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Comaparative study of the antidiabetic and biochemical effects of metformin, glibenclamide and repaglinide in alloxan- induced diabetic rats

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Diabetes mellitus is one of the major global health burden affecting both developed and developing countries. This study examined the antidiabetic effects of metformin (MET), glibenclamide (GLI) and repaglinide (REP) on biochemical parameters in alloxan-induced diabetic rats. The study will assess the efficacy of these standard drugs in managing the complications arising from diabetes mellitus. Alloxan (130 mg/kg BW) was administered as a single dose to induce diabetes. Four (4) groups of rats (n=6) were used; group 1 served as diabetic control while groups 2, 3 and 4 were the diabetic test groups that received MET (25 mg/kg), GLI (2.5 mg/kg) and REP (0.5 mg/kg) respectively. The effects of these agents on blood glucose, total cholesterol (TC), triacylglycerol (TAG), low density lipoprotein (LDL-C) and high density lipoprotein (HDL-C) concentrations were determined. Also, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alanine phosphatase (ALP) activities were assayed. The results showed that the blood glucose concentration of diabetic rats treated with MET, GLI and REP were significantly ($p<0.05$) reduced compared with the diabetic control. Serum total cholesterol, triacylglycerol and low density lipoprotein cholesterol concentrations were significantly ($p<0.05$) reduced while high density lipoprotein cholesterol concentration was significantly ($p<0.05$) higher in the treated diabetic rats compared with the diabetic control. Also, alanine aminotransferase, aspartate aminotransferase and alanine phosphatase activities were markedly ($p<0.05$) reduced compared with the diabetic control group. Findings from this study suggest that the administration of MET, GLI and REP exhibited significant reductions in the blood glucose concentrations; hence, significant improvement in the biochemical parameters altered during diabetic associated manifestations.

Key words: Diabetes mellitus, metformin, glibenclamide, repaglinide, biochemical parameters.

INTRODUCTION

A growing awareness of the complications arising from public health issues has led to the prediction of approximately 150 to 300 million people likely to be diabetic by the year 2025 (World Health Organization,

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2002); this accounts for the leading cause of morbidity and mortality worldwide. Diabetes encompasses a wide range of conditions that affects either the functioning of the eyes, heart, nerves, liver, pancreas or kidney, and includes those that originates due to genetic abnormalities and infectious diseases. Despite the great strides made in the understanding and management of diabetes, the disease and disease related complications are increasing unabated due to multiple defects in its pathophysiology (Ivorra et al., 1985). Efforts are continuously underway to find effective methods to treat diabetes; to slow and prevent its complications. Many therapeutic approaches have been utilized for treatment of this disorder including the use of oral hypoglycaemic agents. An ideal oral treatment for diabetes would be a drug that not only controls the glycaemic level but also prevents the development of atherosclerosis and other complications of diabetes (Al-neaimy, 2011).

Unfortunately, many scientists have worked and are still working to find a lasting solution to abnormalities in lipid metabolism due to diabetes-induced hypertriglyceridaemia and hypercholesterolaemia. While there are numerous studies that showed the potential of oral hypoglycaemic drugs in treating diabetes, there are only few data to support their safety and especially their effects on biochemical parameters. In view of this, it is therefore imperative to investigate the effects of three standard antidiabetic agents belonging to three different classes; metformin (MET), glibenclamide (GLI) and repaglinide (REP) used in the management of diabetes mellitus as well as their effects on biochemical parameters. Metformin, a biguanide antihyperglycaemic agent, is widely used in the management of type 2 diabetes mellitus. They differ significantly from the sulphonylureas in that they do not cause hypoglycaemia in normoglycaemic (non-diabetic) individuals, and do not stimulate pancreatic beta-cells to produce insulin. It lowers the blood glucose concentration without causing hypoglycaemia (Scheen, 1996).

Sulfonylureas such as the glibenclamide are a class of oral drugs that reduce blood glucose levels by stimulating insulin secretion. In the presence of viable pancreatic β-cells, sulfonylureas stimulate the release of endogenous insulin from the pancreatic β-cells, thereby reducing blood glucose levels. The drug molecules bind to a specific receptor (sulphonylurea receptor) identified as adenosine triphosphate (ATP)-sensitive potassium (K_{ATP}) channel that is present on the pancreatic beta-cell membrane causing depolarization by reducing conductance of ATP sensitive K channels (Rachman and Turner, 1995). This results in the opening of voltage-gated calcium channel causing Ca^{2+} influx and degranulation with the release of pre-formed insulin (Karam, 1997; Philipson and Steiner, 2005).

Repaglinide, chemically unrelated to the oral sulfonylurea insulin secretagogue, is a prandial glucose regulator used in the management of type 2 diabetes mellitus (Polonsky et al., 1988). It belongs to the meglitinide class of short-acting insulin secretagogues, which act by binding to β-cells of the pancreas to stimulate insulin release. Like the sulfonylureas, repaglinide produces a hypoglycaemic effect by stimulating insulin secretion from the pancreatic β-cells (Mycek et al., 2000), but in contrast to the sulfonylureas, its action is at least in part glucose-mediated and is effected via a different high-affinity binding site on the β-cell (Gromada et al.,1995; Fuhlendorff et al., 1998; Roudovitch et al., 2001).

