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A comparative evaluation of cardiac and neurological safety status of two commonly used oral hypoglycaemic agents in T2-DM Swiss albino mice model

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ABSTRACT

Background: Diabetes mellitus (DM), along with its associated complications, including diabetic neuropathy and hyperlipidemia, has become a global concern in the last few decades. The main objective of our study is to evaluate the comparative neuro-safety status, serum plasma glucose, and lipid-lowering potential of two widely recognized antidiabetic drugs named metformin and glimepiride.

Methods: The neurological evaluation was done by open field test, hole board test, forced swimming test, dark and lighthouse test, and elevated plus maze test by employing diazepam as standard. Serum blood glucose level of streptozotocin (STZ)-induced diabetic mice was determined by glucose oxidizing method using a glucometer. Total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very-low-density lipoprotein cholesterol (VLDL-C) levels were estimated by using the reference method where atorvastatin was used as standard.

Results: In neurological evaluation, both drugs produce almost the same anxiolytic activity in the open field test, hole board test, light and dark house test, and elevated plus maze test. However, in the forced swimming test, glimepiride produced more antidepressant activity than metformin. Glimepiride was found to remarkably reduce serum glucose and VLDL-C levels more than metformin, whereas, for other parameters, metformin takes over glimepiride sometimes took over the standard atorvastatin.

Conclusions: The results of our study indicate that both oral hypoglycaemic drugs alter the lipid index while producing some anxiolytic effects on the central nervous system. Thus, recommended to be carefully administered to patients with low BMI and might be beneficial to patients suffering from peripheral nerve function and anxiety.

1. Introduction

In the last few decades, continuously increasing reports have brought diabetes mellitus (DM) into a pandemic position and has become a major global issue where 80% of mortality can be attributed to this disease [1]. As a result of diabetes,' widespread complications, and higher risk of mortality, global health care systems and economies are putting under tremendous capital pressure. According to IDF Diabetes Atlas, more than 537 million people ages 20 to 79 experience diabetes in their lifetime. By 2030, this amount is estimated to climb to 643 million and 783 million by 2045, respectively. A person with diabetes dies every 5 s, causing 6.7 million losses of life by 2021 [2]. A group of complications is associated with this disease, including diabetic neuropathy and dyslipidemia, which further increases the risk of cardiovascular disease and other health hazards [3]. Lipid abnormalities are a common scenario in patients with diabetes, characterized by high total cholesterol (TC) levels, higher levels of triglyceride (TG), lower levels of high-density lipoprotein cholesterol (HDL-C), and increased level of low-density lipoprotein

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cholesterol level (LDL-C). In a diabetic state, low-density lipoprotein cholesterol (LDL-C) level may remain normal or moderately increased [4]. Diabetic neuropathy is a peripheral nerve dysfunction and approximately half of all diabetic patients experiences this neurological abnormality. Hyperglycaemia is responsible for this complication which can damage nerve fibres throughout the body [5].

Metformin is a biguanide derivative, one of the most commonly used drugs for the management of type 2 DM, and it has been reported that it reduces diabetes mortality and complications by 30% compared to insulin, glibenclamide, and chlorpropamide [6]. It inhibits crucial enzymes responsible for gluconeogenesis and glucose synthesis in the liver through the activation of adenosine monophosphate kinase (AMPK) but stimulates insulin signalling and glucose transport in the muscle. This AMPK enzyme determines the fate of various metabolic pathways, including glucose metabolism, lipid metabolism, and energy homeostasis [6]. The activation of AMPK introduces the neuroprotective effect of metformin on human neural stem cells [7]. The role of metformin as stimulation of AMPK as well as autophagy has the potential to improve the nerve cell metabolic activity as well as to boost nerve healing, and minimize harmful protein accumulation in neurological illnesses [8]. Furthermore, metformin minimizes the activity of acetylcholinesterase (AChE), which is recognized as a primary enzyme responsible for the hydrolytic degradation of acetylcholine (Ach) to choline, Ach is a neurotransmitter involved in the process of memory and brain function [9]. Metformin exerts not only plasma glucose-lowering effects but is also well recognized for its beneficial effect on reducing serum lipid profiles, reducing inflammatory cell adhesion to the endothelium, and anti-inflammatory, anti-apoptotic and antioxidative profiles [6].

