

# Beneficiary Effect of Combination Therapy of Metformin and Pitavastatin Drug on Alloxan Induced Diabetic Rats Comparing to Single Drug Therapy

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## Abstract

**Background:** The present study was designed to investigate the antihyperglycemic, antidyslipidemic effects and hepatoprotectivity of the fixed dose combination of metformin [850 mg/70 kg body weight (BW)] and pitavastatin [2 mg/70 kg (BW)] on alloxan induced (120 mg/kg BW) diabetic rats.

**Methods:** A well-established method was implemented to evaluate the effects of combination drug therapy of metformin and pitavastatin on alloxan induced diabetic rats. This study has been designed to evaluate the effects of metformin, pitavastatin and combination on blood glucose level, triglyceride (TG) level, LDL cholesterol level, total cholesterol level, liver dysfunction indices (ALT), liver dysfunction indices (AST), effect of drugs and their combination on left ventricle, survival rate of diabetic treated rats, compare weight of different groups and justify among them. The statistical method applied in each analysis was described in each figure. Results were considered to be significant when p values were less than 0.05 ( $p < 0.05$ ).

**Results:** In alloxan induced diabetic rats, combination therapy induced a significant decrease in blood glucose level from  $15.5 \pm 0.01$  to  $6 \pm 0.03$  mmol/L two hours after last dose administration, after daily treatment for two weeks. In case of dyslipidemic effect, combination therapy reduced total cholesterol (33%), triglyceride (36%) and LDL-cholesterol (34%) levels significantly and increased HDL-cholesterol level (67%) in comparison with their respective diabetic control groups. It was also observed that combination therapy decreased LV hypertrophy (47%) in comparison with diabetic control group effectively. It was also observed that combination therapy decreased ALT (46%) and AST (35%) in comparison with diabetic control group effectively.

**Conclusion:** In conclusion pitavastatin potentiates the antihyperglycemic, antidyslipidemic and hepatoprotectivity of metformin on alloxan induced diabetic rats. The results of the present study suggest that, combination of metformin and pitavastatin might be efficacious in patients with diabetic dyslipidemia and increased hepatoprotectivity.

**Keywords:** Alloxan; Combination therapy; Diabetes; Metformin; Pitavastatin

## Abbreviations:

BW: Body Weight; WHO: World Health Organization; TG: Triglyceride; TC: Total cholesterol; CVD: Cardiovascular Disease; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; ALT: Alanine Amino Transferase; AST: Aspartate Amino Transferase

## Introduction

Diabetes mellitus is not a disease, it is a metabolic disorder which resulting protein, carbohydrate, and fat metabolism thus ultimately causing hyperglycemia due to defect in insulin secretion or insulin resistance in body [1]. Free radicals production or impaired antioxidant defenses is the ultimate result of diabetic mellitus which

leads to cell injury like apoptosis, impair beta cell or myocardial and ultimately change gene expression and cellular responses [2-4]. About 200 million people worldwide are affected by Diabetic mellitus and the number is growing rapidly [5]. This disease has now become very common and its frequency is rising dramatically worldwide. Now a day the Indian subcontinent has emerged as the capital of this diabetes epidemic. One research strategy has shown prevalence of diabetes in adults between the ages of 20 and 79 is as follows: Bangladesh 9.85%, India 8.31%, Nepal 3.03%, Sri Lanka 7.77%, and Pakistan 6.72% [6]. Various categories drugs are used to treat diabetes in addition to insulin like, thiazolidinediones, sulfonylureas D-phenylalanine, biguanides, and  $\alpha$ -glucosidase inhibitors. But due to unwanted side effect and doubted efficiency of these drugs there is a demand as well as continuation of these drugs for the treatment of diabetes with CVD [7]. Combination of anti-diabetic and hypolipidemic drug has been suggested as a rich source for treatment [8,9]. As, combination therapy

treatment has an effect on protecting  $\beta$ -cells and smoothing out fluctuation in glucose level and cholesterol biosynthetic pathway. Therefore, the present study was aimed, at providing a strong view on experimental studies in animals, to find out the most effective and commonly used hypoglycemic and lipid lowering drugs combination on long-term alloxan induced diabetes in rats [10].

Four major type of diabetes are classified by National Diabetes Data Group (NDDG) and the World Health Organization (WHO) [11].

**Insulin-dependent diabetes mellitus (IDDM):** This specific type of Diabetes Mellitus (DM) is also known as type 1 DM or juvenile diabetes. Body cannot produce insulin, and needs insulin shots to maintain the blood glucose level. Insulin must be delivered by injection, pump, or inhalation to treat the patient [12].