MATERIALS AND METHODS

Animals and treatment

Twenty-four adult male albino rats (100 to 160 g) were obtained from the animal house of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. All animal experiments were conducted in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No. 85 to 23, revised 1985) and in accordance with the University's Ethics Committee on the use of laboratory animals. The animals were housed in standard cages under standard environmental conditions, with a 12 h light/dark cycle maintained on a regular feed (vital feed) and water *ad libitum.*

The baseline blood glucose levels were determined before the induction of diabetes. The rats were fasted for 12 h with free access to water prior to the administration of alloxan monohydrate (130 mg/kg; i.p.) dissolved in ice-cold normal saline. After 5 days stabilization of diabetes, blood was collected from the tail vein of rats and placed on the sensor pad of the glucometer strip already inserted into the glucometer. Animals having fasting blood glucose level ≥200 mg/dl (11.1 mmol/l) were considered diabetic and used for the investigation. The animals were divided into four (4) groups (n = 6) as follows:

Group 1: diabetic control (untreated group)
Group 2: received MET (25 mg/kg, p.o).
Group 3: received GLI (2.5 mg/kg, p.o).
Group 4: received REP (0.5 mg/kg, p.o).

Drugs/Chemicals

Metformin Hydrochloride (Merck Pharm, Nig. Ltd), Glibenclamide (Swiss Pharma, Nig. Ltd) and Repaglinide (Novo Nordisk Inc., Germany) were used in the study. All drugs (containing the required dose) were freshly dissolved in distilled water before administering to the animals. Alloxan monohydrate was purchased from Sigma-Aldrich Chemical (St. Louis, Missouri, USA). All other chemicals used were obtained commercially and of analytical grade.

Biochemical analysis

At the end of the experimental period, blood samples (about 5 ml) were collected through ocular puncture with the aid of non-heparinised capillary tube and transferred into clean sample bottles. Subsequently, the samples were allowed to clot and centrifuged at 2000 g for 10 min to obtain the serum component. Fasting blood glucose concentration was estimated by one touch glucometer (Accu-check). Serum lipid profiles (total cholesterol, triacylglycerol, LDL and HDL) were all determined by spectrophotometric method.

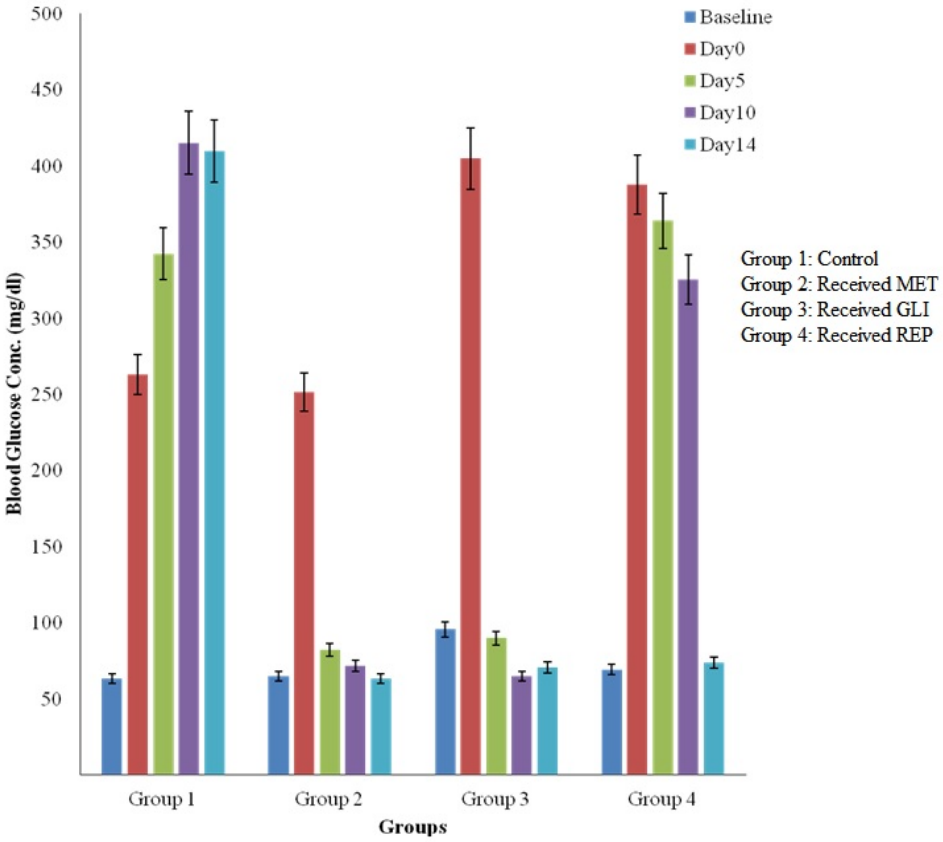

Figure 1. Effects of metformin (MET), glibenclamide (GLI) and repaglinide (REP) on blood glucose concentration.

Assays of alanine aminotransferase activity (ALT, EC, 2.6.1.2), aspartate aminotransferase activity (AST, EC, 2.6.1.1) and alkaline phosphatase activity (ALP, EC, 3.1.3.1) were carried out according to previously described methods (Reitman and Frankel, 1957; Rec, 1972).

