Original Article

Combined effect of Metformin and Pitavastatin on blood glucose in Alloxan induced diabetic rats.

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ABSTRACT

Background

Comparing to general population, the diabetic population is at higher risk of cardiovascular disease. Therefore, along with glycemic control in diabetic patient control of other risk factors for cardiovascular problem is also important. metformin is widely used oral hypoglycemic agent and pitavastatin as an antilipidemic agent. Combination of antidiabetic and hypolipidemic drug has been suggested as a rich source for treatment.

Objective

The current study focused on the efficacy of Metformin, Pitavastatin and their combinations.

Materials and methods

48 healthy male Wister strains of albino rats weighting to 180-220gm aged between 10-12wks were selected for the study. The experimental condition was all set in a very. Alloxan was prepared accordingly. The metformin and pitavastatin solution were prepared every 48hrs to maintain its activity. Data were analyzed using ANOVA in each variable.

Result

Blood glucose level in all six groups after two weeks treatment on day 15, the mean value and its Standard Deviation of fasting blood glucose level in Metformin treated group was (9.37±0.92 mmol/L) which is significant. Whereas the mean ± SD of fasting blood glucose level in Pitavastatin treated group was (13.81 ±1.31mmol/L) not significant. When given in combination Metformin and Pitavastatin these mean ± SD of fasting blood glucose levels mean were (6.93 ±0.84mmol/L) and (5.21±0.93) in Metformin 100 mg and Pitavastatin 2mg treated group and Metformin 200 mg and Pitavastatin 4 mg respectively, which were even reduced more and show highly significant than the Metformin treated group rats.

Conclusion

Combination of Metformin and Pitavastatin is able to improve glucose level more than that in single drug therapy.

Keywords

Metformin; pitavastatin; alloxan; hyperglycemic.

INTRODUCTION

According to the report by IDF on 2021, approximately 537 million individuals globally were affected with diabetes, constituting roughly 10.5% of the world's population[1]. In Bangladesh, Diabetes mellitus is also a public health concern and its prevalence is steadily rising [2]. Diabetes mellitus (DM) is a metabolic disease having chronic hyperglycemia which is due to disturbed insulin secretion or function or both. Diabetes mellitus (DM) is a disease with high morbidity. It significantly deteriorates the quality of health and life. Lack of early diagnostic methods results increased the risk of diabetic complications. Uncontrolled DM is accompanied by dysfunction, damage, and failure of various organs and tissues as well as development of micro and macro vascular complications[3]. Diabetes mellitus along with the

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co-morbidities has been reported as the fifth leading cause of death[4]. Metformin, is highly effective oral antihyperglycemic agent of biguanide class, is the most widely used glucose-lowering drug for type 2 diabetes mellitus (T2DM). It has an excellent safety record[5,6]. It has been proven to improve insulin resistance and dyslipidemia[6]. Metformin has a protective effect on coronary artery disease beyond its hypoglycemic effects[3].Insulin resistance is the major cause for development of dyslipidemia[7]. Which is a significant risk factor for cardiovascular disease [4,8,9,12]. Statins have been found to be a safe and effective approach to reduce dyslipidemia. Statins block substance that liver needs to make cholesterol therefore, statins are prescribed to lower their total cholesterol which reduces their risk of heart attack and stroke[8]. Pitavastatin is a new statin which has a low potential for drug- drug interaction and has powerful LDL cholesterol lowering effect as like atorvastatin[9]. It is an HMG-CoA reductase inhibitor, are widely used as cholesterol-lowering drugs to treat dyslipidemia. Pitavastatin is a highly potent statin [10]. Pitavastatin have little effect on the glycemic profile in patients with and without T2DM[9]. Recent study reported that Pitavastatin is neutral, whereas other statin causes deterioration of glycemic control in patient with type 2 Diabetes Mellitus[10,12,14,18]. Large clinical studies have also shown that statin therapy may reduce the incidence of type 2 diabetes[11]. Statins are frequently prescribed for patient with diabetes and dyslipidemia, so effect of statin is a major concern in dyslipidemia, as on glycemic control in patient with type 2 diabetes. As DM, impaired fasting glucose and pre-diabetes are significant cardiovascular risk factors[9]. Previous studies have reported that pitavastatin had less influence on the development of diabetes mellitus or glucose metabolism than other statins. Both metformin and statins thus act on glucose— as well as lipid metabolism therefore, metformin–statin combination therapy is prescribed to many T2DM patients[5]. In present days, clinical practice assumes multiple drug therapy in managing either a single disease or simultaneously occurring different diseases. Therefore, when two drugs are used simultaneously those having synergistic effect helps to get a good response in managing disease conditions. Studying the mechanism of drug interactions, clinical studies cannot be carried out by human models, hence animal model studies can help in understanding the underlying mechanisms in effect. The present study was intended for studying effect of Metformin and Pitavastatin in diabetic rats. As cardiovascular problem is more common in diabetic patients and simultaneous use of such combination of Metformin and Statin was considered in this study. Alloxan is widely used to induce experimental diabetes therefore has been chosen to induce diabetes in rats. Alloxan causes diabetes by partial degeneration of beta-cells of pancreatic islets and subsequent compromise in the quality and quantity of insulin produced by these cells [13].

