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# Evaluation of toxic effects of metformin hydrochloride and glibenclamide on some organs of male rats

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**Summary:** Metformin hydrochloride (MET) and glibenclamide (GB) are used in the management of Type 2 Diabetes (T2D). This study was designed to investigate the toxic effect of MET and GB in the Liver, kidney and testis of rats. Twenty one rats were divided into three groups of seven rats each; group 1 served as control, groups 2 and 3 received GB and MET at doses of 5 and 30 mg/kg, respectively, for 21 days by oral gavage. Results indicate that MET and GB treatment did not affect body weight-gain in the rats. Also, there were no treatment-related changes in the absolute and relative weights of liver, kidney, and testis in MET and GB-treated rats relative to controls. However, significant increase ($p<0.05$) in testicular lipid peroxidation levels were accompanied by reduction in epididymal sperm count and motility in MET and GB-treated rats, whereas sperm live/dead ratio was unaffected. Specifically, MET and GB decreased sperm count and motility by 34%, 31% and 25%, 28%, respectively. Activities of hepatic and renal superoxide dismutase (SOD) and catalase (CAT), serum alkaline phosphatase, lactate dehydrogenase and alanine aminotransferase were not significantly ($p>0.05$) affected in MET and GB-treated rats, whereas testicular SOD, CAT, glutathione, serum aspartate aminotransferase and conjugated bilirubin were markedly affected by MET treatment. Histopathological results showed marked necrosis, degeneration of seminiferous tubules and defoliation of spermatocytes in testis of MET-treated rats. Taken together, MET and GB induced lipid peroxidation, affected seminal qualities and decreased antioxidant status. These drugs may interfere with normal biochemical processes in testis and liver of the rats.

**Keywords***:* Toxicity, Diabetes, Glibenclamide, Metformin.



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## INTRODUCTION

The prevalence of Type 2 diabetes (T2D) has reached epidemic proportions worldwide and promotes the risk of cardiovascular diseases and mortality. Metformin (1,1- dimethylbiguanide) (MET), a biguanide derivate, is widely prescribed to treat hyperglycaemia in T2D and is recommended, in conjunction with lifestyle modification (such as diet, weight control and physical activity). It is a first-line oral therapy in the recent guidelines of ADA (American Diabetes Association) and EASD (European Association of the Study of Diabetes) (Adler *et al*., 2009; Nathan *et al*., 2009). It has been reported that MET maintain intensive glucose control and decrease the risk of diabetes-related end points and death (Selvin *et al*., 2008; Lamanna *et al*., 2011). MET therapy in T2D is associated with fewer hypoglycaemic attacks when compared with insulin and sulfonylureas. The reduction in cardiovascular mortality by MET compared with other oral diabetic agent has been confirmed in more than 30 clinical trials (Lamanna *et al*., 2011). Recent clinical trials suggest that MET may have therapeutic potential in other conditions, including diabetic nephropathy, cardiovascular diseases, polycystic ovary disease and treatment of cancer (Viollet *et al*., 2012). Despite its clinical usefulness, little is known about the tissue toxicity of MET.

Sulphonylureas (SUs), such as glimepiride and glibenclamide (GB), are commonly used oral antidiabetic drugs which stimulate insulin secretion by blocking an ATP-dependent potassium channel ($K_{ATP}$ channel) on plasma membrane of pancreatic *β*-cells (Mayer *et al*., 2011). Despite the worldwide use of SUs, loss of β-cell mass and function, and hypoglycemic episode have raised concern regarding its use (Kwon *et al*., 2011). Studies have shown that

SUs may induce apoptosis in β-cell lines and rodent islets (Efanova *et al*., 1998), and its therapy failure is common in long-term treatment (Kahn *et al*., 2006). However, some evidence has suggested that chronic use of SUs may lead to endoplasmic reticulum (ER) stress in β-cells, which finally causes exhaustion of β-cell function (Takahashi *et al*., 2007), and the decline in β-cell function causes the progressive deterioration of glycemic control. The use of SUs for the treatment of T2D may therefore have deleterious effect on other tissues in the body. In view of the above, this study was designed to obtain a more comprehensive overview of the toxic effect of MET and GB in the liver, kidney and testis of rats.