Statistical analysis

The data obtained were analyzed by one way analysis of variance (ANOVA) using Statistical Product and Service Solutions (SPSS), version 18. Results were expressed as mean ± SD. Post-Hoc Dunnett's-test at 95% level of significance was used to assess significant difference between the control and treated groups. $p < 0.05$ was considered to be statistically significant.

RESULTS

Effects of metformin, glibenclamide and repaglinide on blood glucose concentration

The glucose response of the animals in all the test groups after administration of alloxan showed significant increase ($p<0.05$) in glucose concentration which is an indication of diabetic condition. The oral administration of MET (25 mg/kg), GLI (2.5 mg/kg) and REP (0.5 mg/kg) in groups 2, 3 and 4 respectively caused significant ($p<0.05$) reduction in the blood glucose concentration of the diabetic rats compared with the diabetic control rats in group 1 (Figure 1).

Effects of metformin, glibenclamide and repaglinide on serum total cholesterol (TC) concentration

There were significant ($p<0.05$) reductions observed in the total cholesterol concentrations of groups 2, 3 and 4 diabetic rats administered MET (25 mg/kg), GLI (2.5 mg/kg) and REP (0.5 mg/kg) respectively compared with the diabetic control (Figure 2).

Effects of metformin, glibenclamide and repaglinide on serum triacylglycerol (TAG) concentration

The TAG concentrations of diabetic rats in groups 2, 3 and 4 administered MET (25 mg/kg), GLI (2.5 mg/kg) and REP (0.5 mg/kg) respectively were significantly ($p<0.05$) reduced compared with that of diabetic control rats in

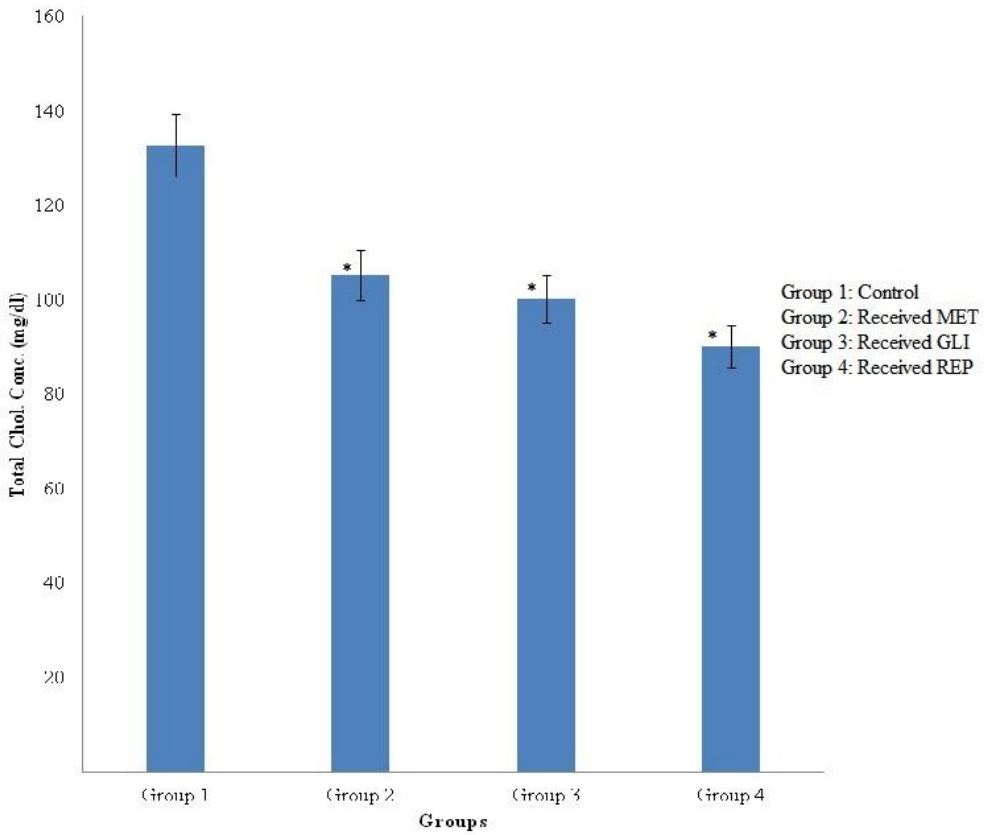

Figure 2. Effects of metformin (MET), glibenclamide (GLI) and repaglinide (REP) on serum total cholesterol concentration. Data are expressed as mean ± SEM (n=6). * *p<0.05* when compared with control group.

group 1 (Figure 3).

Effects of metformin, glibenclamide and repaglinide on serum low density lipoprotein concentration

The LDL-C concentration of diabetic rats in groups 2 and 3 administered MET (25 mg/kg) and GLI (2.5 mg/kg) respectively was significantly ($p<0.05$) reduced compared with the LDL concentration of the diabetic control group. However, no significant ($p>0.05$) reduction was observed in the LDL-C concentration of diabetic rats in group 4 administered REP (0.5 mg/kg) compared with the diabetic control group (Figure 4).

Effects of metformin, glibenclamide and repaglinide on serum high density lipoprotein concentration

The HDL-C concentration was observed to be significantly ($p<0.05$) higher in groups 2, 3 and 4 diabetic rats administered MET (25 mg/kg), GLI (2.5 mg/kg) and REP (0.5 mg/kg) respectively when compared with the diabetic control in group 1. However, the increase in the HDL-C concentration of group 3 rats was most significant (Figure 5).

