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Review Article

# A PROSPECTIVE STUDY TO EVALUATE THE EFFECT OF METFORMIN AND VITAMIN C COMBINATION ON INSULIN LEVEL IN STREPTOZOTOCIN INDUCED DIABETES IN RATS

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**A B S T R A C T**

The metabolic consequences of prolonged hyperglycemia and dyslipidemia, including accelerated atherosclerosis, chronic kidney disease and blindness pose an enormous burden on patients with diabetes milletus and on the public health system. We planned a study to evaluate the effect of metformin and vitamin c combination on insulin level in streptozotocin induced diabetes in rats. Inbred adult male albino rats were obtained from the Central animal house, Madurai Medical College for the study. 24 adult male albino rats each weighing 150 to 200 grams were used in the study. Animals were divided into four groups, of six animals in each group. Animals were allowed to take standard diet (pellet feed) and tap water ad libitum. Animals in the test group 1 received Tab.Metformin200mg/kg and Tab. Vitamin C 50mg/kg for 28 days. Animals in the test group 2 received Tab. Metformin 200mg/kg and Tab. Vitamin C 100mg/kg for 28 days. Fasting blood sugar was measured for all rats on 1st, 3rd, 7th , 14th, 21st and 28th day respectively. Cardiac puncture was on 28th day for all rats to measure the insulin level. Comparison of serum insulin level showed significant increase in the standard, test 1 and test 2 groups . In this test 1 and test 2 showed better results than standard. Test 2 had better results than test 1.



## INTRODUCTION

Diabetes mellitus (DM) is the most common chronic metabolic disorder characterized by hyperglycemia. Several distinct types of DM are caused by a complex interaction of genetics and environmental, inflammation and autoimmune factors. The factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization and increased glucose production DM exhibits enhanced oxidative stress and highly reactive oxygen species(ROS) production in pancreatic islets due to persistent and chronic hyperglycemia, thereby depletes the activity of the antioxidativedefence mechanism and thus promote free radical generation . Oxygen free radicals have been suggested to be a contributory factor in complications of DM. It seems to be an oxidative stress-related disorder and the antioxidants may be useful in preventing it. Therefore supplementation of therapeutics with antioxidants may have a chemoprotective role in diabetes. Prevention and treatment of DM is a major public health challenge. In the Diabetes Prevention Programme (DPP) 10 yr follow-up study, lifestyle management including diet and exercise led to a 31–58% reduction in the incidence of diabetes. Greater understanding of the pathophysiology of DM has contributed to the development of new pharmacological approaches Metformin (Biguanide) is a potent oral hypoglycemic agent now recommended as the first-line therapy for diabetes mellitus (type 2). In addition to its hypoglycemic activity metformin has been shown to elicit marked antioxidant activity, neuroprotective activity and antiepileptic activity.

Ascorbic acid (vitamin C), an antioxidant vitamin, plays an important role in protecting free radical-induced damage. Vitamin C is structurally similar to glucose and can replace it in many chemical reactions and thus is effective for prevention of non enzymatic glycosylation of protein. Ascorbic acid supplementation has been shown to produce antidiabetic activity and antioxidant activity. Vitamin C in diabetes may play a positive role in the prevention of diabetic microangiopathies and atherosclerosis.

The additive effects of hyperglycaemia and hyperlipidaemia (due to lipid peroxidation) may increase the risk of cardiovascular disease in diabetes, Vitamin C may have beneficial effects on serum lipids and glycatedhaemoglobin (HbA1c).

Hence it was considered that supplementation of Vitamin C with Metformin may give good glycemic control and antioxidant effect in diabetes and in preventing long standing complications.

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***Aim***

To evaluate the effect of metformin and vitamin c combination on insulin level in streptozotocin induced diabetes in rats

## MATERIALS & METHODS

The antihyperglycemic effect of metformin and Vitamin C combination was evaluated in adult male albino rats. The study was done after obtaining approval from Institutional Animal Ethical Committee of Madurai Medical College, Madurai, dated31.05.2018 The study was conducted in the central animal house, Institute of Pharmacology, Madurai Medical College,

***Study Center***

Institute of Pharmacology,
Madurai Medical College, Madurai

***Duration of the Study***

This study was conducted for a period of 6 months since November 2018.