## MATERIALS AND METHODS

### *Chemicals*

Metformin hydrochloride and glibenclamide produced by Merck Sante s.a.s., United Kingdom and Sanofi-Aventis Limited, Nigeria, respectively, were purchased from a Local Chemist in Ibadan, Nigeria. Hydrogen peroxide, 5,5'-dithios-bis-2-nitrobenzoic acid (DTNB) and epinephrine were purchased from Sigma Chemical Co., Saint Louis, MO USA. All other chemicals used were supplied by Zayo Company, Jos, Nigeria, which is an accredited supplier for Sigma and BDH.

### *Animals*

Inbred male Wistar rats weighing between 170 and 200g were purchased from the animal house of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria. Animals were kept in ventilated cages at room temperature (28-30$^{o}$C) and maintained on normal laboratory chow (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum*. Rats handling and treatments conform to guidelines of the National Institute of Health (NIH publication 85-23, 1985) for laboratory animal care and use.

### *Study Design*

Twenty-one male albino rats (Wistar strain) were randomly distributed into three groups of seven animals each. Animals were given a period of two weeks for acclimatization before the experiment. The first group served as the control and was given corn oil (Vehicle for drugs). The second group received metformin hydrochloride (MET) at a dose of 30 mg/kg body weight/ day and the third group received glibenclamide at a dose of 5 mg/kg body weight/ day. Both doses are equivalents of human therapeutic doses of the drugs. Drugs were prepared with corn oil and given daily to the animals by oral gavage for twenty-one consecutive days.

### *Preparation of Serum*

Rats were fasted overnight and sacrificed by cervical decapitation 24 hours after the last dose of drugs. Blood was collected from the heart of the animals into plain centrifuge tubes and was allowed to stand for 1 hour. Serum was prepared by centrifugation at 3,000 *g* for 15 minutes in a Beckman bench centrifuge. The clear supernatant was used for the estimation of serum urea, creatinine and enzymes

### *Preparation of tissues*

Liver, kidney and testis from the rats were quickly removed and washed in ice-cold 1.15% KCl solution, dried and weighed. A section of liver and kidney samples were fixed in 10% formalin, while the right testes were fixed in Bouin's solution. Other parts were homogenized in 4 volumes of 50mM phosphate buffer, pH 7.4 and centrifuged at 10,000 *g* for 15 minutes to obtain post-mitochondrial supernatant fraction (PMF). All procedures were carried out at temperature of 0-4$^{0}$C.

### *Biochemical assays*

Protein contents of the samples were assayed by the method of Lowry *et al*., (1951) using bovine serum albumin as standard. Serum urea and creatinine levels were determined by the method of Talke and Schubert (1965) and Jaffe (1886), respectively. The activities of alanine and aspartate aminotransferases (ALT and AST) were assayed by the combined methods of Mohun and Cook (1957) and, Reitman and Frankel (1957). The estimation of serum alkaline phosphatase (ALP) activity was based on the method of Williamson (1972). ALP activity was measured spectrophotometrically by monitoring the concentration of phenol formed when ALP reacts with disodium phenyl phosphate at 680 nm. Serum total bilirubin level was assayed by the method of Rutkowski and Debaare (1966), the method involved the reaction between bilirubin and diazotized sulphanilic acid in alkaline medium to form a blue coloured complex, which was read spectrophotometrically at 546 nm. The activity of serum lactate dehydrogenase (LDH) was determined by the method of Zimmerman and Weinstein (1956). PMF lipid peroxidation levels were assayed by the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde (MDA), an end product of lipid peroxides as described by Buege and Aust (1978). PMF superoxide dismutase (SOD) activity was measured by the nitro blue tetrazolium (NBT) reduction method of McCord and Fridovich (1969). PMF catalase (CAT) activity was assayed spectrophotometrically by measuring the rate of decomposition of hydrogen peroxide at 240 nm as described by Aebi (1974).

