Original Article

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Effect of Metformin on Lipid Profile in Alloxan Induced Diabetic Rats

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ABSTRACT

Background: Diabetes mellitus is a heterogeneous group of metabolic disorders with microvascular and macrovascular complications which are the major causes of morbidity and mortality in diabetic patients. So simultaneous use of multiple drugs have been used to prevent complication of diabetes mellitus. This study aimed to evaluate the effect of Metformin and Atorvastatin on lipid profile in alloxan induced diabetic rats.

Materials and methods: This prospective interventional study was carried out in the Department of Pharmacology and Therapeutics, Sir Salimullah Medical College and Mitford Hospital, Dhaka from July 2017 to June 2018. Total number of 24 Wistar albino rats of either sex (150-200 g) were selected for experiment and six rats were selected for normal control group and 18 were induced diabetes by administering alloxan and they were divided again into three groups, each consisting of six rats. On the 72 hours after Alloxan injection blood samples were collected, serum was isolated and subjected to glucose, triglycerides (TG), Total Cholesterol (TC) Low Density Lipoprotein Cholesterol (LDL-C) and High Density Lipoprotein Cholesterol (HDL-C) estimation. Normal control group I was treated with normal feeding. Group IIA served as diabetic control. To the diabetic IIB was treated with metformin and IIC was treated with atorvastatin for 7 days. On the last day blood samples were collected, serum was isolated and subjected to glucose, Triglycerides (TG) Total Cholesterol (TC) Low Density Lipoprotein Cholesterol (LDL-C) and High Density Lipoprotein Cholesterol (HDL-C) estimation. Body weight was also calculated.

Results: Metformin significantly reduced the serum glucose level in diabetic rats. Metformin also altered the lipid profile of diabetic rats. atorvastatin significantly reduced the lipid profile when compared to Metformin alone.

Conclusion: The observation and result of the study provide information that use of metformine in alloxen induced diabetic rats slightly improved the lipid profile level which is beneficial for the treatment of dyslipidaemia.

KEY WORDS
Alloxan; Atorvastatin; Diabetes mellitus; Dyslipidemia; Metformin.

INTRODUCTION

Diabetes Mellitus (DM) is currently a serious health concern all over the world and the prevalence of diabetes is increasing day by day. The global prevalence of DM among the adults will increase to 7.7%, about 439 million adults by 2030. Between 2010 and 2030, there will be a 69% increasing in the number of adults with diabetes in developing countries and 20% increase in developed countries.[1]

The mortality of relative risk is particularly important in younger age classes: diabetic males and females aged 45–64 years present relative risk for death of 1.7 (95% CI 1.58-1.88) and 2.6 (95% CI 2.29-2.97) respectively.[2] World health organization reveals that in 2014, an estimated 3.4 million people died from the consequence of high blood glucose. More than 80% of diabetic deaths occur in the low and middle income countries. WHO projects that diabetic death will double between 2005 and 2030.[3]

According to WHO (2006) diabetes is a condition primarily defined by the level of hyperglycaemic giving rise to risk of micro vascular damage (Retinopathy, nephropathy and neuropathy). It is associated with reduced life expectancy, significant morbidity due to specific diabetes related micro vascular complication,

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increased risk of macro vascular complications (Ischemic heart disease, stroke and peripheral vascular disease) and diminished quality of life.

Diabetes mellitus is mainly of two types, type 1 and type 2. In type I diabetes there is total absence of insulin secretion and type 2 there is a combined defect in insulin secretion and its action. The prevalence of type 2 DM among adults about 91% depending on the population in question and type 2 DM is growing at an exponential rate due to dietary habits, increasing obesity, sedentariness. Earlier Diagnosisof type 2 diabetes can only be beneficial for reducing mortality and morbidity.[4] Many Type 2 Diabetes Mellitus (T2DM) patients suffer from diabetes-related cardiovascular complications, which are the major cause of death in patients with T2DM. Atherosclerosis, a major risk factor for cardiovascular diseases, which is characterized by lipid accumulation and inflammation.[5]

Goal of treatment includes maintenance of normal blood sugar level and prevention of development of complications. Type I diabetes requires more Insulin treatment, where type 2 can treated with oral anti diabetic drugs or insulin or as a combination of both. Lifestyle modification can delay the onset of diabetic complications and prevent the onset of diabetes among those who are at high risk of developing the disease.[6]

Dyslipidemia and atherosclerosis is the most common cause of death in diabetics. Mean serum levels of TC, LDL, TG and TC: HDL were significantly higher among adults 30-39 years old compared to other age groups, regardless of sex. The proportion of high TC and LDL from 2005 to 2011 among individuals aged 30-39 years old varied widely.[7]An ideal oral treatment for diabetes and dyslipidemia would be a drug that not only controls the glycaemic level but also prevents the development of atherosclerosis and other complications of diabetes.