**Non-insulin-dependent diabetes mellitus (NIDDM):** This type of diabetes is also known as type 2 DM or adult-onset diabetes. Insulin resistance is the main reason for this type of diabetes, insulin resistance is a condition in which cells fail to use insulin properly and sometimes also with an absolute insulin deficiency. Insulin shots may not normalize the glucose level. An oral medication can be added to the treatment plan if first line treatment does not control blood sugar levels effectively. Insulin may also needed in certain circumstances, patients with Type 2 diabetes [13].

**Gestational diabetes mellitus (GDM):** Diabetes of this type occurs when pregnant women without a previous diagnosis of diabetes develop a high blood glucose level.

**Diabetes secondary to other conditions:** This is caused due to affected various organs in body such as pancreatic disease, hormonal disease, drug or chemical exposure, insulin receptor abnormalities, certain genetic syndromes etc.

Different types of complications such as atherogenesis, cardiovascular disorders, neuropathy and retinopathy mainly due to high levels of circulating glucose in blood circulation system [14,15]. Reactive oxygen species (ROS) overpowers the amount of neutralizing agents or antioxidants due to the case of oxidative stress. Endothelial dysfunctions and cardiovascular disease (CVD) which causes premature sickness and death in most countries which cause due to the formation of superoxide and the subsequent increase in oxidative stress [16]. The main purpose of treatment of diabetes is reduction in hyperglycemia by the use of insulin (in case of type 1 diabetics) and oral antihyperglycemics (e.g., biguanides, thiazolidinediones, sulfonylureas DPP-4 and  $\alpha$ -glucosidase inhibitors etc.) either alone or in combination with insulin. But due to adverse effect of drug in human body and their lack of efficacy and complication new remedies are still in great demand. So there is a demand as well as continuation of these drugs for the treatment of diabetes with CVD [17]. It is suggested that Combination therapy has been suggested as a rich, as yet unexplored source of potentially useful antidiabetic and hypolipidemic drugs.

Chemically alloxan or mesoxalylurea is an organic compound based on a pyrimidinediheterocyclic skeleton by nature. This compound has a high affinity for water and therefore exists as the monohydrate. Mechanism of alloxan includes selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase and the glucose sensor of the  $\beta$  cell [18].

## Materials and Methods

### Raw materials

Antidiabetic drug Metformin and antilipid drug pitavastatin were the generous gift from Aristopharma limited, Dhaka-1204, and Bangladesh. Alloxan was purchased from Loba Chemiie, Bombay, India, Total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL) kits were purchased from Human, Germany. SGPT (ALT) and SGOT (AST) kits were purchased from LiNEAR Chemicals, Spain, Phenobarbital Sodium, Normal Saline Solution (Incepta Pharmaceuticals Ltd.). All chemicals in this investigation were of analytical reagent grade.

### Selection of animal

Randomly selected (both male and female) Long Evans rats weighing about 100-150 g, age 2-3 months were purchased from animal's house of ICDDR. Prior to commencement of the experiments, all the rats were acclimatized to the new environmental condition for a period of one week. During the experimental period the rats were kept in a well-ventilated animal house at room temperature of 25°C and were supplied with standard pellets and fresh drinking water. All the rats were kept in cages and maintained with natural 12 hour light and dark cycle.

### Grouping of experimental rats

Long-Evans rats were randomly assigned into group 1, 2, 3, 4 and 5; 5 rats in each group for the respective two weeks treatment protocols for the determination of blood glucose, lipid profile and liver function test studies.

**Group 1:** Normal Control rat group.

**Group 2:** Untreated Group Diabetic Control group.

**Group 3:** Alloxan induced Diabetic + Metformin drug.

**Group 4:** Alloxan induced Diabetic + Pitavastatin drug.

**Group 5:** Alloxan induced Diabetic + Combination of Metformin and Pitavastatin drug.

### Experimental induction of diabetes

After fasting for 12 hours Group 2-5 animals were rendered diabetic by injection intraperitoneally by a freshly prepared solution of alloxan (120 mg/kg BW) in distilled water after base line glucose estimation was done. The alloxan treated animals were allowed to drink 10% glucose solution and feed overnight to overcome drug induced hypoglycemia. Clever Check glucose test meter (Bioland, Germany) were used to check blood glucose after 24 hours by using blood sample collected from the tail vein of the rats. When the condition of diabetes was established animals with blood glucose levels above 11.1 mmol/L was selected for the study.

### Preparation of dosage of active drug(s)

**Pitavastatin drug:** Pitavastatin is an antilipid drug which inhibit HMG-CoA reductase. It is a synthetic lipid-lowering agent administered through oral route. (Plus/+) monocalciumbis {(3R, 5S, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinoly]-3,5 dihydroxy-6-heptenoate} is the chemical name for Pitavastatin.

Physically Pitavastatin is off white powder form and very slightly soluble in water. Water was used to prepare the dosage form in suspension in such a concentration that, each 0.1 ml of solution contained pitavastatin according to the dose of 2 mg/70 kg BW, since pitavastatin is effective in such dose in humans.

