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EVALUATION OF ANTIHYPERGLYCEMIC EFFECT OF METFORMIN AND VITAMIN C COMBINATION IN STREPTOZOTOCIN INDUCED DIABETIC RATS

Pharmacology

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ABSTRACT

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 intraperitoneal injection of streptozotocin. Comparison of blood sugar was done with the control group. The standard, test 1 and test 2 groups showed a significant control of blood sugar on day 3, 7, 14, 21, 28. There was a good glycemic control in test group 1 and 2 than standard. Test group 2 showed better control than test 1. 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. To summarize supplementation of vitamin C in diet as well as in the treatment schedule may help in improving glycemic control and in improving the lipid profile status in type 2 diabetes. The morbidity and mortality of diabetes due to vascular complications may be improved

KEYWORDS

diabetes , anti hyperglycemic effect , animal model , vitamin –c, metformin

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. The metabolic dysregulation and complications associated with DM are due to glucotoxicity, lipotoxicity, formation of Advanced Glycation End products (AGEs), protein kinase C and Hexosamine pathway products. All these comprehensively cause secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system. Diabetes mellitus is associated with both macrovascular (coronary artery disease, stroke and peripheral vascular disease) and microvascular (retinopathy, neuropathy and nephropathy) complications and leading to end organ damage. With an increasing incidence worldwide, Diabetes mellitus will be a leading cause of morbidity and mortality in the future.

The two broad categories of DM are designated as Type 1 and 2. Type 1 DM is the result of complete or near-total insulin deficiency. Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion and increased glucose production.The Global Burden of Disease Study 2013 identified diabetes mellitus (all forms) as the ninth major cause of reduced life expectancy. In 2010, it was estimated that diabetes mellitus caused 3.96 million deaths in adults aged 20–79 years during that year (6.8% of global mortality). This estimate was raised to 5.0 million deaths due to diabetes mellitus and its complications during 2015[4]. Globally, about 1 in 11 adults have diabetes mellitus (90% have type 2 diabetes mellitus (T2DM)), and Asia is the epicentre of this global T2DM epidemic.A national study estimated that India is having 69.1 million people with diabetes and is estimated to have second highest number of cases in the world in 2015.According to International Diabetes Federation estimates around 453 million people had diabetes in 2017 and is expected to rise to 642 million by 2045[5]. The epidemic of diabetes mellitus and its complications poses a major global health threat. 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 glycated haemoglobin (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.

METHODS & MATERIALS

The antihyperglycemic effect of metformin and Vitamin C combination was evaluated in adult male albino rats.The study was done after obtaining approval from InstitutionalAnimal 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

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 . Thefloor 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 GLUCOSE ESTIMATION

Blood glucose was measured by glucometer having glucose oxidize enzyme specific sticks. This is a very easy and reliable method to perform.

The blood sample was placed on the glucose strip which was kept in the glucometer.

The results will be read in the screen in 15 seconds.

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 attached to 1 ml syringe containing the drug to be given. The rat was held in the left hand 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. Inj streptozotocin 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 received.

Tab.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 1^{st}, 3^{rd}, 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively. Cardiac puncture was on 28^{th} day for all rats to measure the insulin level.

Estimation of blood glucose was done on 1^{st}, 7^{th}, 14^{th}, 21^{st} and 28^{th} day. Insulin level was measured on 1^{st} and 28^{th} day.The results are tabulated Statistical analysis was done by using ANOVA test to find out the level of significance between the groups.

Table No 1: Group Study Treatment

GROUP	STUDY	TREATMENT
I	CONTROL	Normal feed and distilled water
II	STANDARD	Normal feed and water +Tab.Metformin 200 mg/kg for 28 days
III	TEST -1	Normal feed and water +Tab.Metformin 200mg/kg and Tab.Vitamin C 50 mg/kg for 28 days
IV	TEST -2	Normal feed and water +Tab.Metformin200mg/kg and Tab.Vitamin C 100mg/kg for 28 days

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 1^{st} Day

Control Group

The mean blood sugar values of diabetic rats of control group were422 ± 22.11 on day 1, 413.83 ± 19.92 on day 3 , 412.33 ± 20.53 on day 7, 411 ± 17.37 on day 14, 409.83 ± 17.02 on day 21, 407.83 ± 15.74 on day 28.

Standard Group

The mean blood sugar values of diabetic rats of standard group were417.50 ± 71 on day 1 , 263.50 ± 22 on day 3 , 174.50 ± 14.3 on day 7 , 156.66 ± 11 on day 14 , 127.16 ± 16 on day 2 1 , 121.00 ± 20 on day 28.

Test Group 1(Metformin 200mg/kg and Vitamin C 50mg/kg)

The mean blood sugar values of diabetic rats of test group 1 were484.66 ± 94 on day 1 , 305.33 ± 36 on day 3 , 139.16 ± 7 on day 7 , 133.33 ± 7 on day 14, 114.50 ± 14 on day 21, 104.16 ± 14 on day 28.

One-way ANOVA (analysis of variance)

In this study four independent groups were compared, so ANOVA was used as the statistical test which is an extension of student t test (done for the comparison of two groups). One-way ANOVA was used as there was a significant difference ($p < 0.0001$) between the groups.

DISCUSSION :

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 intraperitoneal injection of streptozotocin.Comparison of blood sugar was done with the control group. The standard, test 1 and test 2 groups showed a significant control of blood sugar on day 3, 7, 14, 21, 28. There was a good glycemic control in test group 1 and 2 than standard. Test group 2 showed better control than test 1.

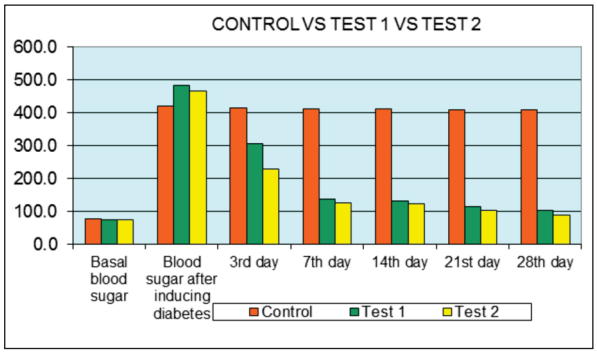

Figure No 1: Blood Sugar Estimation

CONCLUSION :

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.

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