Glimepiride belongs to the second-generation sulfonylurea that stimulates the pancreatic β cells to secrete insulin through interaction with the sulfonylurea receptor (SUR) and concurrently enhances insulin action in peripheral tissues. It can interact with lipid rafts, DIGs (detergent-insoluble glycolipid), at the plasma membrane of adipose and muscle cells, which results in the introduction of insulin-mimetic activity via the activation of a glycosylphosphatidylinositol-specific phospholipase, redistribution of signalling components and positive cross-talk downstream of the insulin signalling cascade. It produces insulin-sensitizing activity through the regulation of adipocytokines released from adipocytes, which may be dependent on or independent of SUR or DIGs [10]. Compared to other sulfonylurea drugs, glimepiride was associated with a lower risk of developing hypoglycaemia and weight gain in clinical trials [11] and fewer cardiovascular effects than other sulfonylurea drugs due to minimal effects on ischemic preconditioning of cardiac myocytes. It is effective in reducing fasting plasma glucose, postprandial glucose, and glycosylated haemoglobin levels and is considered a useful, cost-effective treatment option for managing type 2 DM [7]. Besides having tremendous insulin secretory effects, the neuroprotective effects, i.e., of glimepiride, are attributed to the stimulation of insulin secretion in non-diabetic models. Therefore, the stimulatory effect on insulin secretion provides the neuroprotective profile of glimepiride [12].

In our study, we investigated the comparative impact of metformin and glimepiride on lipid profile in diabetic mice models, along with the cardiac and neurological safety status of these commonly used hypoglycaemic drugs.

2. Materials and methods

2.1. Experimental animals

To conduct the comparative cardiac and neurological safety study of both metformin and glimepiride, Swiss albino mice were imported from the animal house of Jahangirnagar University, Savar, Dhaka, Bangladesh, and kept in transparent polypropylene cages at a temperature of 20–25 °C and 55 ± 10% relative humidity. Feeding of mice was done with ICDDRB formulated rodent food and water at the libitum. Before 3–4 days of the date of the experiment, the animals were allowed to acclimatize to the laboratory condition. The experimental protocol used in this study in the mice models was carried out based on the guideline of the Institutional Animal Ethics Committee.

2.2. Collection of drugs

Active pharmaceutical ingredient (API) samples of atorvastatin, glimepiride, and metformin were kindly provided by BEXIMCO Pharmaceuticals Ltd, Bangladesh, while diazepam API sample was kindly given by SQUARE Pharmaceuticals Ltd, Bangladesh. All other reagents and materials used in the experiment were analytical grade. In this study, we used glimepiride 4 mg/kg dose and metformin 250 mg/kg dose. The dose was selected based on some previous studies where glimepiride at a 4 mg/kg dose and metformin at a 250 mg/kg dose produced maximal effects [13,14].

2.3. Preparation and administration of drug

For oral administration of glimepiride at a 4 mg/kg dose, first, the drug was accurately measured and grounded with a clean and fresh mortar pestle. The powder was dissolved in 0.1 N NaOH solution, and 1 ml of the prepared solution was administered orally. On the other hand, atorvastatin 40 mg/kg and metformin 250 mg/kg dose were prepared as same where distilled water was used to prepare the solution. 1 ml of prepared solution was administered to test animals through oral gavage.

2.4. Induction of type-2 diabetes in a mice model

Exactly 40 healthy mice were randomly selected for the experiment, and they were fasted overnight for at least 12 h. Hyperglycaemia was induced in each mouse through the administration of streptozotocin (STZ) (35 mg/kg suspended in 0.1 mol/L citrate buffer at pH 4.5) [15]. After 48 h of administering STZ, blood glucose was assayed by the glucose oxidase method using a glucometer. Once hyperglycaemia (blood glucose >250 mg/dl) was established within the studied mouse model. The animals were subjected to subsequent treatment for the rest of the analysis.

2.5. Bodyweight, lipid profile, and major organ effect analysis

2.5.1. Study design

Exactly 20 diabetic mice were randomly selected for the experiment and they were separated into 4 different groups.

Group 1 (Control group): All diabetic mice were fed a normal diet.

Group 2 (Atorvastatin group): All diabetic mice were fed 40 mg/kg standard atorvastatin along with the normal diet.

Group 3 (Glimepiride group): All diabetic mice were fed glimepiride 4 mg/kg along with the normal diet.

Group 4 (Metformin group): All diabetic mice were fed metformin 250 mg/kg along with the normal diet.

2.5.2. Animal sacrifice and pathological examination of organ weight

After the completion of the seventh day of treatment, the experimental animals were humanely killed by CO2 and sacrificed by decapitation. Blood was collected by cardiac puncture for analysis. After sacrificing, the liver, the kidney, and accumulated fat were separated from the carcasses and preserved in normal saline. All experimental organ was weighed separately, and the average was compared statistically.