The aim of optimum glycemic control in Type 2 diabetes mellitus is to reduce the risk of long-term macrovascular and microvascular complications[14]. The purpose of the current work was to study the effects of pitavastatins alone and in combination with metformin on blood glucose levels in diabetic rats.

MATERIALS AND METHODS

Animal

A total number of 48 healthy male Wister strains of Albino rats weighting to 180-220 grams and ages between 10-12 weeks were selected for the study and which was collected from BCSIR, Dhaka. They were kept in animal house of Department of Pharmacology, Dhaka Medical College and were feed standard rat pellets collected from ICDDR'B Dhaka. Rats of different Groups were kept in different metallic cages and allowed to drink *ab libitum* and maintained under standard laboratory conditions. These rats were acclimatized for 3 days at room temperature and humidity before commencement of the study. Animal described as fasted were deprived of food for 16 hours but had free access to water. It is to be noted that out of 48 rats 6 rats were dead during the study period.

Chemicals

Drugs

a. Metformin: Metformin was supplied by Square Pharmaceuticals, Dhaka.
b. Pitavastatin: Pitavastatin was supplied by Square Pharmaceuticals, Dhaka.

Reagents

a. Alloxan: Alloxan was supplied by Millon chemicals. Alloxan was dissolved in normal saline and was administered intraperitonially in a dose of 120 mg/kg body weight.
b. Reagents for estimation of blood glucose.
c. Normal saline.

Study design

Type of study: This was an Experimental Study.

Place of study: This study was conducted at Department of Pharmacology, Dhaka Medical College, Dhaka.

Period of study: Total study period was one year extending from January 2018 to December 2018.

Sample size: Sample size was 42 adults (180-220 gm) Wister strains of Albino rats.

Sampling Technique: Stratified Random Sampling was followed for the selection of sample

Alloxan induction in animal model

It was an experimental study, which designed to demonstrate the effect of combination therapy of Metformin and Pitavastatin on blood glucose level comparing to single drug of Metformin and Pitavastatin on Alloxan induced diabetic rats. The rats were divided randomly into two Groups containing 7 rats in Group I (Normal control Group) and 40 rats in Group II (diabetic Group). To induce diabetes, these 40 rats of Group II were kept fasting overnight and 120 mg of Alloxan per kg body weight was injected intraperitoneally to each of the rats. The rats were than kept in cages with 5% glucose bottles to prevent hypoglycemia. After 72 hours of Alloxan injection to the rats, serum blood glucose level was estimated to measure the glycemic status, where blood was collected from the tail vein with aseptic precaution. Thirty-five rats became diabetic after 72 hours and 5 rats were died. All those 35 rats having blood glucose level ≥11.11 mmol/L, and were considered as diabetic, and further divided randomly into five Groups as IIA, IIB, IIC, IID and IIE. Each Group contained 7 rats. The Group I was treated as normal control and IIA was treated as diabetic control and the Groups IIB, IIC, IID and IIE were taken as experimental Groups. The day after 72 hours of Alloxan injection was considered as first day of follow up.

- Group I normal control Group
- Group IIA diabetic control Group
- Group IIB diabetic rats treated with Metformin 100 mg/kg body weight
- Group IIC diabetic rats treated with Pitavastatin 2 mg/kg body weight
- Group IID diabetic rats treated with Metformin 100 mg/kg and Pitavastatin 2 mg/kg body weight
- Group IIE diabetic rats treated with Metformin 200 mg/kg and Pitavastatin 4 mg/kg body weight.

Group-I: This Group contained 7 rats, which were given standard rat diet and water for 15 days. Body weight and fasting blood glucose level were estimated on day 1 and day 15 of the experiment.