Effects of metformin, glibenclamide and repaglinide on serum alkaline phosphatase (ALP) activity

The ALP activity of diabetic rats in groups 2, 3 and 4 administered MET (25 mg/kg) and GLI (2.5 mg/kg) and REP (0.5 mg/kg) respectively were significantly ($p<0.05$) reduced when compared with the ALP activity of the diabetic control rats in group 1 (Figure 6).

Effects of metformin, glibenclamide and repaglinide on serum alanine aminotransferase (ALT) activity

There was no significant ($p>0.05$) reduction observed in the ALT activity of group 2 diabetic rats administered MET (2.5 mg/kg) when compared with the ALT activity of the diabetic control. However, significant ($p<0.05$) reductions were observed in the ALT activity of diabetic rats in groups 3 and 4 administered GLI (2.5 mg/kg) and REP (0.5 mg/kg) compared with the diabetic control

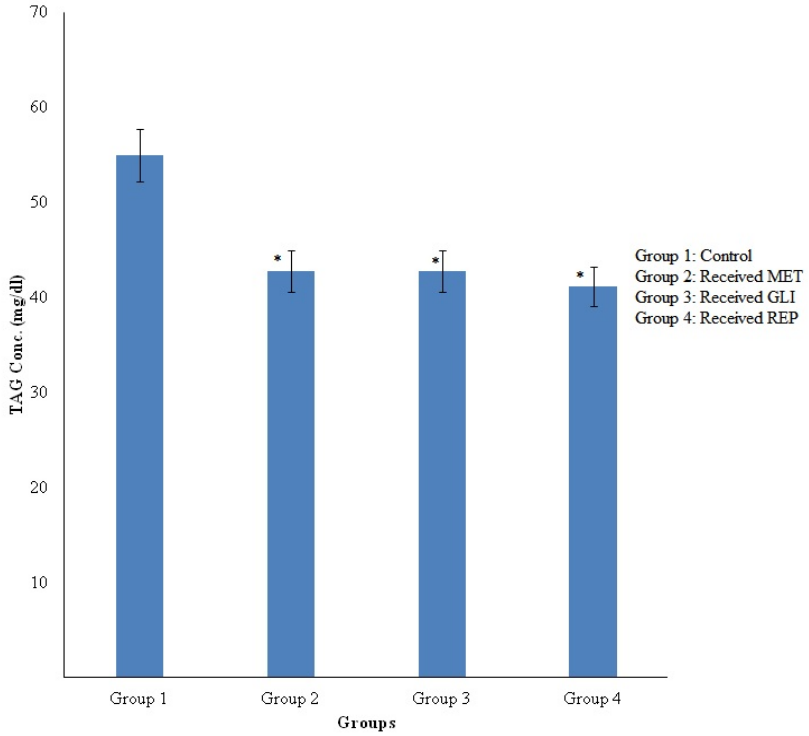

Figure 3. Effects of metformin (MET), glibenclamide (GLI) and repaglinide (REP) on serum triacylglycerol concentration. Data are expressed as mean ± SEM (n=6). * p<0.05 when compared with control group.

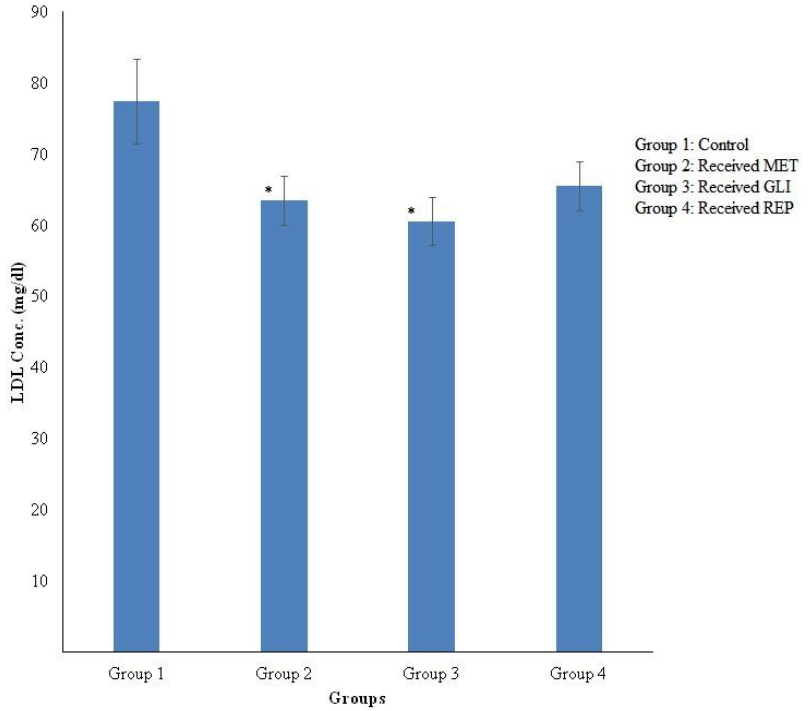

Figure 4. Effects of metformin (MET), glibenclamide (GLI) and repaglinide (REP) on serum low density lipoprotein concentration. Data are expressed as mean ± SEM (n=6). * p<0.05 when compared with control group.