***Sperm analysis***

Sperm motility was assessed by methods described by Zemjanis (1970) and Rezvanfar *et al*., (2008). Epididymal sperm count was determined by the method described by Pant and Srivastava (2003).

***Histopathology of tissues***

Tissues fixed in 10% formalin and bouin's solution were dehydrated in 95% ethanol and then cleared in xylene before embedded in paraffin. Micro sections (about 4 µm) were prepared and stained with haematoxylin and eosin (H&E) dye, and were examined under a light microscope by a Histopathologist who was ignorant of the treatment groups.

***Statistical analysis***

All values were expressed as the mean ± S.D. of seven animals per group. Data were analyzed using one-way ANOVA followed by the post-hoc Duncan multiple range test for analysis of biochemical data using SPSS (10.0). Values were considered statistically significant at $p< 0.05$.

**RESULTS**

Administration of GB and MET at doses of 5 and 30 mg/kg body weight did not significantly ($p>0.05$) affect the weight-gain and relative weights of liver, kidney and testis of the rats (Table 1). Also, there were no significant ($p>0.05$) differences in the levels of serum total and unconjugated bilirubin, protein, creatinine and urea in MET and GB-treated rats (Table 2). MET and GB treatment did not produce significant ($p>0.05$) alteration in the activities of serum alkaline phosphatase, lactate dehydrogenase and alanine aminotransferase relative to controls (Figures 1 and 2). However, MET administration significantly ($p<0.05$) increased the level of serum conjugated bilirubin and aspartate aminotransferase activity in the rats by 307% and 70%, respectively (Table 2 and figure 2). Also, MET and GB administration caused insignificant ($p>0.05$) effect on the ratio of live/dead sperm cells, however, sperm count and motility were significantly ($p<0.05$) decreased in MET- and GB-treated rats (Figure 3). Furthermore, MET and GB treatment caused significant increase ($p<0.05$) in testicular lipid peroxidation (LPO) as assessed by the accumulation of malondialdehyde (MDA). Specifically, MDA levels in the testis increased by 74% and 67% in MET and GB-treated rats, respectively (Figure 4). The increased testicular LPO levels were accompanied by significant ($p<0.05$) decrease in the activities of testicular superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) in MET and GB-treated rats (Figures 5, 6 and 7). However, there

**Table 1.** Effect of metformin hydrochloride(MET) and glibenclamide (GB) on serum protein and some biochemical variables of rats after 21days of treatment

| Groups | Body weight (g) | | Weight-gain (g) | Weight (g) | | | Relative Weight (% of body weight) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Initial | Final | | Liver | Kidney | Testis | Liver | Kidney | Testis |
| Control | 178.2±7.3 | 202.4±5.3 | 24.2±2.4 | 4.7±0.4 | 1.3±0.2 | 2.9±0.2 | 2.3±0.2 | 0.6±0.1 | 1.4±0.2 |
| MET | 180.3±6.1 | 199.6±7.2 | 19.3±5.1 | 4.9±0.3 | 1.2±0.1 | 3.1±0.1 | 2.5±0.3 | 0.6±0.2 | 1.6±0.1 |
| GB | 184.0±5.6 | 206.3±4.9 | 22.3±4.7 | 4.6±0.5 | 1.3±0.3 | 2.8±0.1 | 2.2±0.3 | 0.6±0.2 | 1.4±0.1 |

