Effect of Thiamine on Fasting Blood Glucose Level of Non-Diabetic and Diabetic Albino Rats

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Abstract: The worldwide prevalence of diabetes has risen dramatically over the past two decades from an estimated 30 million cases in 1985 to 382 million in 2013. The traditional anti diabetic drugs have several drawbacks in long term use. Thiamine is a member of the vitamin B family. Thiamine is necessary for normal insulin synthesis and secretion. So it may be beneficial in diabetic persons where insulin synthesis and secretion are disturbed. Albino rats used in this study was divided into four groups; six rats in each group. The groups were normal control, diabetic control, non-diabetic rats treated with thiamine and diabetic rats treated with thiamine. Diabetes was produced by intraperitoneal injection of Streptozotocin in the dose of 60 mg/kg. Fifteen minutes before streptozotocin administration nicotinamide was administered 120 mg/ kg intraperitoneally. Thiamine was given to the respective group for a period of 6 weeks. Fasting blood glucose was estimated at the end of every week. In non-diabetic rats thiamine showed no significant effect in lowering fasting blood sugar level. In diabetic albino rats thiamine normalized fasting blood glucose in six weeks. Thiamine does not affect the fasting blood glucose level of the normal rat but this effect was found significant in streptozotocin induced diabetic albino rats in six weeks period.

Keywords: Diabetes, Antidiabetic drugs, Thiamine, Insulin, Streptozotocin, Nicotinamide.

INTRODUCTION

Diabetes mellitus refers to a group of common metabolic disorders that share the phenotype of hyperglycaemia. The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs especially heart, kidneys, eyes and blood vessels [1]. At present, India is considered as the diabetic capital of the world by WHO. There are approximately 3.5 crore diabetics in India, and this figure is expected to increase up to 5.2 crore by 2025 [2]. The maximum cases of diabetes are of type 2 diabetes. The drugs available for treatment are sulfonylureas, biguanides, thiazolidinediones, GLP-1 analogs, gliptins, alfa glucosidase inhibitors, SGLT-2 inhibitors and amylin analogs. But there are several drawbacks linked with these drugs like tolerance with sulfonylureas, lactic acidosis with metformin, hepatotoxicity and oedema with thiazolidinediones [3].

Thiamine is a member of the B-vitamin family and was the first water-soluble vitamin to be discovered in 1912 and isolated in 1926[4]. Thiamine diphosphate is essential for carbohydrate metabolism as it is the co-factor of transketolase in pentose phosphate pathway and dehydrogenases in glycolysis and creb's cycle [4].

Thiamine is necessary for normal insulin synthesis and secretion [5]. In the thiamine-deficient state glucose undergoes metabolism via alternate pathways which can result in insulin resistance and complications [6, 7]. In 1987, Dr Saito from Japan found decreased plasma thiamine in patients with diabetes. Thornalley and his team in 2007 proved the glucose induced down regulation of thiamine transporter in proximal tubular epithelial cells leading to decreased renal uptake and excess urinary loss of thiamine [8]. This deficiency cannot be replenished by therapeutic low dose of thiamine as in lower dose the absorption of thiamine is dependent on these transporters which are already defective. This can be corrected by giving high dose of thiamine as in higher concentration thiamine can pass any biological membrane by passive diffusion [9]. In experimental and clinical diabetes high dose thiamine therapy not only corrected thiamine loss but also disposed excess glucose via pentose phosphate pathway and revert fasting hyperglycaemia [10]. High dose thiamine also lowered blood glucose in normal individuals after maximal aerobic exercise [11]. On this background the aims and objectives of this research work is to study the effect of thiamine on fasting blood

glucose of non-diabetic and streptozotocin-nicotinamide induced diabetic albino rats.

MATERIALS AND METHODS
Animals

Healthy male Wister rats weighing 150-250 g. were taken for the present study. The animals were kept in clean and dry cages with 12 h: 12 h light-dark cycle at room temperature and humidity. They were acclimatized to the available housing condition and were fed with standard laboratory diet consisting of soaked black gram (Kala Chana) and water was given *ad libitum*. Arrangements were made to ensure regular cleaning of cages and disposal of excreta and urine. The cages were floored with a layer of saw dust for absorption of urine of rats. The whole experiment was conducted in accordance with ethical norms approved by Institutional Animal Ethics Committee (IAEC) guidelines. The drug which was used in this experiment were thiamine hydrochloride 100 mg tablet (TIM 100), Gentech Healthcare Pvt Ltd; Streptozotocin 250 mg powder, Himedia, Mumbai and Nicotinamide 100 gm powder Animed, Kolkata.