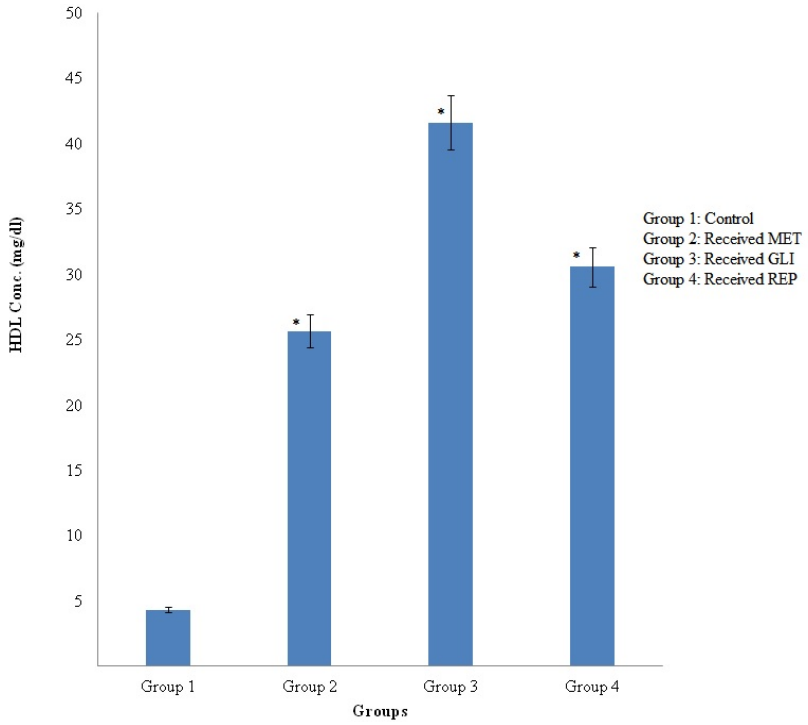

Figure 5. Effects of metformin (MET), glibenclamide (GLI) and repaglinide (REP) on serum high density lipoprotein concentration. Data are expressed as mean ± SEM (n=6). *p<0.05 when compared with control group.

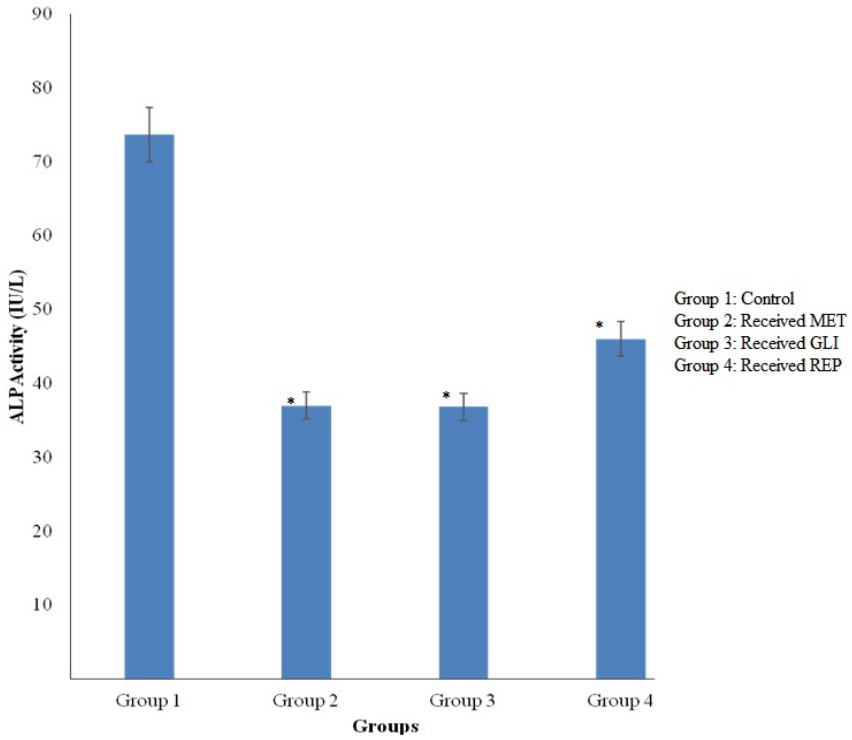

Figure 6. Effects of metformin (MET), glibenclamide (GLI) and repaglinide (REP) on serum alkaline phosphatase activity. Data are expressed as mean ± SEM (n=6). *p>0.05 when compared with control group.