***Number of Animals Used***

24 adult male albino rats weighing about 150 –200 grams

***Materials of the Study***

1. Twenty four Adult Male Albino rats
2. Inj. Streptozotocin
3. Tab.Metformin
4. Tab.Vitamin C
5. Glucometer
6. Glucose strips
7. Oral feeding tube
8. Syringes.
9. Rat insulin ELISA Kit
10. Diethyl ether
11. Cotton
12. Drop jar
13. Blood collection tubes coated with anticoagulant
14. Distilled water

***Animals***

Inbred adult male albino rats were obtained from the Central animal house, Madurai Medical College for the study. 24 adult male albino rats each weighing 150 to 200 grams were used in the study. Animals were divided into four groups, of six animals in each group. Animals were allowed to take standard diet (pellet feed) and tap water ad libitum.

Each group of animals were kept separately with a distinct identity throughout the study by having marking with picric acid. Special care was given for the diabetic rats. The floor of cages were covered with thick layer of saw dust and it was changed daily. The diabetic rats were provided with adequate food pellets and water as the diabetic rats will have polyphagia, polyuria and polydypsia. The bottles were filled with fresh water whenever needed. Adequate hygiene was maintained to prevent infection.

***Streptozotocin***

Streptozotocin manufactured by Sisco Research Laboratories Pvt. Ltd was used to induce diabetes in the rats. After overnight fasting Inj.. Streptozotocin was given intraperitoneally at the dose of 50mg/kg as a single dose.

***Metformin***

Metformin manufactured by USV private limited (Trade name-Glycomet) was used as the standard drug. It is available as 500 mg tablet and the rats were given 200 mg/kg orally once daily.

***Vitamin C***

Vitamin C manufactured by Abbott pharma limited in the name of Limcee was used. It is available as 500mg tablet. It was given in the dose of 50mg and 100mg/kg orally once daily

***Collection of Blood Samples***

Blood was collected by tail vein puncture method. The rat was placed in the restrainer. The tail was cleaned and xylol was applied to make the veins prominent. Lateral veins were identified. Under aseptic precautions, the vein was punctured by using 22G needle, 0.2 ml of blood was collected.

***Method of Insulin Estimation***

Insulin level was estimated in rat serum sample by using rat insulin ELISA Kit manufactured by Novus Biologicals Ltd.

***Method of Collection of Blood For Serum Insulin***

- Blood was collected for serum insulin by cardiac puncture.
- For this the rats were kept fasted overnight. The rats were anasthetised with diethyl ether. The rats were kept individually in the drop jar. Ether was given through inhalation by using cotton soaked with ether which was placed in the jar.
- After getting anesthetized the rat was checked for anesthesia by lack of spontaneous movement, and lack of response to stimuli such as pinching the toe. The rat was placed on its back over the wooden board.
- Under aseptic precautions, the left index finger was placed at the level of the lowest ribs, without applying any pressure,
- The heart beat was located ~ 1 cm above this point and slightly to the right. Following this 5ml syringe with 23G needle was inserted at a 45-degree angle, between the two ribs and was watched for a drop of blood to come into the needle.
- After confirming this, the plunger was pulled to withdraw the blood and 2ml of blood was collected in the collecting tubes coated with anticoagulant heparin. This blood was centrifuged and serum was separated and insulin was measured
- All the rats were kept under warmth till they recover from anathesia and take normal feed.

***Preparation of Drug Solutions and Drug Administration***

Tab. Metformin was dissolved in distilled water and was given in the dose of 200mg/kg orally daily. Tab. Vitamin C was dissolved in distilled water and was given in the dose of 50mg and 100mg//kg daily orally.

***Oral Feeding Technique***

For oral feeding 16 Guage feeding tube of 2-3 inches in length with blunted tip with a small ball soldered at the tip was used. The feeding tube was attachedto1 ml syringe containing the drug to be given. The rat was held in the lefth and gently by holding the nape of the neck.. The oral feeding tube was

insertad laterally through the interdental space and was advanced gently into the oesophagus by gentle rotation of the tube. After reaching the desired level the drug was pushed inside.

## METHODOLOGY

The study was done by following the principles of CPCSEA and utmost care was given while handling of animals and adequate care was given to them.