2.5.3. Determination of serum cholesterol, triglyceride, HDL-C, LD-C, VLDL-C level

After the collection of blood, the serum was separated through centrifugation. Then TC, TG, HDL-C, LDL-C, and VLDL-C were estimated by the enzymatic method using Auto pack kits. These reagents were

manufactured by the standard company Human Diagnostics Worldwide, Germany. TC was determined by an enzymatic colorimetric method (endpoint) [15], and TG was estimated by the enzymatic colorimetric GPO-PAP method [16]. HDL-C was measured by phosphotungstate method [17] by using Mindray BA-88A Semi-Auto Clinical Chemistry Analyzer (Crown Healthcare, Tanzania). VLDL-C was measured indirectly from triglyceride values, and LDL-C was also determined indirectly based on Fried Walds Equation [18].

$$VLDL\text{-}C = TG/5$$

$$LDL\text{-}C = HDL\text{-}C + VLDL\text{-}C$$

2.6. Neuropharmacological safety profile analysis

2.6.1. Study design

Exactly 20 diabetic mice were randomly selected for the experiment and were separated into 4 treatment groups as described below:

Group 1 (Control group; n = 5): All diabetic mice were fed a normal diet.

Group 2 (Diazepam group; n = 5): All diabetic mice were fed 2 mg/kg standard diazepam along with the normal diet.

Group 3 (Glimepiride group; n = 5): All diabetic mice were fed glimepiride 4 mg/kg along with the normal diet.

Group 4 (metformin group; n = 5): All diabetic mice were fed metformin 250 mg/kg along with the normal diet.

Exactly 60 min after treatment, the animals were subjected to different equipment set up to evaluate the effects of hypoglycaemic and diazepam feeding groups. One hour interval was given based on the peak plasma concentration of diazepam, metformin, and glimepiride, which are 1.4–1.5-h, 1.5–3.5-h, and approximately 2.5 h, respectively. After 1 h, 2.4 h, and 2 h of treatment of different drug groups, respectively, the animals were subjected to different equipment set up to evaluate the effects of hypoglycaemic [19–21].

2.6.2. Open field test

To evaluate the locomotor activity, exploration, and anxiolytic behavioral status, an open field test was done with the reference to the method described previously, where the number of squares crossed with all four paws was counted for 30 min. Activities of control and drug-treated mice were monitored in a balanced design to avoid the order effects [22].

2.6.3. Hole board test

The Hole board test determines the anxiolytic activity of experimental animals, which is based on the animal's tendency to snout or poke its nose through the holes. Hole poking or head dipping behavioural test was performed according to the method described previously [23].

2.6.4. Forced swimming test

The forced swimming test is recognized as the most widespread experimental model to evaluate the antidepressant activity of test animals, and we used Porsolt et al. method as the reference to conduct this test [22].

2.6.5. Elevated plus maze test

This test is a widely accepted behavioral assay to determine the anxiolytic effects of pharmacological elements and drugs. This test was conducted according to the reference method described previously [24], where the entry of all four paws inside the arm was described as arm entries.

2.6.6. Dark and light test

The dark and light test is another animal behavioral model for the evaluation of the anti-anxiety properties of experimental drugs. This test was done with the help of the reference method designed by Jacqueline and Frederick [25], where the test animals were allowed to freely run in a two-compartmental system that had a two-thirds illuminated area, and one-third dark area, These two areas were separated with black partitions. Each animal was placed at the centre of the transparent compartment and then the number of entries in each space, as well as the time spent in each compartment, was recorded for 30 min.

2.7. Statistical analysis

The results were expressed as mean ± SEM (n = 5). To perform statistical analysis of the results, a One-Way analysis of variance (ANOVA) followed by Dunnett's post hoc test was used as appropriate. All statistical analysis was performed by Statistical Package for Social science (SPSS) version 20 software. Differences between groups were considered significant at the level of $p < 0.05$.

3. Results

3.1. Body and organ weight variation and biochemical parameter analysis

3.1.1. Analysis of body weight

Table 1 Represents the bodyweight variation of different experimental groups after the treatment of experimental drugs. The results revealed that the metformin group exerted a noticeable downward tendency in weight gaining ($p < 0.05$) when compared with mice of other groups, even the tendency was lower than the standard atorvastatin group ($p < 0.05$). The bodyweight gaining tendency was higher in the glimepiride treatment group which was almost near to the control group.

3.1.2. Analysis of organ weight variation

Data obtained from Fig. 1 Represented that the average weight of the liver significantly decreased in both glimepiride and metformin treatment groups when compared with the control group ($p < 0.05$). The average weight of the right and left kidneys followed the same manner ($p < 0.05$). In the case of the fat deposition profile, the standard atorvastatin group had the lowest amount of deposited fat. The Glimepiride treatment group had the highest amount of deposited fat, and the amount of deposited fat in the metformin group was next to the standard atorvastatin group.

3.1.3. Evaluation of serum blood glucose

Results obtained from serum blood glucose reducing the efficacy of metformin and glimepiride revealed that both metformin and glimepiride significantly ($p < 0.05$) reduced the serum glucose level (Table 2). However, serum blood glucose level reducing the efficacy of glimepiride was more remarkable than metformin even after reaching the peak plasma concentration of the drug. Meanwhile, atorvastatin significantly ($p < 0.05$) increased the serum glucose level at T_{max} while compared to

Table 1
Bodyweight variation of mice of different experimental groups.