Values are the Mean±SD, n=7

**Table 2**. Effect of metformin hydrochloride(MET) and glibenclamide (GB) on the body weights and relative weights of liver, kidney and testis of rats after 21days of treatment

| Groups | SERUM | | | | | |
|---|---|---|---|---|---|---|
| | Protein (mg/ ml) | TB (mmol/L) | DB (mmol/L) | IB (mmol/L) | Creatinine (µmol/L) | Urea (µmol/L) |
| Control | 10.7 ± 2.1 | 324.8±8.8 | 8.9±1.6 | 315.9±5.2 | 2.66 ± 0.15 | 10.7 ± 2.08 |
| MET | 10.7± 2.6 | 329.4±12.2 | 36.2±8.8* | 293.2±7.4 | 2.98 ± 0.29 | 10.7 ± 2.55 |
| GB | 13.1 ± 1.0 | 342.6±16.4 | 7.2±5.2 | 335.4±11.2 | 2.94 ± 0.21 | 13.2 ± 1.00 |

Values are the Mean±SD, n=7, TB=Total bilirubin, DB=Direct/conjugated bilirubin, IB= Indirect/unconjugated bilirubin

were no significant ($p>0.05$) differences in the levels of hepatic and renal LPO, SOD, GSH and CAT in MET and GB-treated rats when compared to controls. Figures 8a, 8b and 8c show representative photomicrographs of liver, kidney and testes from MET and GB-treated animals. Slides from control animals showed normal architecture of the tissues. Mild distortion and slight necrosis could be seen in liver and kidney tissues from MET and GB-treated animals. In figure 8c, MET treatment caused marked necrosis, degeneration of seminiferous tubules and defoliation of spermatocytes in the testis.

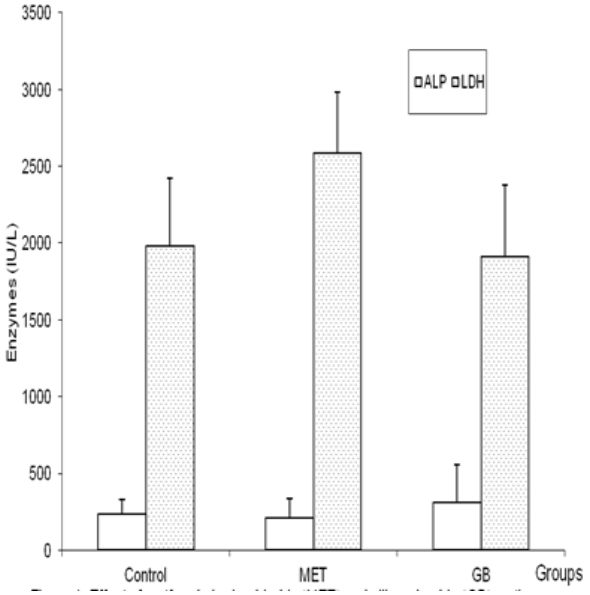


**Figure 1.** Effect of metformin hydrochloride(MET) and glibenclamide (GB) on serum Alkaline Phosphatase (ALP) and Lactate dehydrogenase (LDH) of rats after 21days of treatment

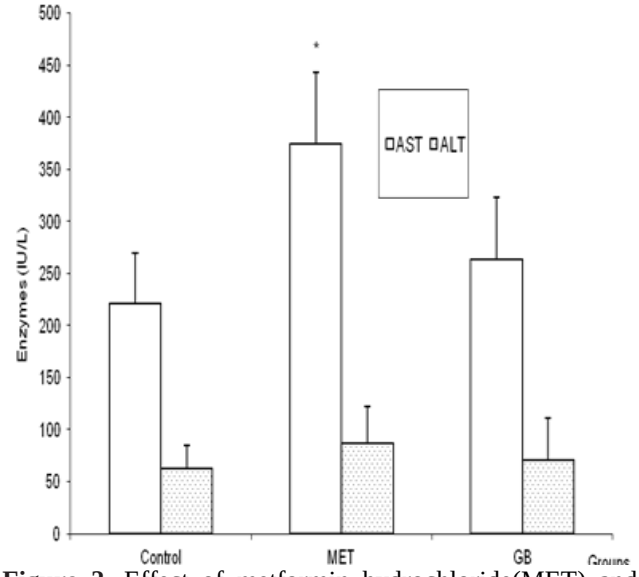


**Figure 2.** Effect of metformin hydrochloride(MET) and glibenclamide (GB) on serum Aspartate amino transferase (AST) and Alanine transferase (ALT) of rats after 21days of treatment. *P<0.05