All the selected rats were kept at overnight fasting. The blood glucose was measured for all the rats by tail venipuncture. The baseline blood sugar levels were found to be normal. Injstreptozotocin in the dose of 50mg/kg was administered intraperitoneally. After 72 hours, blood sugar was estimated for all rats. The rats having blood sugar of >250mg/dl were considered as diabetic and those rats were taken for the study. The diabetic rats were divided into four groups, six animals of each group. They were kept as control, standard, test group 1 and test group 2. Cardiac puncture was done for all group of rats and blood samples were collected for insulin estimation on Day 1 .All the rats received pellet diet water ad libitum. The animals in the control group received normal feed and distilled water for 28 days. Animals in the standard group receivedTab. Metformin 200 mg/kg for 28 days. Animals in the test group 1 received Tab. Metformin200mg/kg and Tab.Vitamin C 50mg/kg for 28 days. Animals in the test group 2 received Tab.Metformin 200mg/kg and Tab. Vitamin C 100mg/kg for 28 days.Fasting blood sugar was measured for all rats on 1st, 3rd, 7th , 14th, 21st and 28th day respectively. Cardiac puncture was on 28th day for all rats to measure the insulin level.

## RESULTS

In this present study, the antihyperglycemic effect of combination of Metformin and Vitamin C was evaluated in 24 adult male albino rats. They were divided into four groups and six animals in each group. They were kept as control, standard, test group 1 and test group 2.Diabetes was induced in rats by intraperitoneal administration of Inj. Streptozotocin at 50mg/kg. After 3 days all the rats were having >250mg/dl. The control group was given normal feed and distilled water. The standard group was given Tab.Metformin 200mg/kg orally. The test group 1 was given Tab.Metformin 200mg/kg and Tab. Vitamin C 50mg/kg orally. The test group 2 was given Tab.Metformin 200mg/kg and Tab. Vitamin C 100mg/kg orally. The blood sugar levels and insulin level of all diabetic rats on Ist Day

**Table No 1** Serum insulin level in diabetic rats in mIU/ml - Day 1

| S. No | Control | Standard | T1 | T2 |
|---|---|---|---|---|
| 1 | 1.24 | 1.52 | 1.25 | 1.40 |
| 2 | 1.02 | 1.59 | 1.30 | 1.71 |
| 3 | 1.50 | 1.45 | 1.22 | 1.22 |
| 4 | 1.22 | 1.46 | 1.35 | 1.33 |
| 5 | 1.31 | 1.51 | 1.42 | 1.41 |
| 6 | 1.18 | 1.55 | 1.59 | 1.01 |

**Table No 2** Serum insulin level in diabetic rats mIU/ml -Day 28

| S. No | Control | Standard | T1 | T2 |
|---|---|---|---|---|
| 1 | 1.35 | 1.75 | 1.94 | 2.22 |
| 2 | 1.30 | 1.71 | 2.05 | 1.99 |
| 3 | 1.61 | 1.92 | 1.09 | 2.32 |
| 4 | 1.50 | 1.88 | 2.00 | 2.09 |
| 5 | 1.54 | 1.95 | 2.10 | 2.05 |
| 6 | 1.48 | 1.76 | 2.06 | 2.24 |

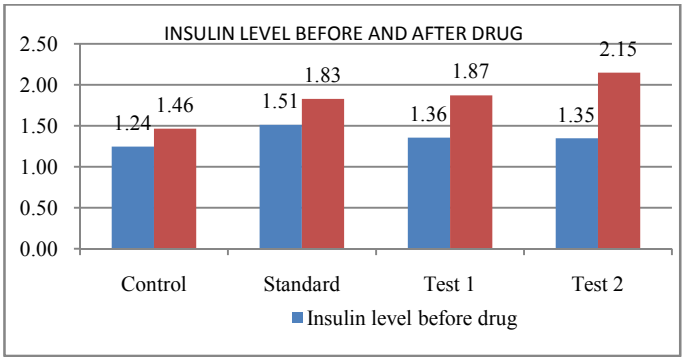


**Figure No 1** compares the serum insulin level of all 4 groups on Day 1 and Day 28

Once it was found that there was difference existing between the means among the four groups, to determine where the difference is present Bonferroni post hoc test was applied. It is used where an array of paired comparison are made that is One group(control) is compared with all the other groups (standard, test 1 and test 2 groups).

## DISCUSSION & CONCLUSION

Diabetes mellitus a chronic metabolic disorder, is an emerging epidemic in the developing countries. It occurs due to deficiency of insulin or due to insulin resistance leads to hyperglycemia. It is the major cause for morbidity and mortality It is associated with long term complications both macrovascular and microvascular like coronary artery disease, retinopathy, nephropathy and neuropathy. The management of diabetes includes pharmacotherapy, life style modification and exercise. Pharmacotherapy includes oral antidiabetic drugs or injection insulin. These synthetic drugs are the mainstay of treatment of diabetes and are effective in controlling hyperglycemia but they have some harmful adverse effects.