Name of the group	Initial body weight (gm)	Final body weight (gm)	Percentage of body weight gain
Control	22.18 ± 0.18	38.41 ± 1.014	37.41 ± 0.18
Atorvastatin (40 mg/kg)	22.62 ± 0.16	28.10 ± 1.304**	27.10 ± 0.10**
Metformin (250 mg/kg)	21.94 ± 0.11	26.21 ± 1.052*	25.21 ± 0.15*
Glimepiride (4 mg/kg)	22.25 ± 0.14	34.75 ± 1.081*	33.75 ± 0.16*

Values are expressed as Mean ± SEM (n = 5) where the level of significance stated as *p < 0.05, **p < 0.01. Body weight variation calculated in percentage based on the weight difference between initial and endpoint.

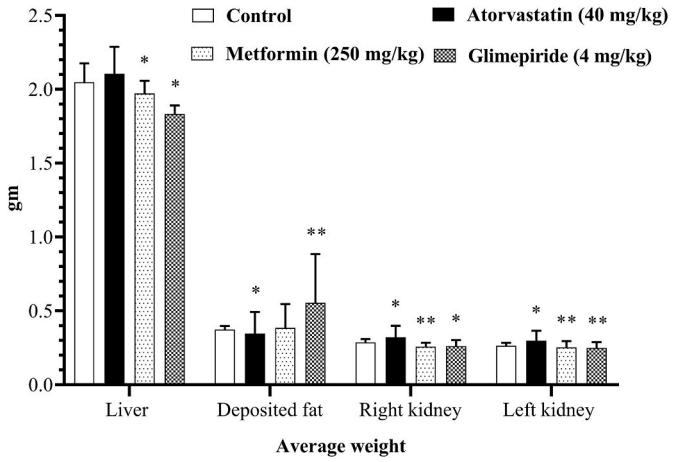

Fig. 1. Organ weight variation among mice of different experimental groups. Values are expressed as Mean ± SEM (n = 5) where the level of significance stated as *p < 0.05, **p < 0.01. Body weight variation calculated in percentage based on the weight difference between initial and endpoint.

Table 2
Effect of atorvastatin, metformin, and glimepiride on serum blood glucose level.

Group	Initial day reading (before treatment)	After 7 days of treatment reading	The plasma glucose level at drug T_{max} (mg/dl)
	After 12 h fasting (mg/dl)	After 12 h fasting (mg/dl)	
Control	421.56 ± 67.73	453.34 ± 23.08	
Atorvastatin (40 mg/kg)	338.85 ± 58.84	395.94 ± 21.40	443.31 ± 22.62***
Glimepiride (4 mg/kg)	413.64 ± 32.63	329.09 ± 20.33**	241.49 ± 20.80
Metformin (250 mg/kg)	329.90 ± 79.53	287.49 ± 23.73***	214.84 ± 28.76

Values are expressed as Mean ± SEM (n=5) where level of significance stated as *p < 0.05, **p < 0.01, ***p < 0.001. T_{max} value of the metformin, glimepiride, atorvastatin is 2.5 h; 3 h, 2 h based on the supplier data sheet.

the hypoglycaemic drug effects at T_{max} (Table 2).

3.1.4. Lipid profile analysis

Fig. 2 Showed the treatment effects on different parameters of the lipid index of mice in different groups. It was great that the downstream changes in the serum levels of TC, TG, LDL-C, and VLDL-C in the glimepiride and metformin treatment groups were comparable to the standard atorvastatin (p < 0.05) where metformin effect on serum TC was better than the standard atorvastatin treatment. Besides, all these drugs improved the value of HDL-C compared to the control group. Among the two diabetic drug treatments, the metformin group had much better effects compared to the glimepiride treated group. However, in the case of VLDL-C estimation, the effects of the glimepiride treatment took over the metformin treatment and were almost the same as that of the atorvastatin treated group.

3.2. Neuropharmacological effects analysis

3.2.1. Open field test

The following diagram represents the comparative anxiolytic activity status of glimepiride and metformin. The results revealed that the group treated with glimepiride 4 mg/kg showed comparatively higher anxiolytic activity as the experimental animals crossed the lower number of squares with all four paws when compared to the control group (p < 0.05) (Fig. 3A).

