Original Article

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Metformin and Pitavastatin Alone and in Combination on Renal Function in Alloxan Induced Diabetic Rat

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ABSTRACT

Background: Type 2 diabetes has a strong association with dyslipidemia.Combination of antidiabetic and hypolipidemic drug are commonly practiced. Metformin, Pitavastatin and their combinations, widely used as therapeutic agents for diabetes and dyslipidemia. This study aimed to evaluate the renal safety by measuring serum creatinine levels in all groups of experimental rats.

Materials and methods: 48 healthy male Wister strains of albino rats weighting to 180-220 gm aged between 10-12 wks 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.

Results: In all six groups after two weeks treatment on day 15 The ranges of serum Creatinine in control group I were (0.3-0.9) mg/dl, diabetic control group IIA (0.7-1.2) mg/dl, metformin treated group IIB (0.3-1.1) mg/dl, pitavastatin treated group IIC (0.3-0.9) mg/dl, combination of metformin and pitavastatin treated group IID (0.3-0.8) mg/dl and group IIE (0.2-0.7) mg/dl. Here, significant difference in mean serum creatinine level in group IIA (0.97±0.18) against group I (normal control group-0.57±0.23), other groups were insignificant when compare to group I (Normal control group). The ranges of serum Creatinine Although levels were within normal limit in all groups.

Conclusion: Metformin and pitavastatin combination might be an effective treatment in patients with both diabetes and dyslipidemia. As The ranges of serum Creatinine although levels were within normal limit in all groups.

KEY WORDS
Metformin; Pitavastatin; Renal function; Serum creatinine.

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder of altered carbohydrate, fat and protein metabolism is become a global public health issue.[1] Prevalence of diabetes mellitus is currently increasing, sedentary life style and obesity, a major risk factor for diabetes.[3] It is accompanied by damage, dysfunction and failure of various organs.[2] About 5 million adults died from DM and its complications, in 2019.[1] Metformin is one of the safest and most effective anti-hyperglycemic agents currently used as first-line oral therapy recommended by both the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) since 2009.[3,4] Metformin is a biguanide, improves glycemic control by enhancing insulin sensitivity in liver and muscle. Metformin also has a beneficial effect on several cardiovascular risk factors including dyslipidemia.[5] It is estimated that 30-60% of patients with T2DM have dyslipidemia.[6] Dyslipidemia is one of the major modifiable risk factors for CVD in DM patients. In DM, important enzymes and lipid metabolism pathways are affected, therefore, dyslipidemia is more common.[1] The one of the major goals of the treatment of dyslipidemias are the prevention of cardiovascular disease.[7] Statins are considered to be the first-line pharmacological treatment for dyslipidemia worldwide and reducing the risk of coronary heart disease.[8] Pitavastatin is the latest which is highly potent and has emerged with optimum efficacy and improved safety.[9,10] Most statins increase the risk of new-onset diabetes. Unlike other statins, pitavastatin is reported to exert neutral effects on serum

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glucose level.[11,12,13] Therefore, pitavastatin may be considered as good choices for patients with DM and hypercholesterolemia.[14] Both metformin and statins act on glucose as well as lipid metabolism therefore, metformin–statin combination therapy is prescribed to many T2DM patients.[15] When two drugs are used simultaneously those having synergistic effect helps to get a good response in managing disease conditions, as well as safety is a concern also. Hence animal studies can help in understanding the safety effect rather than human models. 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.[16] The present study was intended for studying safety effect of Metformin and Pitavastatin alone and in combination in serum creatinine level in alloxan induced 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:

- Metformin: Metformin was supplied by Square Pharmaceuticals, Dhaka.
- Pitavastatin: Pitavastatin was supplied by Square Pharmaceuticals, Dhaka.

Reagents:

- 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.
- Reagents for estimation of serum creatinine.
- Normal saline.

This was an Experimental study was conducted at Department of Pharmacology, Dhaka Medical College, Dhaka. Total study period was one year extending from January 2018 to December 2018. Sample size was 42 adults (180-220 gm) Wister strains of Albino rats. 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. Serum SGPT and serum creatinine were also measured on day 15.