Statins are widely used to reduce cardiovascular risk and Metformin, an anti-diabetic drug, was reported to possess anti-atherosclerotic effects.[8] Therefore, the experiment designed to evaluate whether combined use of metformin and atorvastatin can achieve beneficial effects. In rats, we evaluated the effects of the metfomin therapy on triglyceride, total cholesterol, low density lipoprotein, high density lipoprotein.

An ideal oral treatment for diabetes and dyslipidemia would be a drug that not only controls the glycaemic level but also prevents the development of atherosclerosis and other complications of diabetes. The present study aimed to evaluated the effect of Metformin and Atorvastatin on lipid profile in alloxan induced diabetic rats.

MATERIALS AND METHODS

This quasi experimental study was carried out at Sir Salimullah Medical College and Mitford Hospital in collaboration with Institution of Nutrition and food Sciences (INFS) University of Dhaka between From July 2017 to June 2018. Study approval was obtained from the medical ethical committee of Sir Salimullah Medical College, Dhaka, Bangladesh. A total 24 healthy wister strains of Albino rats weighing between 150-200 gramsof both sex were collected from the animal house of BSMMU, Dhaka, Bangladesh. They were kept in metallic cages in the animal house of INFS, University of Dhaka in a well ventilated room and a temperature of about 26-28C. They were allowed to feed standard pellets of mice and drink water ab libitum, except during the day of blood sampling when animals were kept an overnight fasting. Alloxan Monohydrate was supplied by INFS, University of Dhaka.

At first rats were divided into two groups containing 6 rats in Group I and 18 rats in Group II. In order to induce diabetic, the 18 rats in group II were kept fasting overnight and 120 mg of alloxan per kg body weight was injected intraperitoneally to each of the rats. After 72 hours of alloxan injection, serum blood glucose level and lipid profile were estimated from tail blood of rats.[9] Rats with blood glucose level 250-350 mg/dl considered as diabetic and further divided them three groups as IIA, IIB and IIC. The group I was treated as normal control and IIA was treated as diabetic control and the groups IIB, IIC were taken as experimental groups. The day after 72 hours of Alloxan injection was considered as 1st day of follow up.

In Group I the rats were given normal feed and water for 7 days from first day of follow up and fasting blood glucose, serum lipid profile were estimated after 7 days. In group IIA, IIB and IIC diabetic rats were treated with normal feed, metformin 100 mg/kg[9] body weight and atorvastatin 10 mg/kg[9] body weight orally for 7 days respectively. Than fasting blood glucose and serum lipid profile were estimated by ELISA.[10] Result was expressed as mean±SD. The inter-group comparison was analyzed by paired student's t-test. Ap value <0.05 was considered statistically significant. Statistical analysis was done by using SPSS program (Version 22).

RESULTS

Table IA shows the five experimental animal groups with serum glucose level at day 1 and day 7. Group I normal control was given normal feed only. Group IIA Alloxan induced Diabetic control rats. Group IIB alloxan induced diabetic rats treated with metformin.

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Group IIC alloxan induced diabetic rats treated with atorvastatin. Group IID diabetic rats treated with both Atorvastatin and metformin.

Table IA Glycemic status of the rats at onset and end of the drug administration.

Group		Serum glucose level (Fasting) (mg/dl)	
		Day 1	Day 7
Group I	Normal control	95.2±11.6	94.2±6.50
Group IIA	Alloxan induced Diabetic control	290.4±9.1	320.6±9.1
Group IIB	Alloxan induced diabetic rats treated with metformin	295.1±7.4	119.5± 6.2
Group IIC	Alloxan induced diabetic rats treated with atorvastatin	301.2±5.4	270.3±5.5

Table IA and IB shows that at the onset of study mean blood glucose level significantly higher in Alloxan induced rats group than those of group I. After 7 days of follow up mean blood glucose level of group IIB was significantly lower than IIC.

Table IB Comparison of hypoglycemic activity of Metformin and Atorvastatin in different groups of rats at the day 1 (Onset) and day 7 (End) of treatment.