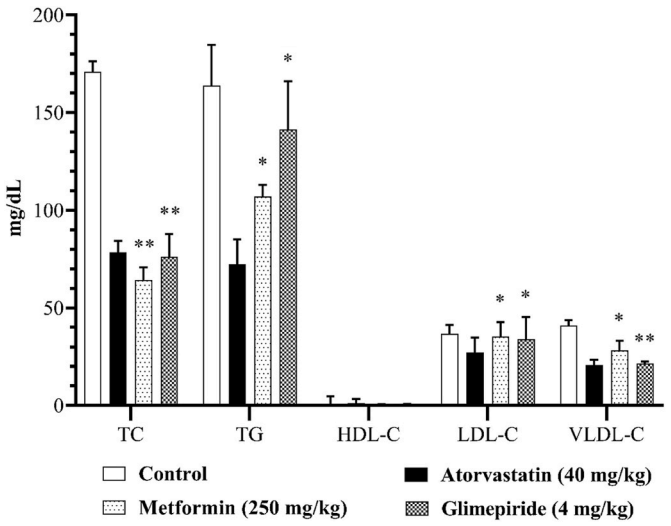

Fig. 2. Analysis of serum biological parameters of different experimental groups. Values are expressed as the mean ± SEM (n = 5), where the level of significance stated as *p < 0.05, **p < 0.01, ***p < 0.001. TC = Total Cholesterol, TG = Triglyceride, HDL-C = High-density lipoprotein cholesterol, LDL-C = Low density lipoprotein cholesterol, and VLDL-C = Very low-density lipoprotein cholesterol.

3.2.2. Hole board test

In the hole board test, the tendency of head dipping was decreased in mice treated with glimepiride 4 mg/kg after 30 min of oral administration when compared to the control and standard diazepam group. However, the group treated with metformin 250 mg/kg showed a reduced head dipping tendency than the glimepiride treatment group. Both data have been found to be significant when compared with the standard drug diazepam (p < 0.05) (Fig. 3B).

3.2.3. Forced swim test

Results obtained from the forced swim test revealed that glimepiride at a dose of 4 mg/kg body weight reduced the immobility time but increased the mobility time of experimental mice when compared to other experiment groups (Fig. 4). This event put the evidence of having a more significant (p < 0.05) antidepressant activity of glimepiride than metformin.

3.2.4. Elevated plus maze test

In the elevated plus-maze, the mice treated with metformin at a dose of 250 mg/kg body weight not only increased the time spent in the open arm but also increased the percentage of time spent when compared to the glimepiride group, while the finding was statistically significant while compared to standard diazepam treatment (p < 0.05) (Fig. 5). The result represented in Fig. 5 indicates that metformin produces a more anxiolytic effect than glimepiride (p < 0.05).

3.2.5. Dark and lighthouse test

Results of the dark and lighthouse test revealed that metformin-treated mice significantly increased both times spent in the light compartment and the number of entities in the light compartment when compared with the control and glimepiride-treated group and the data was comparable to the treatment effects on the standard diazepam group (p < 0.05) (Fig. 6).

4. Discussion

DM and its associated complications, including diabetic neuropathy and hyperlipidaemia, have become a global concern in the last few decades. Multiple lines of evidence suggest that hyperglycaemia,

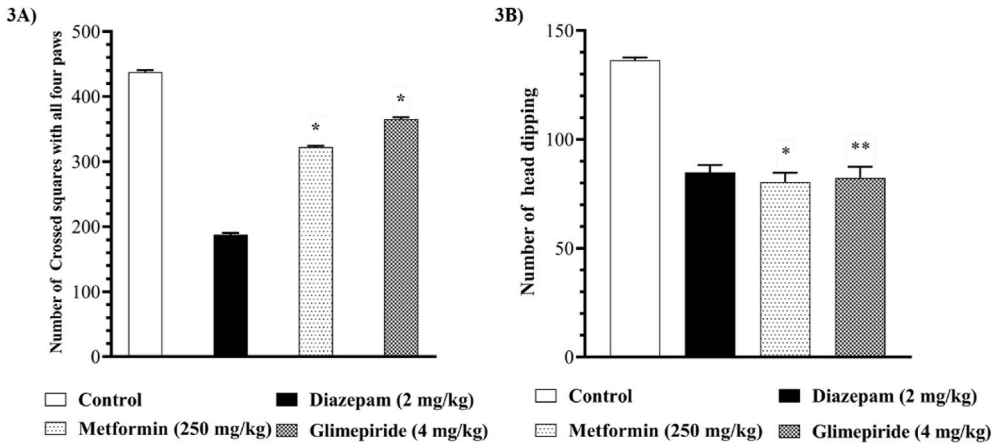

Fig. 3. The anxiolytic effect of different experimental groups in the open field test (A). The effect of glimepiride and metformin in the hole board test (B). Values are expressed as the mean ± SEM (n= 5), where the level of significance stated as *p < 0.05, **p < 0.01.