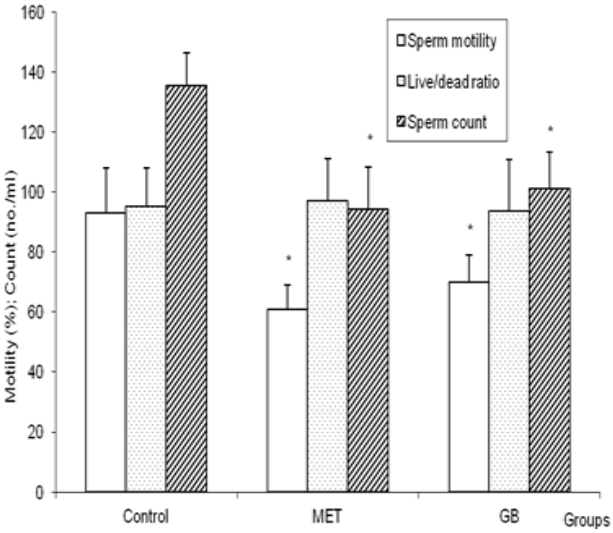


**Figure 3.** Effect of metformin hydrochloride (MET) and glibenclamide (GB) on sperm motility, live/dead ratio and sperm count in males rats after 21days of treatment. *P<0.05

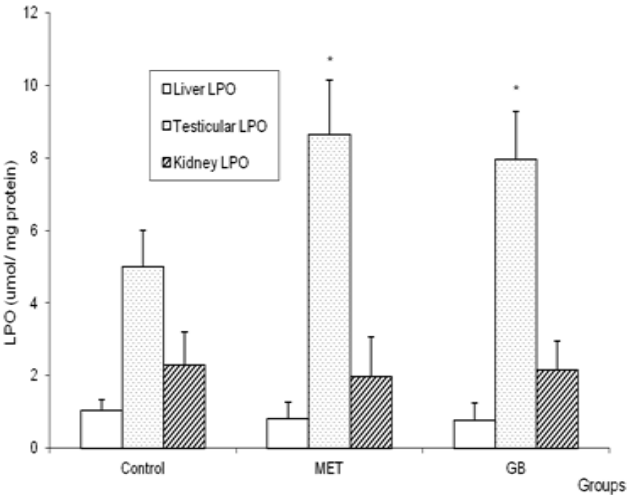


**Figure 4.** Effect of metformin hydrochloride (MET) and glibenclamide (GB) on the level of liver, testicular and kidney lipid peroxidation (LPO) rats after 21days of treatment. *P<0.05

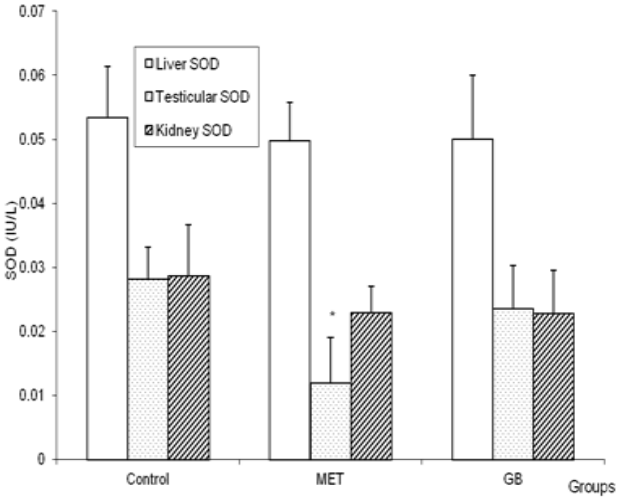


**Figure 5.** Effect of metformin hydrochloride (MET) and glibenclamide (GB) on the level of liver, testicular and kidney Superoxide dismutase (SOD) rats after 21days of treatment. *P<0.05