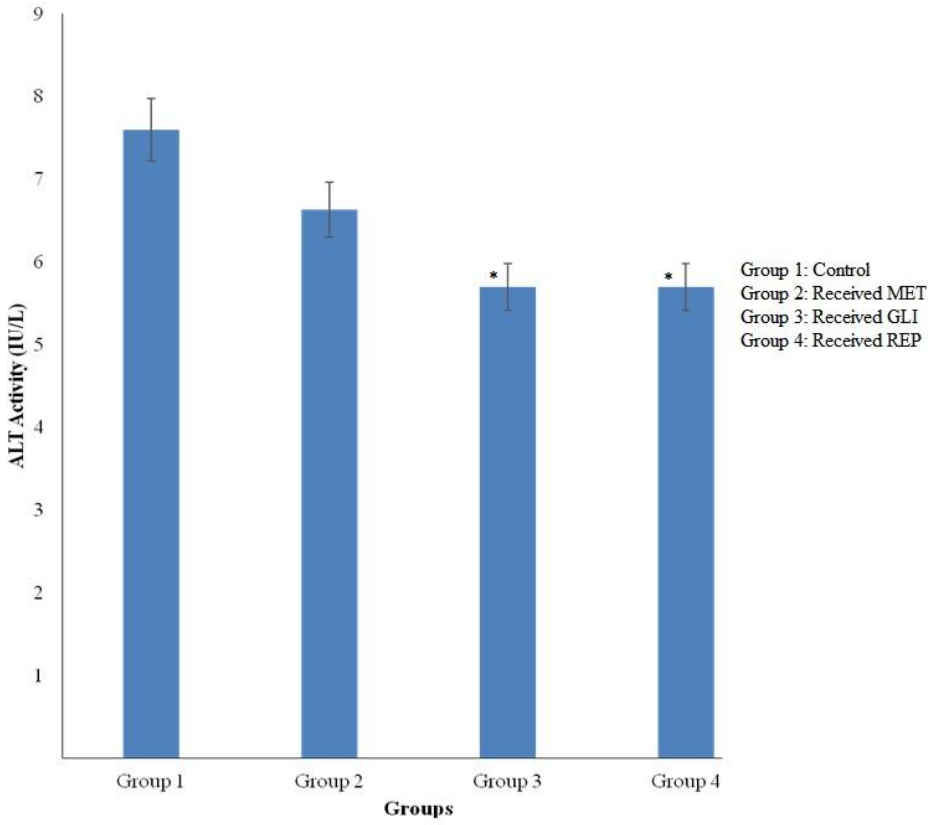

Figure 7. Effects of metformin (MET), glibenclamide (GLI) and repaglinide (REP) on serum alanine aminotransferase activity. Data are expressed as mean ± SEM (n=6). *p<0.05 when compared with control group.

(Figure 7).

Effects of metformin, glibenclamide and repaglinide on serum aspartate aminotransferase (AST) Activity

Significant (p<0.05) reduction was observed in the AST activity of diabetic rats in groups 3 and 4 administered GLI (2.5 mg/kg) and REP (0.5 mg/kg) respectively compared with the diabetic control. However, no significant (p>0.05) reduction was observed in the AST activity of group 2 diabetic rats administered MET (2.5 mg/kg) when compared with the AST activity of the diabetic control (Figure 8).

Effects of metformin, glibenclamide and repaglinide on the mean body weight

There was significant (p<0.05) increase in the mean body weights of diabetic rats in groups 2 and 3 administered MET (25 mg/kg) and GLI (2.5 mg/kg) respectively compared with the mean body weights of the diabetic control in group 1. However, no significant (p>0.05) difference was observed in the mean body weights of diabetic rats in group 4 administered with REP (0.5 mg/kg) compared with the diabetic control rats in group 1 (Figure 9).

DISCUSSION

This study investigated the effects of three standard antidiabetic agent belonging to three different classes; MET, GLI and REP used in the management of diabetes mellitus as well as their effects on biochemical parameters. The glucose response of the animals in all the test groups after administration of alloxan showed significant increase in glucose concentration which is an indication of diabetic condition. This is in line with the work of Etuk and Muhammed (2010).

Alloxan, a beta cytotoxin, induces diabetes in a wide variety of animal species by destroying the beta cells of the Islets of langerhans in pancreas leading to reduction in synthesis and release of insulin (Szkendelsky, 2001). Insulin deficiency leads to various metabolic aberrations in animals, such as increase in blood glucose, decreased protein content and alteration in the lipid profile (Ribes et