**Metformin drug:** Metformin is a noble oral antidiabetic drug which falls in biguanide group of antidiabetic drug. Metformin is most popular first-line drug of choice for the treatment of type 2 diabetes. It is prescribed in overweight and obese people and those with normal kidney function. Physically Metformin was in white crystal form and freely soluble in water. The dosage was prepared in solution from using water in such a concentration that, each 0.1 ml of solution contained metformin according to the dose of 850 mg/70 kg BW, since metformin is effective in such dose in humans.

With combination of drug (metformin and pitavastatin): The combination of metformin and pitavastatin was prepared in solution from using water in such a concentration that, each 0.1 ml of solution contained pitavastatin and metformin according to the dose of 1 mg/70 kg BW and 425 mg/70 kg BW respectively since pitavastatin and metformin are effective in such dose in humans.

### Blood serum collection

In our research we had collected blood sample from rats to get the serum. After completing the two weeks treatment, phenobarbital sodium was used to anesthetize the rats. Then after sacrificing the rats and cutting the abdominal skin, thoracic artery was opened. 3-5 ml of blood was collected directly from thoracic artery by heparinized syringe. At last the blood was centrifuged at 4000 rpm for 30 minutes and the serum was obtained.

### Monitoring of oral blood glucose tolerance test

In our research, after overnight fasting, all animals (group number, n=5) in control group received glucose (1.5 gm/kg per oral) and 0, 0.5, 1, 2 hour interval blood glucose was estimated using glucometer [19].

### Effect of anti hyperglycemic in blood serum

From the five groups four of them that is 2, 3, 4, and 5 were prepared for testing anti-hyperglycemic effect after alloxan induction. For about 16 hours all the rats were starved. Baseline glucose level was tested for all the rats. Diabetic control group that is group 2 did not receive any drug. The group 4 stands for Alloxan induced Diabetic + Pitavastatin drug in which pitavastatin is administered orally at a dose of 2 mg/70 kg BW. The group 3 stands for Alloxan induced Diabetic + Metformin drug in which metformin is administered orally at a dose of 850 mg/70 kg BW. Group 5 receive the combination of drugs (pitavastatin and metformin) according to above mentioned dose.

### Treatment of Metformin, Pitavastatin and their combination for one week

For one week repeatedly drug Metformin, Pitavastatin and their combination were administered in the alloxan induced diabetic (blood glucose >13 mmol/lit) rats. Blood glucose was determined two hours after last dose using glucometer after one week treatment.

### Treatment of Metformin, Pitavastatin and their combination for two week

For two weeks repeatedly drug Metformin, Pitavastatin and their combination were administered in the alloxan induced diabetic (blood glucose >13 mmol/lit) rats. Blood glucose was determined two hours after last dose using glucometer after one week treatment. In addition serum lipid profiles and ALT and AST test were assessed after two weeks in the metformin, pitavastatin and combination drug treated diabetic rats. Diagnostic kits were used for carrying out all the assays.

### Blood Hypolipidemic Effects

**Method of total cholesterol (TC) test:** From thoracic artery blood serum was collected after sacrificing the rats which was used for testing the serum TC. The concentrations were analyzed by taking absorbance by UV spectrophotometer, using diagnostic kits (Human, Germany).

**Method of triglyceride (TG) test:** From thoracic artery blood serum was collected after sacrificing the rats which was used for testing the serum TG. The concentrations were analyzed by taking absorbance by UV spectrophotometer, using diagnostic kits (Human, Germany).

**Method of determination LDL-cholesterol (LDL) in blood serum:** From thoracic artery blood serum was collected after sacrificing the rats which was used for testing the serum LDL-Cholesterol levels. By using UV spectrophotometer the concentrations were analyzed by taking absorbance, using diagnostic kits (Human, Germany).

**Method of determination HDL-cholesterol (HDL) in Blood Serum:** From thoracic artery blood serum was collected after sacrificing the rats which was used for testing the serum HDL-Cholesterol levels. By using UV spectrophotometer the concentrations were analyzed by taking absorbance, using diagnostic kits (Human, Germany). By using UV spectrophotometer the concentrations were analyzed by taking absorbance, using diagnostic kits (Human, Germany).

**Method of hepatoprotective activity in blood serum:** Collection of blood serum: The blood serum which was collected from thoracic artery after sacrificing the rats was used for testing the serum SGPT (ALT) and SGOT (AST) test levels. The concentrations were analyzed by taking absorbance by UV spectrophotometer, using diagnostic kits (LiNEAR Chemicals, SPAIN).

Kinetic method is used for the determination of SGPT (ALAT) and SGOT (AST) activity according to the recommendations of the Expert Panel of the IFCC (International Federation of Clinical Chemistry) without pyridoxal phosphate activation.