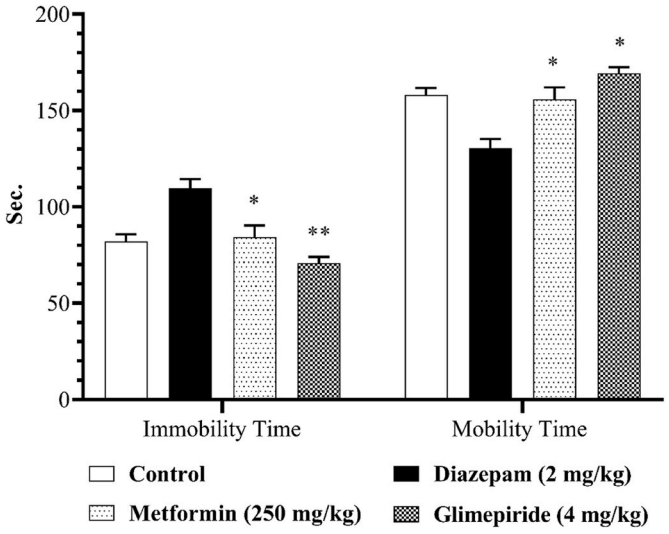

Fig. 4. Effect of glimepiride and metformin on the forced swim test. Values are expressed as the mean ± SEM (n= 5), where the level of significance stated as *p < 0.05, **p < 0.01.

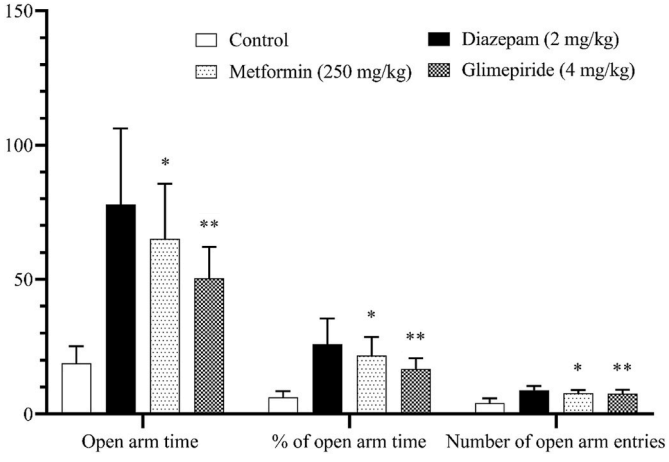

Fig. 5. The effect of glimepiride and metformin in the elevated plus-maze test. Values are expressed as the mean ± SEM (n= 5), where the level of significance stated as *p < 0.05, **p < 0.01.

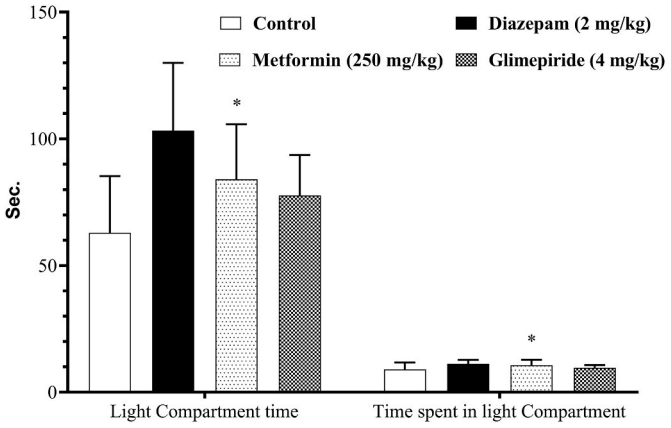

Fig. 6. The effect of glimepiride and metformin in the dark and lighthouse test. Values are expressed as the mean ± SEM (n= 5), where the level of significance stated as *p < 0.05.

dyslipidaemia, neurological disorder, and cardiovascular diseases are well connected with type 2 diabetes and contribute to global morbidity and mortality [1,3]. Therefore, it is necessary to find out the safest antidiabetic drug with a lesser extent of adverse consequences and has a better impression on treating diabetes. Our study investigated the comparative neurological, and hyperlipidaemic effects of the two most used antidiabetic drugs named metformin and glimepiride. In the diabetic state, the liver abundantly produces VLDL-C, which plays a crucial role in elevating TG levels [4]. The abundance of fatty acids promotes TG synthesis in the liver, which determines the fate of VLDL-C secretion through the alteration of intra-hepatic degradation of Apolipoprotein (Apo) B-100 [26]. In the diabetic condition, the insulin level is decreased, or insulin activity is diminished due to insulin resistance, and insulin is considered a potent suppressor which suppresses the lipolysis of triglycerides to free fatty acid in the adipose tissue. The abolishment of insulin activity blunts the inhibition of TG lipolysis and promotes TG conversion to free fatty acid, which is further accumulated in the liver. Multiple studies supported that patients suffering from type 2 DM have shown hyperinsulinemia mediated increased fatty acid synthesis levels in the liver. When the liver becomes resistant to insulin-mediated glucose metabolism, the liver remains sensitive to insulin-mediated lipid synthesis. In this case, insulin stimulates the expression of sterol regulatory element-binding protein 1 (SREBP-1c), a transcription factor responsible for ameliorating the activity of enzymes linked with fatty acid synthesis. The hyperglycaemic condition brings another

transcription factor in front named carbohydrate responsive element-binding protein (ChREBP), which also stimulates the enzymes responsible for fatty acid synthesis. In diabetic conditions, patients have poor clearance of chylomicrons from blood after dietary fat [27]. Therefore, chylomicron secretion is enhanced, storing more fatty acids in the liver. This stored fatty acid could act as the leading stage player to protect.