The limitations with adverse effects, and cost of the currently available oral antidiabetic agents to control blood glucose made the researchers to discover and develop novel anti-diabetic agents with fewer side effects and potential therapeutic benefits. In this study with the available antidiabetic drug, the most commonly used drug metformin which belongs to biguanide group along with Vitamin C were taken in graded doses and evaluated for its anti hyperglycemic effect in streptozotocin induced diabetic rats. Twenty four adult male albino rats were selected and divided into four groups with six animals in each group. They were kept as control, standard, test 1 and test 2 groups respectively. Diabetes was induced in all animals by Comparison of serum insulin level showed significant increase in the standard, test 1 and test 2 groups. In this test 1 and test 2 showed better results than standard. Test 2 had better results than test 1.Stastistical analysis was done by using ANOVA between the groups. There was no significant difference at the baseline values. It showed a significant difference with a p value of <0.001 between the groups. Post hoc analysis was done . It showed more statistical difference in test group 2 > test group 1 > standard in comparison with control group.In type 2 diabetes there will be increased production of damaging free radicals. Glucose auto oxidation, protein glycosylation, formation of advanced glycation end products and polyol pathway are involved in the generation of oxidative stress both in type 1 and 2 DM . The protection against such damage can be offered by free radical-scavenging antioxidant like Vitamin C.Vitamin C is an important antioxidant in human, being capable of

scavenging oxygen-derived free radicals. It is structurally similar to glucose and can replace it in many chemical reactions and thus is effective in prevention of non-enzymatic glycosylation of proteins. Oxidative stress plays an important role in the pathogenesis of vascular complications of diabetes. Diabetic nephropathy is one of the most common complication, in which oxidative stress is high and antioxidant status is low as has been documented. Lipid peroxidation increases in the kidney of diabetic animals; this might be due to reduced level of antioxidant vitamins and enzymes. Diabetic nephropathy is the leading cause of end stage renal disease. It is characterized clinically by proteinuria and albuminuria and pathologically by glomerular hypertrophy, mesangial expansion and tubulointerstitial fibrosis; these are closely related to loss of kidney function. Administration of antioxidant has been reported to have potential beneficial effects in restoring the renal function. In addition Vitamin C also acts as a regulator of catabolism of cholesterol to bile acid in guinea pig and has been demonstrated to be an important factor in lipid metabolism. Most patients with diabetes are associated with lipid metabolism disorders Ness *et al* showed the beneficial effects of vitamin C on lipids in human. Several studies have shown reduced level of basal vitamin C in diabetic patients and also have suggested that oxidative stress is increased in diabetes. Forghani *et al* showed a significant decrease in serum LDL levels in patients supplemented with vitamin C. LDL particles are small in size and dense in type 2 diabetes and are susceptible to oxidation. These LDL particles are protected from oxidative attack by tocopherol which is a lipid soluble antioxidant. Vitamin C is essential for the regeneration of tocopherol and may prevent LDL oxidation. Diabetes is a chronic metabolic condition associated with increased morbidity and mortality. It is becoming an emerging epidemic affecting middle age people to elderly people. Sedentary life style and urbanization are the important contributing factors for the increasing prevalence of diabetes. Despite adequate treatment, complications are increasing and contribute to morbidity and mortality. Diabetes is being a social and economic burden to country. There are several screening procedures and control programs to diagnose diabetes at the earliest and to reduce the complications. There are three major components to the treatment of diabetes: diet, drugs like insulin and oral hypoglycemic agents and exercise. Pharmacotherapy is the mainstay of treatment for diabetes .Since many drugs are available in the market, they are not free from side effects. Compliance of patient is also affected due to these side effects. For better patient compliance, safety and tolerability there is a need for the research of new drug which will give good glycemic control and least side effect. In this study the commonly used antidiabetic drug metformin in combination with Vitamin C which is easily available and cheaper drug was evaluated for its antihyperglycemic effect in adult male albino rats. Treatment with Vitamin C 50mg/kg and 100mg/kg along with metformin showed a significant fall in blood sugar level and significant increase in insulin level. It shows good glycemic control.

The outcome of the study has been achieved. Further studies are required in future to evaluate the mechanism at molecular level for its antihyperglycemic effect in human.

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