Group II: Fasting blood glucose levels and body weights of 40 rats were checked on 1st day of experiment before induction of diabetes. Then the rats were given intraperitoneal injection of Alloxan at a dose of 120 mg/kg b.w. After Alloxan injection rats were provided with 15% glucose solution for 24 hours to prevent hypoglycemia along with standard pellet diet and water ad labitum. Out of 40 rats, 5 rats were died within 3 days after Alloxan induction. Fasting blood glucose level was estimated 72 hours after Alloxan injection. Rats having blood glucose level ≥11.11 mmol/L were considered as diabetic and used for this study. All experimental rats became diabetic and further Grouped as IIA, IIB, IIC, IID and IIE. Each group contained 7 alloxan induced diabetic rats.

Group II A: In this Group, the diabetic rats were left untreated. Again, on day 15 body weight, fasting blood sugar were estimated and the rats were sacrificed for histopathology of the pancreas.

Group II B: In this Group, Alloxan induced diabetic rats were treated with Metformin 100 mg/kg orally by ryles tube for 15 days and body weight, fasting blood glucose were estimated on 15th day.

Group II C: In this Group, Alloxan induced diabetic rats were treated with Pitavastatin 2 mg/kg orally by ryles tube for 15 days and body weight, fasting blood glucose were estimated on 15th day.

Group II D: In this Group, Alloxan induced diabetic rats were treated with Metformin 100 mg/kg and Pitavastatin 2 mg/kg orally by ryles tube for 15 days body weight, fasting blood glucose were estimated after 15 days.

Group II E: In this Group, Alloxan induced diabetic rats were treated with Metformin 200 mg/kg and Pitavastatin 4 mg/kg orally by ryles tube for 15 days and fasting blood glucose were estimated On day 15th.

Collection of Blood

Blood was collected aseptically from each animal from the tip of the tail with a sharp sterile blade after an overnight fasting condition.

Biochemical parameters analysis

Fasting blood glucose as a biochemical parameter was analyzed on six Groups.

Estimation of blood glucose level on day 1

Blood glucose levels were estimated by Glucometer. This machine took 5 seconds to obtain the results. Each strip was inserted into the instrument followed by a drop of blood was collected aseptically from tail vein with a sharp sterile blade. The drop of blood was applied to the test area of the strip; the instrument was kept in 'on' position and waited for 5 seconds. Then the result was displayed on the monitor.

Sacrifice of animals and Histopathological study

The animal's blood was collected by cardiac puncture followed by the opening of the abdomen and the pancreas was dissected out from each animal for histopathological examination under anesthesia.

The histopathological study was done in department of Pathology, Dhaka Medical college, Dhaka, Bangladesh.

Estimation of Blood Glucose Level on day 15

Estimation of serum glucose concentration was obtained by using a glucose oxidase and peroxidase (GOD-POD) method.

Reference value: 4.71-7.33mmol/L.

Data collection and statistical analysis

All the results were appropriately recorded in data collection form. Statistical analysis was done by SPSS version 22.0. The variables were expressed as mean ±SD. The inter-Group comparison was analyzed by one-way ANOVA. Student`s t-test was done for comparison of means. Statistical significance was considered at 5% level of significance

Ethical clearance

Dhaka Medical College ethical committee approved the study

(no: MEUDMC/ECC/2018/220(R)

RESULT

Blood glucose level:

Table-1: Comparison of hypoglycemic activity of Metformin and Pitavastatin between group IIA (diabetic control group) with other groups of rats at day 1 (onset) and day 15 (end) of drug administration.

Groups	Fasting Blood glucose (mmol/L)			
	Day 1 Mean±SD	p-value	Day 15 Mean±SD	p-value
Group IIA	15.07±2.37		17.73±1.74	
vs Group IIB	15.31±1.87	0.835^{ns}	9.37±0.92	0.000**
vs Group IIC	15.34±2.08	0.824^{ns}	13.81±1.31	0.000**
vs Group IID	15.14±2.06	0.953^{ns}	6.93±0.84	0.000**
vs Group IIE	15.47±2.03	0.740^{ns}	5.21±0.93	0.000**

Table:1 revealed all mean result showed insignificant difference in day 1 against the group IIA (15.07±2.37) but all the mean values are significantly decreased in group IIB, IIC, IID, IIE (9.37±0.92, 13.81±1.31, 6.93±0.84, 5.21±0.93) respectively on day 15 compare to group IIA (17.73±1.74). On day 1 blood glucose ranges were group IIA (12.3-18.5) mmol/L, group IIB (13.2-18.8) mmol/L, group IIC (12.9-19.2) mmol/L, group IID (11.4-18.9) mmol/L and Group IIE (12.9-18.1) mmol/L and mean values were -15.07±2.37, 15.31±1.87, 15.34±2.08, 15.14±2.06 and 15.47±2.03 in group IIA, IIB, IIC, IID, IIE respectively. Whereas in day 15, blood glucose ranges were in group IIA (14.5-19.2) mmol/L, IIB (8.1-10.6) mmol/L, IIC (12.4-15.2) mmol/L, IID (5.5-7.8) mmol/L and IIE (4.0-6.3) mmol/L.