**Measurement of left ventricular hypertrophy:** Phenobarbital sodium was used to anaesthetize rats. After sacrificing. The heart was excised from the chest cavity and immersed briefly in phosphate buffer solution at room temperature in order to wash out blood from the chambers. After that each heart was removed from the fixative and excessive fat trimmed off. Then completely separate atria from the ventricles. The weight of the left ventricle was taken. Then left ventricular hypertrophy is calculated by the following formula: Left Ventricular Hypertrophy = Weight of Left Ventricle\Body weight.

### Statistical Analysis

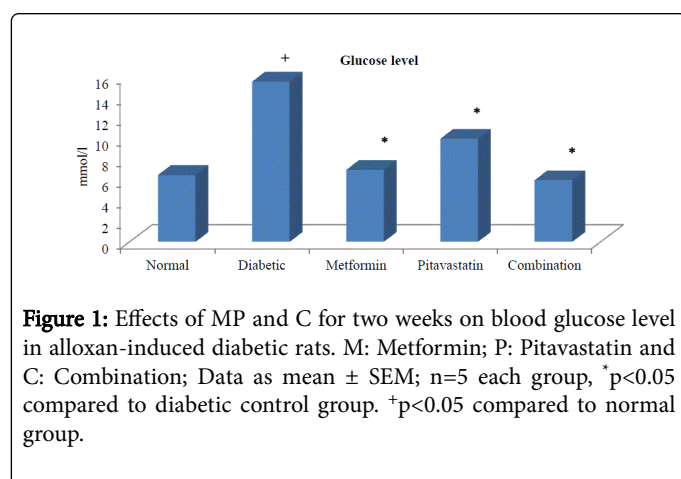
Graph Pad Prism (version 4.0) computer program (Graph pad Software San Diego, CA, USA) was used to calculate the results which are expressed as mean  $\pm$  SEM. In our research one-way analysis of variance (ANOVA), post-hoc test or students paired or unpaired t-test

was followed by Dunnett's test. The statistical method applied in each analysis was described in each figure. Results were considered to be significant when p values were less than 0.05 ( $p < 0.05$ ).

## Results

After two weeks of drug treatment the parameters of blood glucose (BG) level, TC, TG, LDL, HDL, liver function test (AST and ALT), LV hypertrophy, survival rate and body weight were measured to study the effects of drugs alone and combination of them (Metformin and Pitavastatin) on alloxan-induced diabetic rats. Metformin and Pitavastatin were used as standard antidiabetic agent and lipid lowering agent accordingly.

**Effect of Metformin, Pitavastatin and combination on blood glucose level:** After two weeks treatment, we measured blood glucose of all the rats of five groups and found out that in Metformin group, glucose level (from  $15.5 \pm 0.01$  to  $7 \pm 0.015$  mmol/L) was less than the Pitavastatin group (from  $15.5 \pm 0.01$  to  $10 \pm 0.025$  mmol/L), but in combination group of Metformin and Pitavastatin this glucose level (from  $15.5 \pm 0.01$  to  $6 \pm 0.03$  mmol/L) was even lower than the pioglitazone group rats, compared to the untreated diabetic group rats ( $15.5 \pm 0.01$  mmol/L) (Figure 1).



**Figure 1:** Effects of MP and C for two weeks on blood glucose level in alloxan-induced diabetic rats. M: Metformin; P: Pitavastatin and C: Combination; Data as mean  $\pm$  SEM; n=5 each group, \* $p < 0.05$  compared to diabetic control group. + $p < 0.05$  compared to normal group.

**Effect of metformin, pitavastatin and their combination on triglyceride (TG) level:** The following Figure 2 graphically represented the effect of Metformin, Pitavastatin and their combination on triglyceride (TG) level after two weeks treatment. It also showed the values those had significantly changed in comparison with diabetic control group. After two weeks treatment it was found that Metformin, Pitavastatin and their combination reduced TG cholesterol level 25%, 32% and 36% respectively.

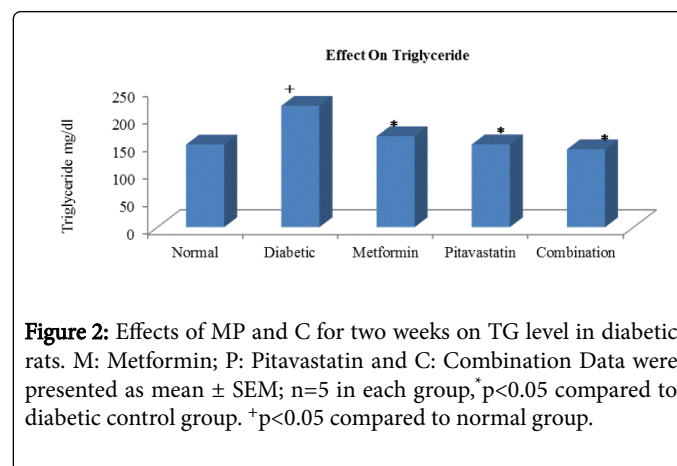
**Effect of metformin, pitavastatin and their combination on HDL cholesterol level:** The following Figure 3 graphically represented the effect of Metformin, Pitavastatin and their combination on HDL cholesterol level after two weeks treatment. It also showed the values those had significantly changed in comparison with diabetic control group. After two weeks treatment it was found that Metformin, Pitavastatin and their combination increased HDL cholesterol level 28%, 51% and 67% respectively.