Group II : Fasting blood glucose levels 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

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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. On day 15 serum creatinine levels were estimated and the rats were sacrificed.

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 serum creatinine were estimated on 15th day.

Group II C: In this Group, Alloxan induced diabetic rats were treated with Pitavastatin 2 mg/kg orally by and serum creatinine 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 serum creatinine was 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 serum creatinine were estimated on day 15th.

Collection of blood

Blood was collected aseptically from each animal after an overnight fasting condition.

Biochemical parameters analysis

Serum creatinine was analyzed on six Groups.

Estimation of serum Creatinine Level on day 15

Serum creatinine is an important laboratory marker for renal function. Kinetic colorimetric method was done to estimate serum creatinine level.

Principle of the method

The procedure was based upon a modification of the original picrate reaction (Jaffe, 1886). Creatinine under alkaline conditions reacted with picrate ions forming a reddish complex measured through the increase of absorbance in a prefixed interval of time was proportional to the concentration of creatinine in the sample.

Creatinine + Picric acid ---> red addition complex (Here, pH>12 and temperature 37^{o}C)

Reference value: 0.2-0.9 mg/dl.

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

Prior to commencement of the study, after the departmental review, the research protocol was approved by the Ethical Review Committee of Dhaka Medical College, Dhaka. The present study was experimental one involving animal. Proper permission regarding animal purchase, transport and experiment was obtained. Permission was also be taken for housing, administrating foods on a standard rat pellet, collection of blood sample for biochemical analysis and sacrifice of animals under light anesthesia and collection of pancreases.

Ethical clearance: Dhaka Medical College ethical committee approved the study
(no: MEUDMC/ECC/2018/220(R).

RESULTS

The study was carried out to compare the blood glucose level before and after treatment in Alloxan induced diabetic rats with single and combination therapy with Metformin and Pitavastatin. For this study six experimental groups were selected. Group I normal control group, was given normal feed only. Group II was Alloxan induced Diabetic rats. Group two was further divided in Group- IIA, IIB, IIC, IID, IIE. Intraperitoneal injection of Alloxan significantly increased blood glucose level (15mmol/L) when compared with normal rats (4-6.5mmol/l). To assess the safety serum creatinine levels was also measured. Results were presented in tables and figures. Figure showed photographic and graphical representations of the relations, similarities and differences among different Groups. All the observations and results are described below.

The experimental rats were healthy and showed normal spontaneous activities throughout with normal respiration, responding appropriately to normal stimuli. On dissection, none of the rats from any group showed any gross abnormality of the internal organs like lungs, heart, liver, kidneys on naked eye examination.

Table I Comparison of mean serum creatinine levels among all groups at the end of drug administration

Groups	Mean±SD (mg/dl)	F-value	p value
Group I	0.57±0.23	4.625	0.002**
Group IIA	0.97±0.18		
Group IIB	0.64±0.30		
Group IIC	0.53±0.24		
Group IID	0.54±0.21		
Group IIE	0.44±0.19		

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Table I showed that group IIA (Diabetic control) had highest (0.97±0.18) and group IIE had lowest (0.44±0.19) mean serum creatinine level among all the six groups. Whereas significant difference among mean serum creatinine level were showed. The ranges of serum Creatinine were in group I (0.3-0.9) mg/dl, group IIA (0.7-1.2) mg/dl, group IIB (0.3-1.1) mg/dl, group IIC (0.3-0.9) mg/dl, group IID (0.3-0.8) mg/dl and group IIE (0.2-0.7) mg/dl. Although levels were within normal limit in all groups.