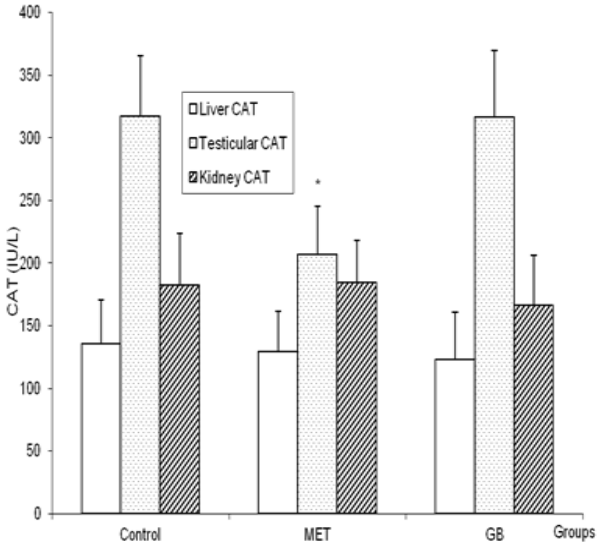


**Figure 6.** Effect of metformin hydrochloride (MET) and glibenclamide (GB) on the level of liver, testicular and kidney Catalase (CAT) rats after 21days of treatment. *P<0.05

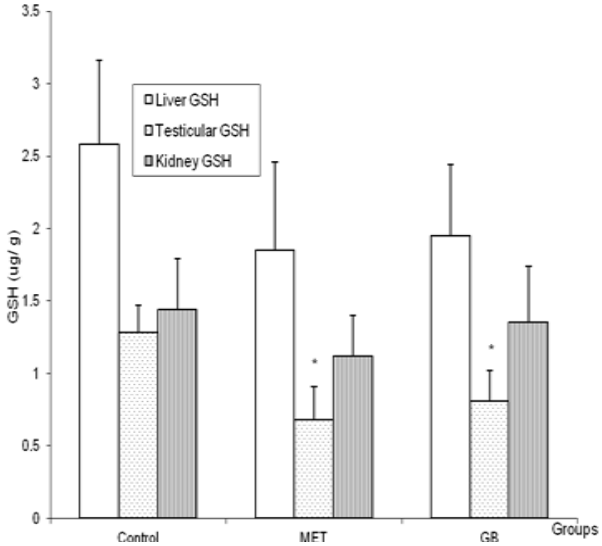


**Figure 7.** Effect of metformin hydrochloride (MET) and glibenclamide (GB) on the level of liver, testicular and kidney Glutathione (GSH) rats after 21days of treatment. *P<0.05

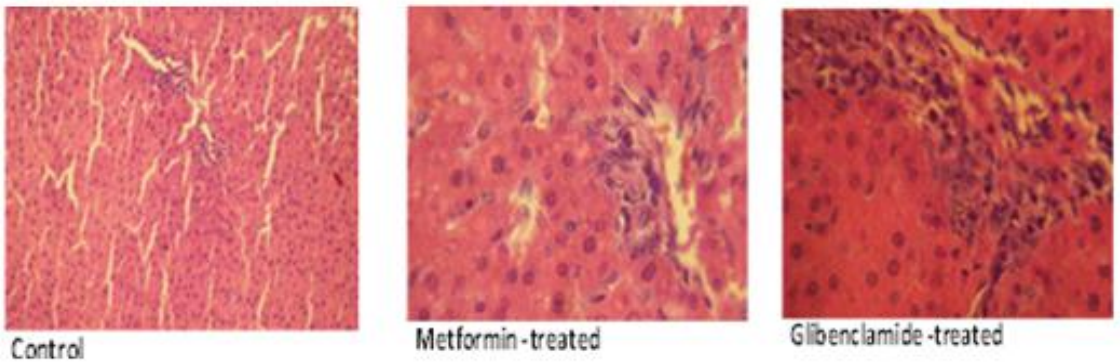


**Figure 8a.** Representative photomicrographs of liver slides from control, metformin- and glibenclamide-treated rats (X100) H&E. Normal architectural layout with no visible lesions (control); mild distortion with marked necrosis in glibenclamide and metformin-treated groups.