**Effect of metformin, pitavastatin and their combination on LDL cholesterol level:** The following Figure 4 graphically represented the effect of Metformin, Pitavastatin and their combination on LDL level. It also showed the values those had significantly changed in

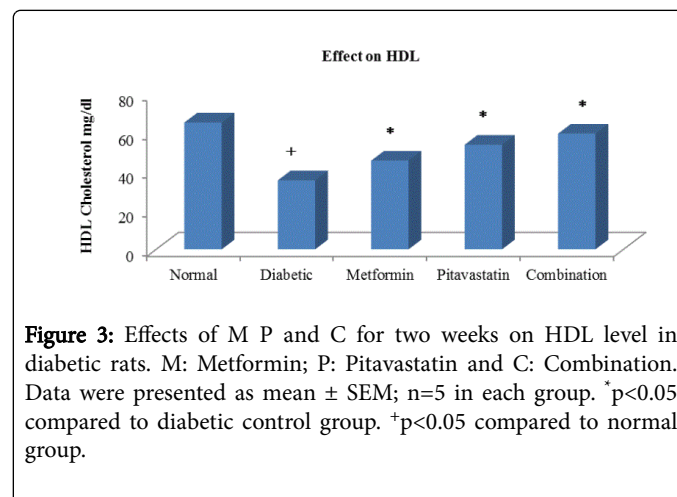
comparison with diabetic control group. After two weeks treatment it was found that Metformin, Pitavastatin and their combination reduced LDL cholesterol level 10%, 30% and 34% respectively.

**Effect of metformin, pitavastatin and their combination on total cholesterol level:** The following Figure 5 graphically represented the effect of Metformin, Pitavastatin and their combination on total cholesterol level. It also showed the values those had significantly changed in comparison with diabetic control group. After two weeks treatment it was found that Metformin, Pitavastatin and their combination reduced total cholesterol level 13%, 26% and 33% respectively.

**Effect of drugs and their combination on liver dysfunction indices (ALT):** The following Figure 6 graphically represented the effect of Metformin, Pitavastatin and their combination on ALT level. It also showed the values those had significantly changed in comparison with diabetic control group. After two weeks treatment it was found that Metformin, Pitavastatin and their combination reduced ALT level 16%, 33% and 46% respectively.

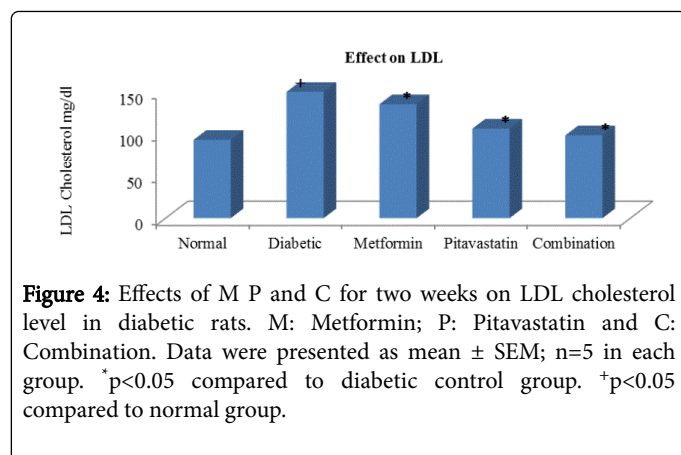


**Figure 2:** Effects of MP and C for two weeks on TG level in diabetic rats. M: Metformin; P: Pitavastatin and C: Combination. Data were presented as mean  $\pm$  SEM; n=5 in each group, \* $p < 0.05$  compared to diabetic control group. + $p < 0.05$  compared to normal group.