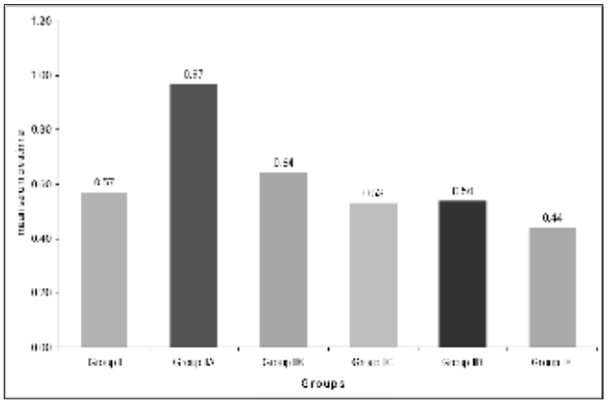

Figure 1 Simple bar diagram showing the mean serum creatinine levels in different groups

Figure 1 reveals comparison of mean serum creatinine levels in different groups. Group IIA showed highest level (0.97) of serum creatinine level and group IIE showed lowest level (0.44) of serum creatinine. The ranges of serum Creatinine were in group I (0.3-0.9) mg/dl, group IIA (0.7-1.2) mg/dl, group IIB (0.3-1.1) mg/dl, group IIC (0.3-0.9) mg/dl, group IID (0.3-0.8) mg/dl and group IIE (0.2-0.7) mg/dl.

Table II Comparison of serum creatinine between group I (Normal control group) with other groups at the end of drug administration.

Groups		Mean±SD (mg/dl)	t-value	p value
Group I		0.57±0.23		
	vs Group IIA	0.97±0.18	3.635	0.003**
	vs Group IIB	0.64±0.30	0.496	0.629^{ns}
	vs Group IIC	0.53±0.24	0.345	0.736^{ns}
	vs Group IID	0.54±0.21	0.245	0.811^{ns}
	vs Group IIE	0.44±0.19	1.143	0.275^{ns}

Table II showed significant difference in mean serum creatinine level in group IIA (0.97±0.18) against group I (Normal control group-0.57±0.23) other groups were insignificant when compare to group I (normal control group). The ranges of serum Creatinine were in group I (0.3-0.9) mg/dl, group IIA (0.7-1.2) mg/dl, group IIB (0.3-1.1) mg/dl, group IIC (0.3-0.9) mg/dl, group IID (0.3-0.8) mg/dl and group IIE (0.2-0.7) mg/dl.

DISCUSSION

This experimental study was carried out to evaluate the effect of Metformin and Pitavastatin when administered alone and in combination on renal function in Alloxan induced diabetic rats. The serum creatinine is a potent renal marker which increase conditions that reveal renal damage. Comparison of mean serum creatinine levels in different groups reveals that there was significantly increased serum creatinine in study group II A (Diabetic control group) in comparison to group I (Normal control group) and the result is statistically significant at 1% level of probability ($p<0.001$). Group IIA diabetic control group showed highest level (0.97) of serum creatinine level.[17] But there was no significant change of serum creatinine among group I (control group) and study group IIC, group IID and study group IIE. Group IIE (Diabetic rats treated with Metformin 200 mg/kg and Pitavastatin 4 mg/kg body weight.) showed lowest level (0.44) of serum creatinine. The ranges of serum Creatinine were in group I (0.3-0.9) mg/dl, group IIA (0.7-1.2) mg/dl, group IIB (0.3-1.1) mg/dl, group IIC (0.3-0.9) mg/dl, group IID (0.3-0.8) mg/dl and group IIE (0.2-0.7) mg/dl. After two weeks of treatment there was significant difference in mean serum creatinine level in group IIA (0.97±0.18) against group I (Normal control group-0.57±0.23), other groups were insignificant when compare to group I (Normal control group).Group II B(Metformin treated group) show not significant result in compare to group I (Normal control group), group II C (Pitavastatin treated group) show not significant result in compare to group I (Normal control group) and is Reno protective.[18,19] Combination group IID and group IIE also show no significant change in serum creatinine level. Hyperglycemia gives rise to many complications of diabetes which includes nephropathy. Serum creatinine levels among different groups were measured. At the termination of treatment, there was no statistically significant difference found in the serum creatinine level of all groups and were found within normal ranges.

CONCLUSION

Serum creatinine level among all groups of experimental rats were within normal limit at the end drug administration of Metformin, Pitavastatin given alone and when given in combination.

DISCLOSURE

All of the authors declared no competing interests.

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