Table-2: Comparison of hypoglycemic activity of Metformin and Pitavastatin between group IIB (Metformin treated group) with other groups of rats at day 1 (onset) and day 15 (end) of drug administration.

Groups	Fasting Blood glucose (mmol/L)			
	Day 1 Mean±SD	p-value	Day 15 Mean±SD	p-value
Group IIB	15.31±1.87		9.37±0.92	
vs Group IIC	15.34±2.08	0.979^{ns}	13.81±1.31	0.000**
vs Group IID	15.14±2.06	0.873^{ns}	6.93±0.84	0.000**
vs Group IIE	15.47±2.03	0.883^{ns}	5.21±0.93	0.000**

Table :2 showed significantly decreased mean blood glucose level in Groups IID and IIE (6.93±0.84 and 5.21±0.93) respectively compared to Group IIB (9.37±0.92) on day 15. Whereas on day 1 mean blood sugar levels showed no significant difference. On day 1 ranges of blood glucose were Group IIB (13.2-18.8) mmol/L, Group IIC (12.9-19.2) mmol/L, Group IID (11.4-18.9) mmol/L and Group IIE (12.9-18.1) mmol/L and mean values were 15.31±1.87, 15.34±2.08, 15.14±2.06 and 15.47±2.03 in Group IIB, IIC, IID, IIE respectively. Whereas in day 15, blood glucose ranges were in Group IIB (8.1-10.6) mmol/L, IIC (12.4-15.2) mmol/L, IID (5.5-7.8) mmol/L and IIE (4.0-6.3) mmol/L.

Table-3: Comparison of hypoglycemic activity of Metformin and Pitavastatin between group IIC (Pitavastatin treated group) with other groups of rats at day 1 (onset) and day 15 (end) of drug administration.

Groups	Fasting Blood glucose (mmol/L)			
	Day 1 Mean±SD	p-value	Day 15 Mean±SD	p-value
Group IIC	15.34±2.08		13.81±1.31	
vs Group IID	15.14±2.06	0.860^{ns}	6.93±0.84	0.000**
vs Group IIE	15.47±2.03	0.909^{ns}	5.21±0.93	0.000**

Table :3 showed significantly decreased mean blood glucose level in group IID and IIE (6.93±0.84 and 5.21±0.93) when compared to group IIC (13.81±1.31) on day 15. Ranges of blood glucose on day 15 were IIC (12.4-15.2) mmol/L, IID (5.5-7.8) mmol/L and IIE (4.0-6.3) mmol/L. Whereas on day 1 mean blood glucose levels were almost similar.

Table-4: Comparison of hypoglycemic activity of Metformin and Pitavastatin between group IID (combination of Metformin 100 mg and Pitavastatin 2 mg) and group IIE (combination of Metformin 200 mg and Pitavastatin 4 mg) of rats at day 1 (onset) and day 15 (end) of drug administration.

Groups	Fasting Blood glucose (mmol/L)			
	Day 1 Mean±SD	p-value	Day 15 Mean±SD	p-value
Group IID	15.14±2.06		6.93±0.84	
vs Group IIE	15.47±2.03	0.769^{ns}	5.21±0.93	0.003**

Table:4 showed significantly decreased mean blood glucose level in group IIE (5.21±0.93) compared to group IID (6.93±0.84) at the end of drug administration. Ranges of blood glucose on day 15 were IID (5.5-7.8) mmol/L and IIE (4.0-6.3) mmol/L. Whereas on day 1 mean blood glucose level showed insignificant differences in between them.