Group Comparison	Blood Glucose at day 1(mg/dl)	Blood Glucose at day 7 (mg/dl)
I vs IIA	1.000^{ns}	1.000^{ns}
I vs IIB	1.000^{ns}	$<0.001^{***}$
I vs IIC	1.000^{ns}	1.000^{ns}
IIA vs IIB	1.000^{ns}	$<0.001^{***}$
IIA vs IIC	1.000^{ns}	1.000^{ns}
IIB vs IIC	1.000^{ns}	1.000^{ns}

*significant, NS: Non-significant.

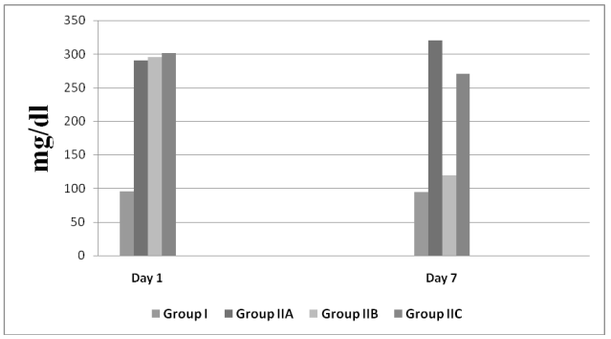

Figure 1 Bar diagram of Blood sugar level at day 1 and day 7

Group I : Normal control
Group IIA : Alloxan induced Diabetic control
Group IIB : Alloxan induced diabetic rats treated with metformin.
Group IIC : Alloxan induced diabetic rats treated with atorvastatin.

Table IIA shows that Alloxan treatment not only increase blood glucose levels but also increases the levels of TG, TC and LDL in diabetic rats. Diabetic rats show increase in the serum levels of TG, TC and LDL and decrease HDL level when compared to control. Atorvastatin decrease the lipid profile near to normal control, which is statistically significant ($p<0.05$). Metformin also slightly decreased the level of lipid profile.

Table IIA Antilipidaemic activity of Atorvastatin and Metformin on Alloxan induced diabetic rats at the onset and end of drug administration

Group	TG		TC		LDL-C		HDL-C	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
Group I	106.3±9.8	106.3±9.8	40.1±5.4	140.1±5.4	60.5±6.4	60.5±6.4	55.62±6.1	55.62±6.1
Group IIA	169.1±1.4	168.4±2.4	202.7±5.5	201.6±6.5	132.7±6.3	132.42±5.3	30.9±5.6	31.9±6.4
Group IIB	161.7±5.3	160.5±8.3	180.1±2.5	179.1±5.4	101.9±5.6	101.2±9.2	47.8±2.8	48.8±5.6
Group IIC	167.5±7.3	126.3±9.2	199.3±2.4	160.3±6.4	130.4±4.6	93.4±11.2	31.6±3.0	41.6±5.0

*signifinat, NS: Non-Significant

Group I : Normal control
Group IIA : Alloxan induced Diabetic control
Group IIB : Alloxan induced diabetic rats treated with metformin.
Group IIC : Alloxan induced diabetic rats treated with atorvastatin.

From the Table IIA and IIB it was evident that there were increase in the serum level of TG, TC and LDL-C and decrease HDL-C level in group IIA (Diabetic control rats). Metformin treated group also slightly decrease TG, TC, LDL and increase HDL-C as compared to diabetic rats (Group IIA). Atorvastatin significantly decrease (0.001) the serum lipid profile as compared to diabetic rats (Group IIA).

Table IIB Comparison of antilipidemic activity of Metformin and Atorvastatin in different groups of rats at end of treatment (Day 7)

Group Comparison	TG	TC	LDL-C	HDL-C
I vs IIA	1.000^{ns}	1.000^{ns}	1.000^{ns}	1.000^{ns}
I vs IIB	1.000^{ns}	1.000^{ns}	1.000^{ns}	1.000^{ns}
I vs IIC	$<0.001^{**}$	$<0.001^{**}$	$<0.001^{**}$	$< 0.001^{**}$
IIA vs IIB	1.000^{ns}	1.000^{ns}	1.000^{ns}	1.000^{ns}
IIA vs IIC	$<0.001^{**}$	$<0.001^{**}$	$<0.001^{**}$	$<0.001^{**}$
IIB vs IIC	$<0.001^{**}$	$<0.001^{**}$	$<0.001^{**}$	$<0.001^{**}$

*significant, NS: Non-significant.