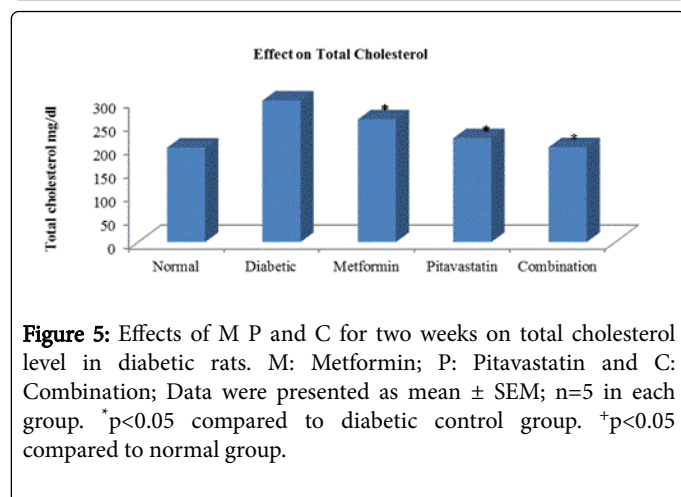


**Figure 3:** Effects of M P and C for two weeks on HDL level in diabetic rats. M: Metformin; P: Pitavastatin and C: Combination. Data were presented as mean  $\pm$  SEM; n=5 in each group, \* $p < 0.05$  compared to diabetic control group. + $p < 0.05$  compared to normal group.

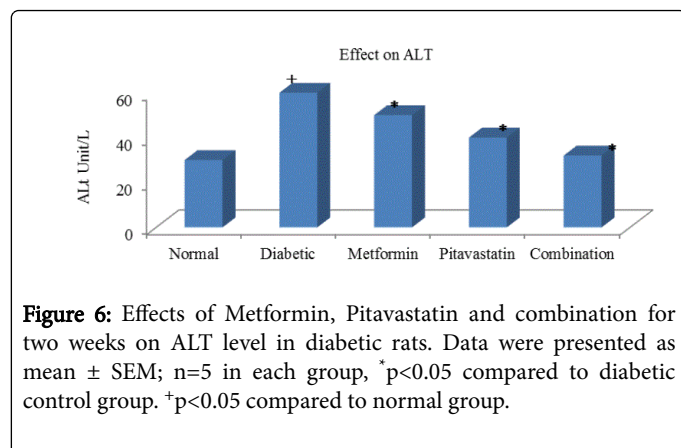




**Figure 4:** Effects of M P and C for two weeks on LDL cholesterol level in diabetic rats. M: Metformin; P: Pitavastatin and C: Combination. Data were presented as mean  $\pm$  SEM; n=5 in each group. \*p<0.05 compared to diabetic control group. +p<0.05 compared to normal group.

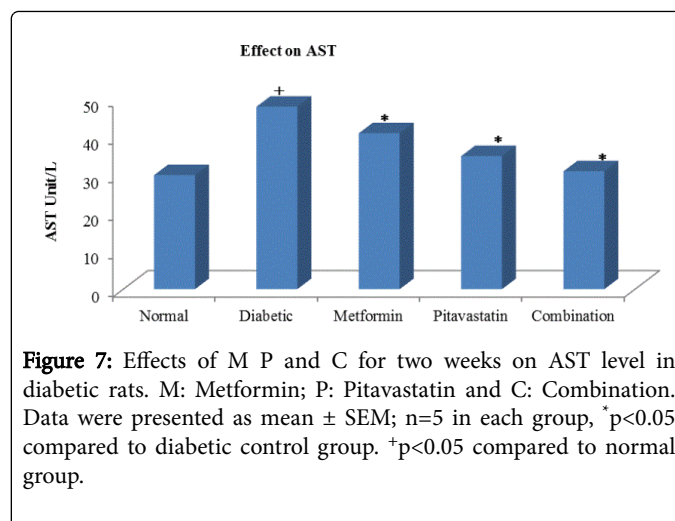


**Figure 5:** Effects of M P and C for two weeks on total cholesterol level in diabetic rats. M: Metformin; P: Pitavastatin and C: Combination; Data were presented as mean  $\pm$  SEM; n=5 in each group. \*p<0.05 compared to diabetic control group. +p<0.05 compared to normal group.



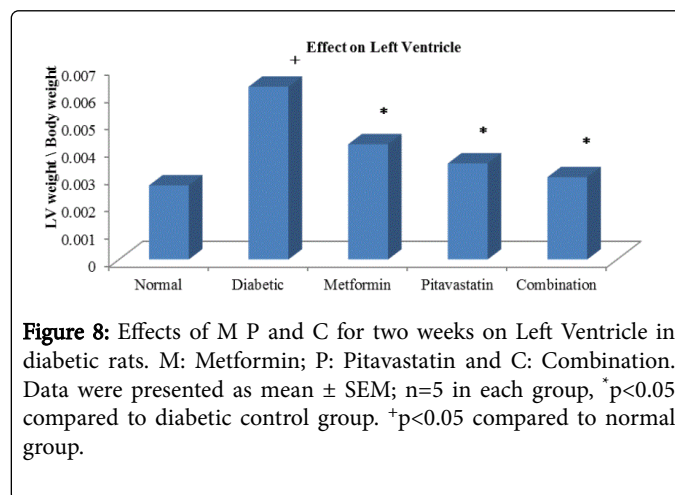
**Figure 6:** Effects of Metformin, Pitavastatin and combination for two weeks on ALT level in diabetic rats. Data were presented as mean  $\pm$  SEM; n=5 in each group, \*p<0.05 compared to diabetic control group. +p<0.05 compared to normal group.