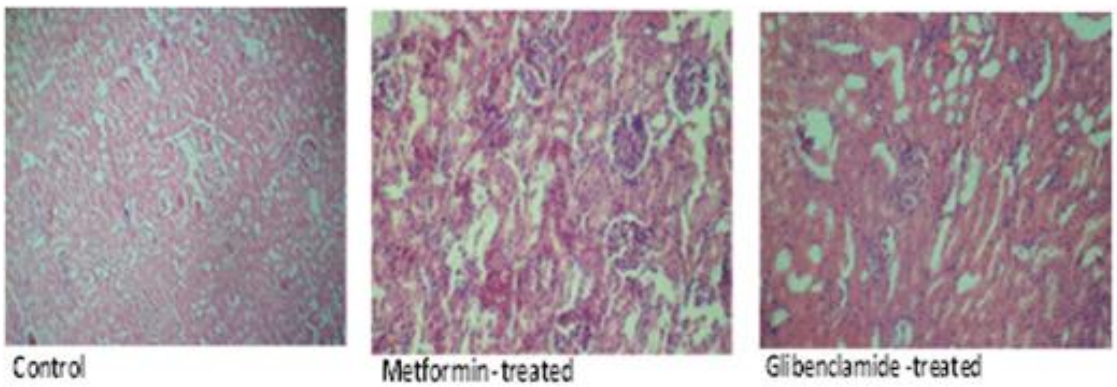


**Figure 8b.** Representative photomicrographs of kidney slides from control, metformin- and glibenclamide-treated rats (X100) H&E. Normal architectural layout with no visible lesions (control); mild distortion with marked necrosis in glibenclamide and metformin-treated groups.

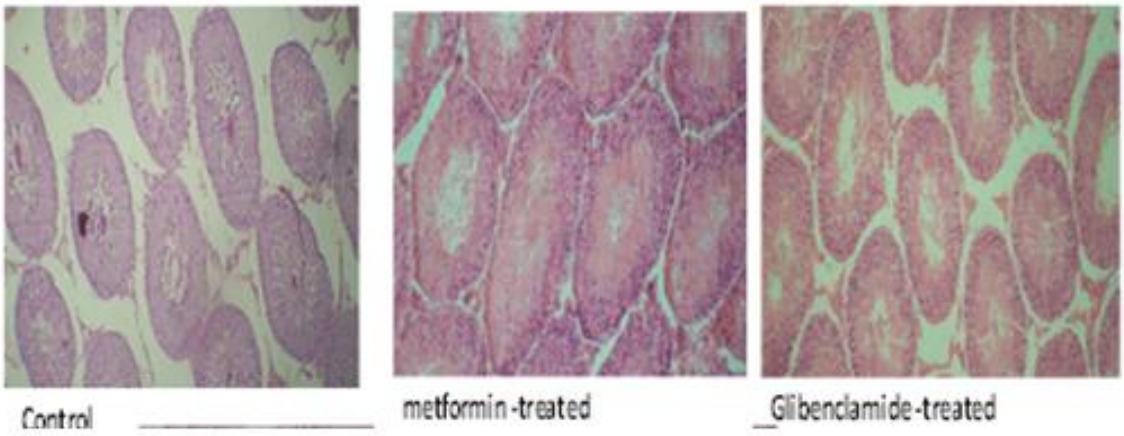


**Figure 8c.** Representative photomicrographs of testicular slides from control, metformin- and glibenclamide-treated rats (X100) H&E. Normal architectural layout with no visible lesions (control and metformin-treated); major distortion with marked necrosis in glibenclamide-treated groups.

## DISCUSSION

In the present study, we found that MET and GB caused testicular dysfunction that was mainly mediated by increased lipid peroxidation and decreased antioxidant status in testis of the rats. The significant decreases in sperm progressive motility and count, and testicular injury revealed by histopathologic examination in the treated groups are the major findings of the present study.