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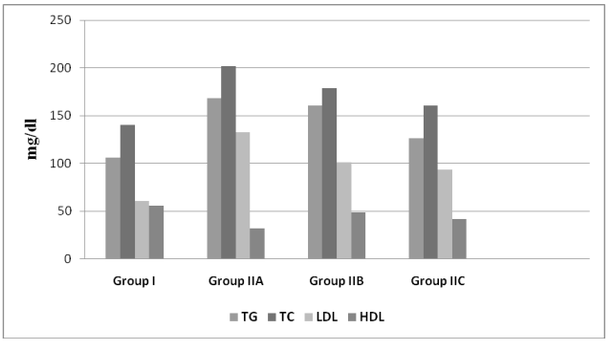

Figure 2 Fasting lipid profile after 7 days treatment

Group I : Normal control
Group IIA : Alloxan induced Diabetic control
Group IIB : Alloxan induced diabetic rats treated with metformin.
Group IIC : Alloxan induced diabetic rats treated with atorvastatin.

DISCUSSION

Polypharmacy is becoming more important in clinical practice these days. Every day, new compounds are developed, and clinicians face new challenges in managing single or many diseases due to drug interactions, which may be the seventh leading cause of death in the United States.[11]

Because dyslipidemia is more common in diabetics, the current study compared the effects of metformin and statins in rats. The blood glucose level and fasting lipid profile in rats were measured 72 hours after alloxan was given to them.[9] In this study, intraperitoneal administration of a single dose of alloxan (120 mg/kg) significantly increased blood glucose levels. The mean±SD fasting blood glucose of group IIA on the 1st day of followup after Alloxan injection was 290.4±9.1 and similar observations were reported by a number of researchers.[9] They discovered that blood glucose levels in the wister albino rat increased 72 hours after an intraperitoneal injection of freshly prepared alloxan monohydrate solution at a dose of 120 mg/kg body weight.In the present study, the rise in blood glucose levels in experimental hyperglycaemic rats was also highly significant as compared to normal control.

In this experiment, blood glucose level and the mean±SD of blood glucose level in group IIB were 119.5±6.2 mg/dl in those who received a normal diet and were treated with metformin, whereas the mean ± SD of blood glucose level in group IID was 112.6±6.9 mg/dl in those who were treated with both metformin (100 mg/kg) and atorvastatin (10 mg/kg) for seven days. There was a change in the mean value of fasting blood glucose level in both groups of alloxan induced diabetic rats and it was statistically significant ($p<0.05$) which was similar to the findings of Anitha N etal, Sakthivel K etal and Trivedi N etal.[9,12,13]

In the other parts, the fasting lipid profile (Mean±SD) in group IIC was TG 126.3±9.2 mg/dl, TC 160.3±6.4 mg/dl, LDL 93.4±11.2 mg/dl, HDL 41.6±5.01 mg/dl, which were treated with atorvastatin (10 mg/kg) only. Whereas the fasting lipid profile (Mean±SD) in group IIB was TG 160.5±8.3 mg/dl, TC 179.1±5.4 mg/dl, LDL 101.2±9.2 mg/dl, HDL 48.8±5.6 mg/dl, whose were treated with metformin (100 mg/kg) for seven days.[9] There was change in the mean value of fasting lipid profile level of both group of alloxan induced diabetic rats and statistically significant ($p<0.05$) in Group IIC which was similar to findings of Anitha N etal.[9]

Metformin and atorvastatin effects were seen in alloxan-induced diabetic rats in this stage of the experiment. In group IIC, the reduction in mean fasting lipid profile was higher than in group IIB.

This finding was consistent of Anitha N etal who administered Metformin and Atorvastatin to Alloxan-induced diabetic rats and found a significant reduction in blood sugar and lipid profile during Metformin and Atorvastatin treatments.[9]

The results in this study are therefore in correlation with the findings of other researchers.[9,12,13] As a result, both metformin and atorvastatin have a positive influence on cholesterol lowering activities. The findings suggest that using metformin and atorvastatin at the same time in diabetic patients improves their lipid profile.

LIMITATION

Sample size in the study, thought collected within the period of one year was relatively smaller in numbers.

CONCLUSION

The findings show that using metformin in alloxen-induced diabetic rats improves their lipid profile marginally, which is advantageous for the treatment of dyslipidaemia.

RECOMMENDATION

Similar study with large sample size with multicentre can be done for proper picture.

DISCLOSURE

All the authors declared no competing interest.

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