Apo B-100 form the degradation, which in turn increases the production and secretion of VLDL-C. The decrease in the level of insulin or insulin activity impedes the posttranslational degradation of Apo B-100 in the liver, which promotes the survival of Apo B-100 and allows the secretion of more VLDL-C. Lipoprotein lipase (LpL) is one of the active players that metabolizes TG-rich lipoproteins into free fatty acids. This enzyme interacts with circulating triglyceride-rich lipoproteins, say VLDL-C and chylomicrons, in the luminal side of capillary endothelial cells and helps in their metabolism [28]. It has been reported that the LpL activity is downregulated in type 1 and type 2 diabetic patients [29]. Insulin stimulates the expression of LpL, and downregulation of insulin reduces the LpL activity in diabetic patients, which results in the amelioration of VLDL-C secretion and production [27]. Apo C-III is an inhibitor of LpL activity, and it is upregulated in patients with type 2 DM. Insulin is a potent inhibitor of Apo C-III; reducing insulin level promotes Apo C-III activity, which further inhibits LpL and reduces the clearance of TG-rich lipoproteins. Several studies hypothesised that loss of function or mutation in Apo C-III leads to decreasing serum TG levels and subsequently reducing the risk of cardiovascular disease and other complications development [30]. In the diabetic state, hypercholesterolemia develops because insulin has an inhibitory action on β-hydroxy-β-methylglutaryl coenzyme-A (HMG-CoA) reductase. This key rate-limiting enzyme is responsible for the metabolism of cholesterol-rich LDL particles [31].

In our study, we observed that the metformin-treated group's weight gaining tendency was lower compared to other groups. Deposited fat was also lower in the metformin group than in the glimepiride group. However, when we focus on the lipid profile, the TC, HDL-C, and LDL-C levels were almost similar in these two groups. Nevertheless, metformin remarkably reduces the serum TC and TG levels while increasing the HDL-C level between these two groups. A recent study demonstrated that metformin reduces ChREBP transcription, which provides a barrier in the nuclear translocation responsible for reduced modulation of glucose and triglyceride metabolism. Furthermore, interruption in ChREBP transcription diminishes liver PCSK9 (proprotein convertase subtilisin/kexin type 9) expression, which raises LDL-R (low-density lipoprotein receptor) in three folds [32]. Metformin is also reported to increase the expression of AMPK, which blocks lipogenesis through downregulation of SREBP-1 and diminishes the function of acetyl-CoA carboxylase [33]. Therefore, lipid-lowering potential is likely to the ChREBP mediated PCSK9 regulation and AMPK mediated inhibition of lipogenesis. It is possible that in our study, metformin demonstrated lipid-lowering activity as the exact mechanism. However, glimepiride was found better than metformin in bad VLDL-C downregulation. Based on our findings, glimepiride reduces fasting plasma glucose levels more significantly than metformin. A study by Hongmei et al., 2013 also suggested that in the case of reducing fasting plasma glucose levels, glimepiride exhibited a more remarkable result than metformin [34]. It has been suggested that weight gain is a well-recognized side effect of sulfonylurea drugs. However, glimepiride has a less tendency to gain weight when compared with other drugs of the sulfonylurea group. Therefore, it was not surprising to observe weight gain in the glimepiride group rather than weight loss as seen with metformin [35]. Based on the previous findings, glimepiride improves the HDL-C level of type 2 diabetic patients by improving adiponectin, which acts as an independent factor for the change in HDL-C levels [36]. The elevated adiponectin level played a significant role in improving glycometabolism and hyperinsulinemia, which in turn ameliorates insulin sensitivity and improves insulin resistance in type 2 diabetic patients. It has been reported that treatment with glimepiride decreases TC, TG, and LDL-C levels but markedly increases HDL-C to a significant level [37]. The lipid-lowering activity of both drugs was comparable to standard atorvastatin. It is possible that metformin and glimepiride produced a lipid-lowering effect as atorvastatin does. Further study is recommended to find out the exact mechanism.