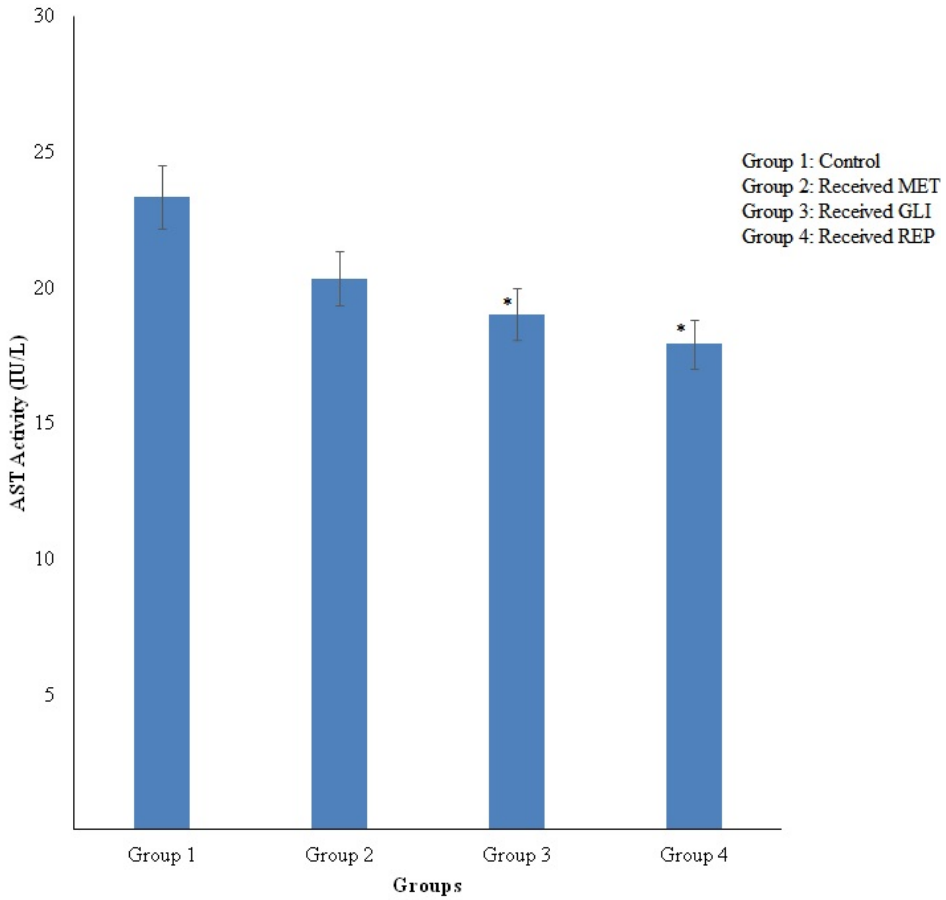

Figure 8. Effects of metformin (MET), glibenclamide (GLI) and repaglinide (REP) on serum aspartate aminotransferase activity. Data are expressed as mean ± SEM (n=6). * *p<0.05* when compared with control group.

al., 1987; Gosh and Suryawanshi, 2001). Administration of MET, GLI and REP caused significant ($p<0.05$) reduction in the blood glucose concentration of the diabetic rats compared with the diabetic control group. The reduction in blood glucose concentration observed in MET and GLI treated diabetic rats is similar to previous reports (Abdel et al., 2004; Stalin et al., 2012; Shareef et al., 2013; Bamidele et al., 2014). The degree of effects of these standard anti-diabetic agents on the blood glucose concentration was in the order of MET > GLI > REP.

The mechanism by which they reduced blood glucose concentration in the rats may be by either increasing the pancreatic secretion of insulin from the cells of Islets of Langerhans or its release from bound insulin as documented in literature. The biguanides (for example, MET) does not affect insulin secretion but requires the presence of insulin to be effective. The exact mechanism is not clear, but it does decrease hepatic glucose production and increase peripheral glucose uptake (Hundal et al., 1992; Galuska et al., 1994; Stumvoll et al., 1995). The sulphonylureas (for example, GLI) may potentiate insulin effects, either by increasing insulin secretion, increasing release of bound insulin, enhancing transport of blood glucose to peripheral tissues or inhibiting the degradation of insulin in the vascular endothelial cells (Farougue and Meredith, 2003; Abdel et al., 2004). The drug molecules bind to a specific receptor (sulphonylurea receptor) identified as adenosine triphosphate (ATP) sensitive potassium (K_{ATP}) channel that is present on the pancreatic beta-cell membrane causing depolarization by reducing conductance of ATP sensitive K channels (Rachman and Turner, 1995). This results in the opening of voltage-gated calcium channel causing Ca^{2+} influx and degranulation with the release of pre-formed insulin (Karam, 1997; Philipson and Steiner, 2005). The meglitinides (for example, REP) stimulates insulin secretion by closing ATP-dependent potassium channels in pancreatic β-cells. They are incapable of stimulating insulin secretion in nutrient starved β-cells, but in the presence of glucose, they demonstrate hypoglycaemic effects by augmenting the release of insulin (Thomas and Thomas, 1997).

Results of the study showed that the lipid profile markers such as total cholesterol, triacylglycerol and

