g) and decreased in simvastatin group (4.85±0.022 g) but the combination therapy showed a significant weight increasing effect of liver (0.58±0.019 g) as normal compared with normal control group. The weight of kidneys was increased during treatment and the combination therapy was more efficient than the other therapies.

Table 1: Effects of long term repeated dose treatment of glibenclamide, simvastatin and combination therapy on different organs on alloxan-induced diabetic rats

Parameters	Normal control	Diabetic control	Diabetic+glibenclamide	Diabetic+simvastatin	Diabetic+combination
Body weight	194.0±1.83	174.0±1.29	178.0±0.6292*	182.7±0.577	189.0±1.09**
Heart	0.645±0.003	0.608±0.0023	0.617±0.0011*	0.631±0.0011	0.639±0.0021**
Liver	5.210±0.065	4.840±0.024	4.900±0.24	4.850±0.022	5.130±0.025*
Kidney	0.600±0.021	0.530±0.019	0.550±0.077	0.540±0.022	0.580±0.019*

Effects of long term repeated dose treatment of glibenclamide, simvastatin and combination therapy on different organs in alloxan-induced diabetic rats. Data were presented as Mean±SEM, n = 10 in each group, *p<0.05 compared to diabetic control group (ANOVA followed by Dunnett's test), *p<0.05 vs normal, **p<0.05 vs alloxan

DISCUSSION

The present study was performed to investigate the hypoglycemic effects of glibenclamide and simvastatin when administered alone and in combination in alloxan induced diabetic rats. Alloxan is a known cytotoxic agent that induces diabetes by damaging insulin secreting β-cells which depletes insulin release thus decreasing the utilization of glucose by the tissues (Islam *et al*., 2012).

The statins are a powerful group of drugs used to lower cholesterol. They work by interrupting the final step in the chemical pathway that creates cholesterol in the liver. Research shows that statins can dramatically reduce the risk for a heart attack, stroke or death, even in people who have normal cholesterol levels and do not have heart disease.

Bile acid sequestrants also lower LDL and can be used alone or in combination with statins. Nicotinic acid lower LDL, triglycerides and raises HDL. Fibric acids lower LDL somewhat but are used mainly to treat high triglyceride and low HDL levels. Cholesterol absorption inhibitors lower LDL and can be used alone or in combination with statins. Once LDL goal has been reached, doctor may prescribe treatment for high triglycerides and/or a low HDL level, if present. The treatment includes losing weight if needed, increasing physical activity, quitting smoking and possibly taking a drug (Williams, 1988).

Simvastatin is a competitive inhibitor of HMG-CoA reductase. The HMG-CoA reductase catalyzes the reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate, which is the rate-limiting step in hepatic cholesterol biosynthesis. Inhibition of the enzyme decreases *de novo* cholesterol synthesis, increases expression of low-density lipoprotein receptors (LDL receptors) on hepatocytes. This increasing LDL uptake by the hepatocytes, decreases the amount of LDL-cholesterol in the blood. Like other statins, simvastatin also reduces blood levels of triglycerides and slightly increases levels of HDL-cholesterol (Rikitake and Liao, 2005).

Our study showed that glibenclamide produced a significant decrease in blood glucose level in alloxan-induced diabetic rats whereas simvastatin did not produce any significant change as it is a lipid lowering agent. The findings of our study are consistent with previously published reports (Anitha *et al*., 2008; Balasubramanian *et al*., 2008). Interestingly, it is observed that the combination therapy demonstrated a significant controlling effect of diabetes than the glibenclamide mono therapy in long-term alloxan induced diabetic rats. It is often observed in patients with type 2 diabetes that monotherapy often fails to achieve glycemic targets across a wide range of baseline levels (Cook *et al*., 2007). Moreover, monotherapy always fail to address the multiple pathophysiological causes of diabetes, whereas combination treatment can address this situation by acting via multiple mechanisms (Phung *et al*., 2014). The SGOT and SGPT are potent liver marker enzymes which are increased during diabetic conditions that reveal hepatic damage but following treatment with glibenclamide in combination regimen it was observed that the level of liver marker enzymes reached their normal level. On the contrary simvastatin monotherapy failed to return the liver enzymes to their normal level. A significant fall in enzyme level was also observed in long term treatment protocol as compared to short term treatment protocol. These findings were supported to the findings of previously published reports (Islam *et al*., 2012). Serum SGOT and SGPT level in combination therapy treated rats reduced significantly but the effect is slightly more effective than previous study (Minarul *et al*., 2014).

In diabetic conditions there is a reduction in body weight and internal organs weight. Weight gain was observed in tested animals undergoing monotherapy and combination therapy. Interestingly, the combination therapy demonstrated a significant weight gaining effect as compared to simvastatin monotherapy. The animals treated with alloxan in long term treatment protocol appeared ill-looking with significant loss of body weight. One of the deleterious effects of alloxan is a reduction of body weight and it also causes DNA alkylation due to the toxic effects of alloxan that produced hyperglycemia as well as necrotic lesions. The present observations are coherent with previous findings (Piyachaturawat *et al*., 1988; Habibuddin *et al*., 2008; Lee *et al*., 2008). The findings of this study suggest that a combination therapy of glibenclamide and simvastatin has a greater implication than simvastatin

monotherapy in lowering blood glucose and elevated enzyme levels in alloxan induced diabetic rats.

CONCLUSION

Based on current study it can be concluded that the combination of glibenclamide with simvastatin at the dose level tested exhibits significant glucose and liver enzymes lowering effects. Increased effect in combination regimen may be due to potentiation or synergism and it needs molecular and tissue based study for further clarification. So, combination therapy has a greater potential to reduce plasma glucose level and liver enzymes dysfuntionality in alloxan induced diabetic rats.

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