Today, there is considerable interest in free radical mediated damage to biological systems due to xenobiotics. A large number of xenobiotics have been identified to have potential to generate free radicals in biological systems (Jaeschke *et al*., 2012). Free radicals have become an attractive means to explain the toxicity of numerous xenobiotics. Some of these free radicals interact with various tissue components, resulting in dysfunction and injury to the tissues. Lipid peroxidation has been suggested as one of the molecular mechanisms involved in drug-induced tissue injuries (Jeng *et al*., 2011). In the present study, increased levels of malondialdehyde (MDA), an index of lipid peroxidation, were observed in tissues of the treated rats. This may be due in part to radical species generated during metabolism of MET and GB, which attack cell membrane phospholipids and other circulating lipids. Mammalian sperm cells present a specific lipid composition, with a high content of polyunsaturated fatty acids, plasmalogens and sphingomyelins. The lipids in spermatozoa are the main substrates for peroxidation, which explained why the process of lipid peroxidation is very high within testis in the present study. Aitken *et al*., (1989) have shown that excess amounts of reactive species produced during metabolism of xenobiotics may have adverse effects on sperm motility and fertility. Thus, oxidative damage to lipids of spermatozoa may be associated with declining in progressive motility and diminished fertility of sperm (Chen *et al*., 1997; Kao *et al*., 1998). This observation was, however, confirmed in the present study in which GB- and MET-treated rats had reduced sperm count and motility when compared to the control. Our study also strongly suggests the possibility of involvement of oxidative stress in MET- and GB-induced testicular dysfunction due to the decline in the testicular GSH levels in the animals. GSH is a tripeptide, powerful nucleophile and a major defence against radical-induced cellular damage, and also controls the transcription of SOD (Gardner and Fridovich, 1987). It has been repeatedly reported that drugs- toxicity enhances the depletion of GSH in cells (Shah and Iqbal 2010). In the present study, the decline in GSH content in testis of treated rats may be due to its utilization to challenge the prevailing oxidative stress under the influence of radicals generated by MET or GB. In addition, GSH can protect the membrane polyunsaturated fatty acids from free radical-mediated LPO by abstracting the hydrogen of the thiol group instead of the methylene hydrogen of unsaturated lipids (Kosower and Kosower, 1979).

It is known that some xenobiotics may inhibit the activity of antioxidant enzymes system, which probably may lead to intracellular accumulation of reactive species with subsequent development of tissue injury. These reactive species may cause decline in the activity of cellular antioxidants, in particular SOD, CAT and GSH (Prakash *et al*., 1997). SOD is the first line of defence against the deleterious effects of oxygen radicals in cells, and it scavenges

reactive species by catalyzing the dismutation of superoxide radicals to hydrogen peroxide (Okado-Matsumoto and Fridovich, 2001). The inhibition of SOD activity during MET and GB treatment may result in an increased flux of superoxide radicals in the animals, which explain, in part, the increased LPO in tissues of treated rats. CAT acts as a preventive antioxidant in cells. Both CAT and SOD are co-regulated in tissues in response to toxic assaults (Lew and Quintanilha, 1991). Therefore, the observed decline in the activities of testicular CAT and SOD following MET and GB-treatment further give support to the adverse effect of these xenobiotics on seminal qualities. Testicular dysfunction in the treated groups was also revealed in histological examination by marked necrosis and degeneration of seminiferous tubules as well as defoliation of spermatocytes. Seminiferous tubule atrophy and the decrease in spermatogenic cells observed in MET and GB-treated rats are indicators of spermatogenesis failure.

To our knowledge, this study is the first that report the direct effect of metformin hydrochloride and glibenclamide on male reproductive system, especially on the sperm quality. Taken together, the results of our study suggest that MET and GB may cause testicular dysfunction and further aggravate the complications of diabetes. This study therefore suggests the need to carefully monitor humans on MET and GB therapy.

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