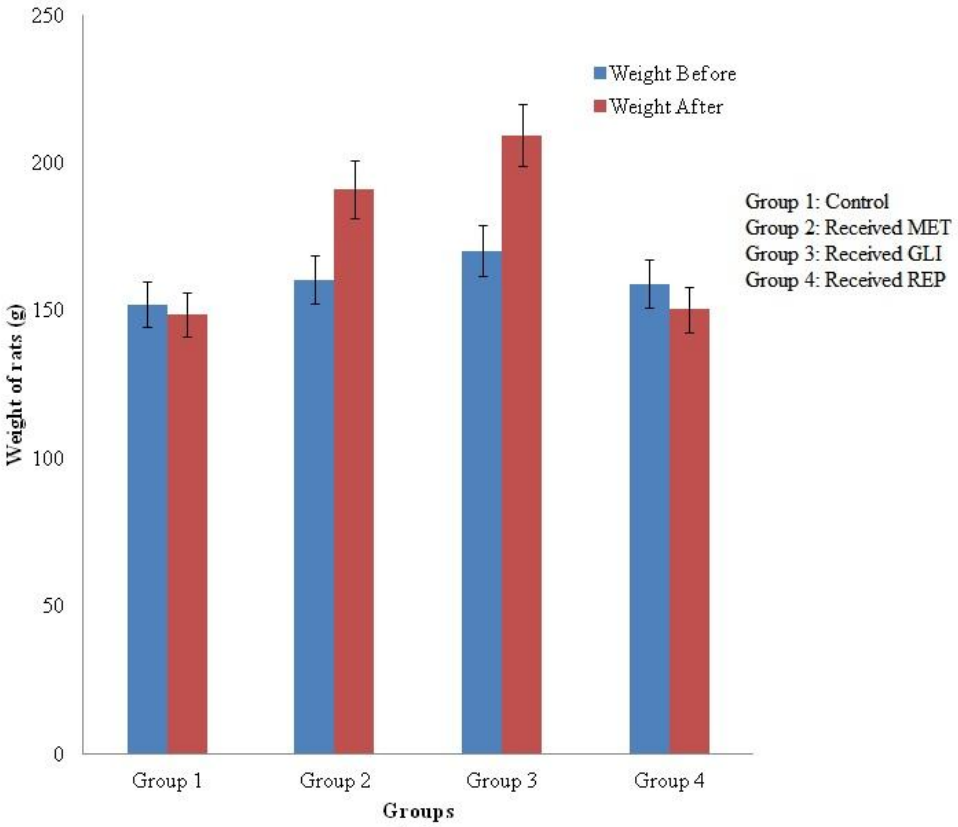

Figure 9. Effects of metformin (MET), glibenclamide (GLI) and repaglinide (REP) on the mean bodyweight.

LDL-cholesterol concentrations were observed to be higher except for the HDL-cholesterol concentration which was reduced in the diabetic control group. It is well known that in uncontrolled diabetes mellitus, there will be an increase in total cholesterol, triacylglycerol and LDL-C with a concomitant decrease in the HDL-C which contributes to coronary artery disease (Arvind et al., 2002; Selvan et al., 2008). This could be attributed to abnormalities in lipid metabolism due to diabetes-induced hypertriglyceridaemia and hypercholesterolaemia (Mitra et al., 1995). The increase in blood cholesterol and triacylglycerol concentrations may be due to the action of hormone sensitive lipase, which promotes lipolysis and subsequently increases the level of free fatty-acids and triacylglycerol in circulation. The free fatty acids are catabolized to acetyl-CoA which is further channeled to cholesterol synthesis; thus, increasing blood cholesterol level (Oyedepo, 2012).

The results of this study showed that administration of MET, GLI and REP considerably reduced the LDL-C, triacylglycerol and total cholesterol concentration with a concomitant increase in the HDL-C concentration compared with the diabetic control. Interestingly, similar observation has been reported with diabetic rats treated with MET and GLI respectively in previous study (Defronzo and Goodman, 1995; Chehade and Mooradian, 2000; Zannah et al., 2014). This could be due to increased breakdown of the cholesterol in the liver, and decreased absorption of cholesterol via the chylomicrons due to inhibition of α-glucosidase enzymes. The above result suggests that the administration of MET, GLI and REP may improve lipid dysfunction and hence retard the development of diabetic complications. This could be attributed to their promotion of utilization of glucose and hence depressed mobilization of fats.

The liver is an important insulin-dependent tissue which plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes (Seifter and England, 1982). Results of this study showed that the administration of alloxan to rats caused a marked elevation in the levels of serum AST, ALT and ALP which is indicative of hepatocellular damage. These enzymes are usually found in large quantities in the liver where they play an important role in the metabolism of amino acids. However, as a result of damage or toxicity to the liver, these enzymes leak from the hepatocytes into the blood circulation where their concentrations become elevated (Whitehead et al., 1999; Harris, 2005). Therefore, the high levels of AST, ALT and ALP in the diabetic control group suggest hepatocellular damage.

Several studies have reported similar elevation in the activities of serum AST, ALT and ALP during alloxan administration (Etuk and Muhammed, 2010; Owolabi et al., 2011). Administration of MET, GLI and REP reversed the higher levels of these enzymes caused by alloxan administration. This suggests that these standard anti-diabetic agents have the potential to prevent liver damage by maintaining the integrity of the plasma membrane thereby suppressing the leakage of the enzymes through the membrane, exhibiting hepatoprotective activity.

The result on the mean body weights of the animals after the experiment revealed a general increase in the body weights of the animals treated with MET and GLI while the group treated with REP elicited no significant ($p>0.05$) difference compared with the diabetic control. The reduction in the weights of the animals in the diabetic control group may be attributed to the effects of the alloxan resulting to depletion of fluid, accelerated breakdown of fats and adipose tissue and consistent low levels of feed intake due to lack of appetite (Oyedepo, 2012). The destruction of the pancreas results in the utilization of non-carbohydrate moieties such as protein for the synthesis of glucose.The loss of structural proteins like muscle protein in increased gluconeogenesis together with increased lipolysis and increased synthesis of ketone bodies results in severe weight loss. However, the increase in the body weights of the rats administered MET and GLI may be attributed to facilitated glucose utilization by peripheral tissues.

Conclusion

From this study results, it could be concluded that metformin (MET), glibenclamide (GLI) and repaglinide (REP) showed profound reduction in the blood glucose concentration and considerable improvement in the other biochemical parameters assayed in the rats. The degree of effects of these standard anti-diabetic agents on the blood glucose concentration was in the order of MET > GLI > REP. MET and GLI exhibited significant anti-hyperglycaemic response in the animals treated better than REP. However, the degree of effects of these standard anti-diabetic agents on the altered biochemical parameters was in the order of GLI > MET > REP. The evidence from this study therefore suggests that MET and GLI may be better agents in achieving and/or maintaining glycaemic control and possibly biochemical complications often associated with diabetic manifestations. However, repaglinide still appears to be a promising antidiabetic agent for the management of diabetes mellitus.

Conflict of Interests

The authors have not declared any conflict of interests.

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