**Effect of drugs and their combination on liver dysfunction indices (AST):** The following Figure 7 graphically represented the effect of Metformin, Pitavastatin and their combination on AST level. It also showed the values those had significantly changed in comparison with diabetic control group. After two weeks treatment it was found that Metformin, Pitavastatin and their combination reduced AST level 45%, 27% and 35% respectively.



**Figure 7:** Effects of M P and C for two weeks on AST level in diabetic rats. M: Metformin; P: Pitavastatin and C: Combination. Data were presented as mean  $\pm$  SEM; n=5 in each group, \*p<0.05 compared to diabetic control group. +p<0.05 compared to normal group.

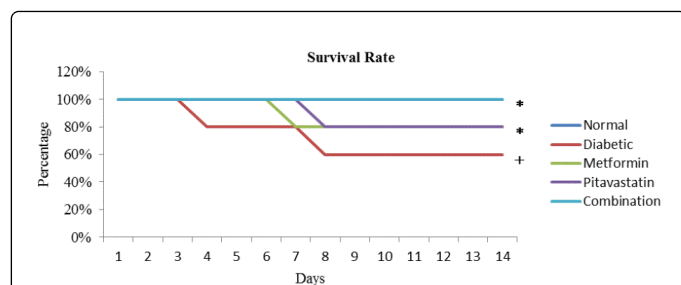
**Effect of drugs and their combination on left ventricle:** The following Figure 8 graphically represented the effect of Metformin, Pitavastatin and their combination on Left Ventricle. It also showed the values those had significantly changed in comparison with diabetic control group. After two weeks treatment it was found that Metformin, Pitavastatin and their combination reduced LV hypertrophy 33%, 44% and 47% respectively.



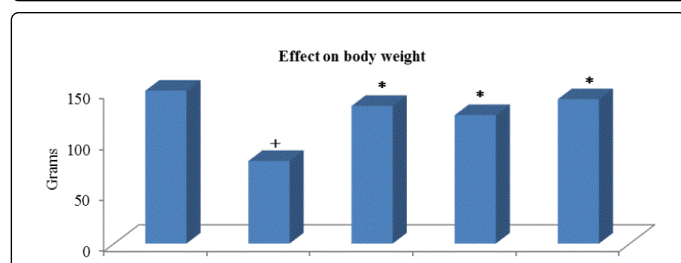
**Figure 8:** Effects of M P and C for two weeks on Left Ventricle in diabetic rats. M: Metformin; P: Pitavastatin and C: Combination. Data were presented as mean  $\pm$  SEM; n=5 in each group, \*p<0.05 compared to diabetic control group. +p<0.05 compared to normal group.

**Survival rate of diabetic treated rats:** The following Figure 9 graphically represented that the survival rate of normal, diabetic and diabetic treated rat groups. After two weeks treatment it is found that combination therapy is much more effective and having full percentage (100%) of survival rate rather than other groups of metformin (80%) and pitavastatin (80%) compared to diabetic group (60%).

**Compare weight of different groups of rats:** The following Figure 10, graphically represented the weight of diabetic treated rat groups. After two weeks treatment it is found that in combination treating group, rats are healthy and gain weight (141.66  $\pm$  0.04 grams) compare to the Metformin (135  $\pm$  0.02 grams), Pitavastatin (126  $\pm$  0.03 grams) groups compared to diabetic groups (81  $\pm$  0.02 grams) that indicate that the combination therapy is effective on rat's health.



**Figure 9:** Combination therapy improves the survival. The following curve shows that combination therapy improved long term survival compared with the monotherapy of pitavastatin and metformin on diabetic rats. Data were presented as mean  $\pm$  SEM; n=5 in each group, \*p<0.05 compared to diabetic control group. +p<0.05 compared to normal group.



**Figure 10:** Effects of Metformin, Pitavastatin and combination of Metformin and Pitavastatin for two weeks effect on body weight in alloxan-induced diabetic rats. The values are expressed as mean  $\pm$  SEM. n = 5 in each group. +p<0.05 compared with normal control group. \*P<0.05 compared with diabetic control group.