Comparison of hypoglycemic activity of Metformin and Pitavastatin between group I (normal control group) with other groups of rats at day 1 (onset) and day 15 (end) of drug administration

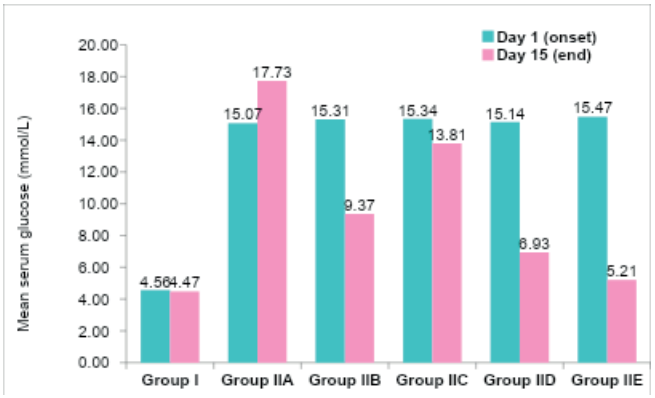

Figure 1: Multiple bar diagram showing the mean glucose in different Groups.

Figure 1 elaborated comparison of mean blood glucose level in different groups of rats at onset and end of drug administration. At the end of drug administration on day 15, group IIB (8.1-10.6mmol/L), IID (5.5-7.8mmol/L) and IIE (4.0-6.3mmol/L) showed statistically highly significant decreased in blood glucose level, where the maximum decreased in mean blood glucose occurred in group IIE (15.47±2.03 to 5.21±0.93). Whereas group IIC showed statistically insignificant result in mean blood glucose level (15.34±2.08 to 13.81±1.31) compared to day 1. Group IIA showed significant increase in mean blood glucose level (15.07±2.37 to 17.73±1.74) which ranged from (14.5-19.2mmol/L) on day 15th.

DISCUSSION

In addition, with hyper-glycemia, dyslipidemia is common complication of diabetes mellitus[16]. Therefore, searching of most effective drug is now the top concern. This experiment is done by using most popular available antidiabetic drug metformin and anti-lipid drug Pitavastatin. Rats blood screening was done to evaluate the hypoglycemic effects of Metformin and Pitavastatin

when administered alone and in combination in Alloxan induced diabetic rats.

Other than the normal control group, diabetic groups were administered Alloxan intraperitonially in a single dose of 120 mg/kg body weight to overnight fasted animal. Which produces significant increase in blood glucose level observed 72 hours following administration of Alloxan [15,16,19]. The animals with blood glucose level 11.11 mmol/L or over were considered to be diabetic [15,16,17]. The mean fasting blood glucose level of group II on the first day follow up was (15.07±2.37) mmol/L. It was found that hyperglycemia occurs by intraperitoneal administration of Alloxan at a dose of 120 mg/kg body weight in the experimental rats. Here, the rise in blood glucose level in experimental hyperglycemic rats were also highly significant as compared to normal control Group.

We measured blood glucose of all the rats of the five groups after two weeks treatment. This study showed that Metformin produced a significant decrease in blood glucose level in Alloxan-induced diabetic rats. Blood glucose level in all six Groups after two weeks treatment the mean value and its Standard Deviation of fasting blood glucose level in Group IIB on day 15 was (9.37 ± 0.92 mmol/L) who received normal diet and treated with Metformin [16,17,20].

Whereas the mean ± SD of fasting blood glucose level in Group IIC on day 15 mean was (13.81± 1.31 mmol/L) who received normal diet and Pitavastatin. Here the blood glucose level in Group IIB is significantly less than Group IIC [9,12,18,19].

But in combination Groups of Metformin and Pitavastatin these mean ± SD of fasting blood glucose levels were (6.93±0.84 mmol/L) and (5.21 ± 0.93 mmol/L) in Group IID and IIE respectively, which were even reduced more than the Metformin treated group rats when compared to untreated diabetic Group rats (17.73±1.74 mmol/L)[16 (Figure 1)].

So, from analyzing these parameters we can conclude that combination therapy of Metformin and Pitavastatin is much more effective than single drug therapy in lowering blood glucose level. The findings of our study were consistent with previously published reports[16].

CONCLUSION

It can be concluded that Metformin significantly reduces the blood glucose level in Alloxan induced diabetic rats at the end of drug administration. On the other hand, Pitavastatin alone produce mild decrease in blood glucose level. Combination of Metformin and Pitavastatin reduce blood glucose level more than that in single drug therapy.

Conflict of interest

No conflict of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Author Contribution

Study design: Dr. Sarwat Jahan

Data gathering: Dr. Sarwat Jahan,

Data analysis: Dr. Sarwat Jahan, Dr. Qazi Alifa Jahan

Writing and submitting manuscript: Dr. Sarwat Jahan, Dr. Siddika Khatun

Editing and approval of final draft: Prof. Zinnat Ara Begum, Prof. Syeda Fahmida Afrin, Dr. Sultana Marufa Shefin.

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