Neuropathy is another most common and intractable health consequence associated with DM, affecting approximately 50% of patients with this disease [3]. Dyslipidaemia is considered a potential contributing factor to the development of diabetic neuropathy through the introduction of oxidative stress in root ganglia, and sensory neurons and lipid-lowering drugs may bring about a beneficial effect in the treatment of diabetic neuropathy [38]. It has been reported that strict long-term glycaemic control helps prevent or improve diabetic peripheral neuropathy. However, short-term control may improve vibratory sensation, and metabolic changes in glucose and lipid metabolism are responsible for improving peripheral nerve function [5]. Andrea et al. (2009) reported that dyslipidaemia is responsible for DRG neuron injury through oxidative stress and acts as an independent risk factor for the development of diabetic neuropathy [39]. In a diabetic state, oxidized low-density lipoprotein (oxLDL) level has been reported to increase, which plays a crucial role in the progression of various diabetic complications. This oxLDL may enter the dorsal root ganglia, where it binds to a cell surface receptor named lipoxygenase-1 (LOX-1), which is linked with an intracellular signalling pathway that leads to the activation of NADPH oxidase, which has a strong association with the generation of superoxide and oxidative stress. This NADPH oxidase is a common pathway for cellular injury and dyslipidaemia [40]. In our study, several assays have been used to evaluate the neurological activity of metformin and glimepiride. In the open field test and hole board test, glimepiride produced a slightly more anxiolytic effect than metformin, as glimepiride treatment reduced the number of holes crossed and increased the head dipping tendency in experimental animals. However, in the forced swimming test, glimepiride produced more antidepressant activity than metformin. In the elevated plus-maze test and light and dark house test, metformin produces a more anxiolytic effect than glimepiride. It has been reported that metformin stimulates the transcriptional activity of forkhead box O3a (FoxO3a), which promotes the activation of AMPK, resulting in increased expression of GABAA and GABAA receptor-associated binding protein (GABARAP) and induces rigorous anxiolytic activity [41]. In another study, it was found that metformin can improve neurological function and oxidative stress through the regulation AMPK/mTOR signalling pathway [42]. Metformin is also supposed to generate neuroprotection by decreasing α-synuclein phosphorylation and aggregation, penetrating the blood-brain barrier, and providing neuroprotection from several neurological disorders [43]. In our study, metformin produced significant effects in neurological test models, which may be through any of the mentioned mechanisms. Although the neuroprotective mechanism of glimepiride is not clear, in our study, it produced comparable neurological effects to metformin.

Considering the results of our present study as a basis, it can be summarised that both studied oral hypoglycaemic drugs showed their antihyperlipidemic and anxiolytic properties. Therefore, it might be helpful for diabetic patients concurrently suffering from anxiety and useful in individuals if there is a risk of experiencing diabetic neuropathy while also warranted for prescribed in revised dose in individuals drastically reducing their body weight or persons diagnosed with DM with low BMI.

5. Conclusion

The results of our study indicate that both oral hypoglycaemic drugs alter the lipid index while producing some anxiolytic effects on the central nervous system. Thus, recommended to be carefully administered to patients with low BMI and might be beneficial to patients suffering from diabetic neuropathy with impaired peripheral nerve

function and anxiety. Thus, these findings should be considered and tested further in other higher animal or human subjects to announce more comprehensive suggestions to advise the patients during taking these medications.

Decelerations

Conflicts of interest disclosures

The authors declare that they have no competing interests.

Ethics approval

Ethical committee of Noakhali Science and Technology University (NSTU-2018-189).

Consent for publication

Not applicable.

Availability of data and materials

The data used to analyse the findings of this study are available from the corresponding author upon request.

CRediT authorship contribution statement

Md. Ohidur Rahman: have experimented, Formal analysis, data interpretation were aided. **Shaheen Ahmed:** have experimented, Formal analysis, data interpretation were aided. **Tanoy Mazumder:** Formal analysis, data interpretation were aided, Writing – original draft, Writing – review & editing. **Md. Abdus Salam:** have experimented. **Prodip Kumar Baral:** Conceptualization, designed the experiments, contributed to reagents, materials, Formal analysis, Writing – review & editing. **Md. Faruk Rana:** Writing – original draft. **Shuvo Mitra:** Writing – original draft. **Sayem Hossain:** Writing – original draft. **Rubiya Rahman:** review & editing, data interpretation were aided, All authors read and approved the final manuscript. **Md. Saddam Hussain:** Conceptualization, designed the experiments, contributed to reagents, materials, Formal analysis, Writing – review & editing, made the necessary corrections in the write-up and gave final approval for the submission of the final version.

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Abbreviations

AMPK	adenosine monophosphate kinase
Apo	apolipoprotein
ChREBP	carbohydrate responsive element-binding protein
DM	diabetes mellitus
HDL-C	high-density lipoprotein cholesterol
LDL-C	low-density lipoprotein cholesterol
LpL	lipoprotein lipase
oxLDL	oxidized low-density lipoprotein
PCSK9	proprotein convertase subtilisin/kexin type 9
SEM	standard error mean
SREBP-1c	sterol regulatory element-binding protein
TC	total cholesterol
TG	triglyceride
VLDL-C	very-low-density lipoprotein cholesterol

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