## Discussion

Recent Research data suggest that in addition with hyper-glycemia some other parameters like Hyper-cholesterolemia and hyper-triglyceridemia are common complications of diabetes mellitus [20]. High lipid level in blood which is associated with Diabetes is the major cause of concern in western and developing countries [21]. Drugs which are available in market for treating diabetes possess some state of adverse effects. For this reason searching of most effective drug is now the top concern. In our work we experiment on diabetes rats by using most popular available antidiabetic drug metformin and antilipid drug Pitavastatin and screening rats blood to find out this two drugs effect both single and combindly.

### Effect of Metformin and Pitavastatin on blood glucose level

We measured blood glucose of all the rats of the five groups after two weeks treatment and we find out that in Metformin group, glucose level ( $7 \pm 0.015$  mmol/L) is less than the Pitavastatin group ( $10 \pm 0.025$  mmol/L), but in combination group of Metformin and Pitavastatin this glucose level ( $6 \pm 0.03$  mmol/L) even more less than this Metformin group rat compared to untreated diabetic group rats ( $15.5 \pm 0.01$  mmol/L) (Figure 1). So from analyzing these parameters we can conclude that combination therapy of Metformin and Pitavastatin is

much more effective than single drug dose treatment in lowering blood glucose level.

### Effects of Metformin and Pitavastatin and their combination therapy on lipid profile

We find out through our two weeks research that treating with alloxan not only increased blood glucose levels but also increased the levels of various parameters like TG, TC, and LDL cholesterol, total cholesterol level and decreased the levels of HDL cholesterol in diabetic rats and also other single treated drug rat group. After two weeks we find out after analyzing blood serum of rats that the TG level (46%), TC level (50%), LDL-Cholesterol level (61%) were increased and HDL-Cholesterol level (46%) were reduced in alloxan induced diabetic rats in comparison with their respective normal drug treated rats groups after two weeks (Figure 2-5).

Research shows that factors those influencing the glucose metabolism also responsible for increasing lipid metabolism as well [22]. We noticed after two weeks treatment there is no appropriate improvement in serum TC, TG, LDL-Cholesterol and HDL-Cholesterol levels by the treatment with Metformin and Pitavastatin (Figure 2-5) alone in comparison with single drug therapy of respected drug. But in case of group which was treating with combination of drug (Metformin and Pitavastatin) showed better reduction in serum TC (33%), TG (36%), LDL-Cholesterol levels (34%) and largely increasing in HDL-Cholesterol level (67%) compared to diabetic control group after the both two treatment protocols.

Some recent research data suggest that in diabetes mellitus the serum lipids were usually increased. But due to the capability of high lipid lowering characteristic of this combination therapy, we proposed after our research work that the drugs of the combination therapy of the Metformin and Pitavastatin may act as inhibitors for cholesterol biosynthesis by inhibiting enzyme such as hydroxyl-methyl-glutaryl-CoA reductase [23].

**Hepatoprotective effect of drugs and combination therapy:** In our study we observed after two weeks of treatment that there were significant increments in ALT (93%) and AST (60%) level in diabetic control group compared to the normal control group (Figure 2-5). It is observed that combination therapy of Metformin and Pitavastatin significantly reduce ALT (46%) and AST (35%) level rather than single therapy of Metformin and Pitavastatin monotherapy after two weeks treatment. ALT and AST enzymes are responsible for production of ketone bodies from amino acids and subsequently produce high concentration of glucose level. As these combination therapies decrease the ALT and AST level, subsequently they decrease the blood glucose level.

**Effect of Metformin and Pitavastatin on Left Ventricle:** In our study it was observed that there were significant increments in LV hypertrophy (133%) in diabetic control group compared to the normal control group (Figure 8). It is observed that combination therapy of Metformin and Pitavastatin significantly reduce LV hypertrophy (47%) rather than single therapy of Metformin and Pitavastatin monotherapy after two weeks treatment.

**Effect of combination therapy on survival rate and body weight:** In our study we have observed that combination therapy improves survival rate of rats. After two weeks treatment the survival rate of diabetic, Pitavastatin and Metformin is 60%, 80%, and 80% (Figure 9) but the combination therapy of Metformin and Pitavastatin group has

significantly improved survival rate. The survival rate of combination treated group is 100% compared to normal group. We also measured body weight after two weeks treatment (Figure 10). Body weight is significantly reduced in diabetic group rats (untreated group) ( $81.5 \pm 0.2$  grams) compared to the normal group ( $150 \pm 0.02$  grams) but body weight of combination group ( $155.5 \pm 0.04$  grams) is significantly increased compared to other Metformin ( $135.5 \pm 0.02$  grams) and Pitavastatin groups ( $126 \pm 0.03$  grams).

### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

### Acknowledgement

The authors are thankful to the Department of Pharmacy, Southeast University, Banani, Dhaka-1213, Bangladesh for their kind permission to use their Animal House and Animal Laboratory.

### Ethical Approval

The guidelines followed for animal experiment were accepted by the institutional animal ethics committee [24].

### Funding

Self-Funded Research Work.

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