Preparation of testing materials
Citrate buffer

Two separate solutions were made. The first solution was made by dissolving 1.921 gm of citric acid powder in 100 ml of deionized water and named solution 'A'. The second one was made by dissolving 2.94 gm of sodium citrate in 100 ml of deionized water and named solution B. To make the 0.1 M citrate buffer with desirable pH, the following parts of solution A and B were mixed to make a volume of 50 ml and finally adjusted to 100 ml by adding deionized water.

The ideal pH for stability of streptozotocin is 4-4.5, for this 31.5 ml of solution 'A' and 18.5 ml of solution 'B' were mixed together in a measuring cylinder and the final volume was adjusted upto 100 ml by adding deionized water. The pH of this solution was 4.2 [12].

Preparation of streptozotocin

Streptozotocin (250) mg used in this experiment was of HIMEDIA Company, Mumbai. During transportation it was stored in dry ice and after delivery it was stored at -20^0C. For induction of moderate type 2 diabetes streptozotocin was used in the dose of 60 mg/kg [13]. For that purpose 480 mg of streptozotocin was dissolved in 40 ml of 0.1 M citrate buffer (pH of 4.2). Now during diabetes induction dose was adjusted according to the body weight of the rat.

Gum acacia

1% Gum acacia suspension was prepared by mixing 1 gm of Gum acacia and small amount of distilled water in a mortar pestle and then making a final suspension in 100 ml distilled water .This was prepared every day just before the administration.

Thiamine

The 50 mg/kg dose [14] of thiamine was used for the study. 100 mg of thiamine was dissolved in 10 ml of gum acacia to have strength of 10 mg/ml. Now during feeding by gavage tube the dose of thiamine was adjusted according to the body weight of the rat.

Nicotinamide [13]

The dose of nicotinamide for this study was 120mg/kg. Thus 2400 mg of nicotinamide was dissolved in 100 ml of normal saline to have strength of 24 mg/ml. Now during diabetes induction the dose of nicotinamide was adjusted according to the body weight of the rat.

Study design

The experiment was carried out in "Department of Pharmacology and Therapeutics, Rajendra Institute of Medical Sciences, Ranchi". Fasting blood sugar before the initiation of study was within the range of 200- 250 mg/dl. Study animals were divided into four groups with six animals in each group. The groups were normal control, diabetic control, non-diabetic with thiamine, diabetic with thiamine. The rats were kept in four animal cages. All the cages were appropriately labelled. Animals in each cage were also labelled separately and colour coded with the help of permanent marker. Rats were given different treatment orally once daily for 42 days in the morning hour at 09:30-10:30 am.

Induction of diabetes mellitus

Diabetes was induced by freshly prepared single intraperitoneal injection of streptozotocin in the dose of 60 mg/kg. Just 15 minutes before the streptozotocin injection, nicotinamide was injected intraperitoneally in a dose of 120 mg/kg [13]. To administer streptozotocin and nicotinamide intraperitonealy, animal was held with its ventrum exposed and head pointed downward. This caused the freely movable abdominal organs to move towards the animal's diaphragm making accidental puncture of organs less likely. A 26 gauge needle with 1 ml syringe was inserted into the abdominal cavity in the lower right quadrant to avoid the caecum and urinary bladder. The needle was directed towards the animal's head at an angle of 15-20 degrees and inserted approximately 5 mm. Following insertion of needle the drug solutions were gently released. After intraperitoneal injections animals were allowed to drink 5% glucose solution overnight to overcome streptozotocin induced hypoglycaemia.

The fasting blood glucose level was determined after 72 hours of streptozotocin injection. The rats having blood glucose level in between 200-250

mg/dl were used for the study. The diabetic animals were allowed free access to tap water, normal laboratory diet, and were maintained at room temperature in their cages.

Though diabetes was confirmed after 72 hours of streptozotocin induction, thiamine was started on 8th day from induction in non-diabetic and diabetic groups and this 8th day was considered as day '0'. Then the treatment continued for 42 days. The blood samples were collected from all groups before induction of diabetes, after 72 hrs of diabetic induction as well as on day 0, 7, 14, 21, 28, 35, 42 day to determine the glucose level by glucose oxidase method.

Estimation of blood sugar [15]

For the estimation of fasting blood sugar, the rats were kept deprived of food overnight and were allowed free access to water. Blood samples were collected from the tail of rat, since it is the most venous part of body of rat. The tail of rat was cleaned with spirit cotton and then with the help of sterilized blade, it was cut 0.5mm just enough to allow one drop of blood to ooze out. The Glucometer was made ready before hand with the test strip attached to it. One drop of blood was allowed on the appropriate reaction zone of the strip. Within a few seconds, the level of fasting blood sugar appeared on the display that was noted down in the master chart. After taking the blood sample betadine ointment was applied to tail of each rat to prevent the infection.

STATISTICAL ANALYSIS

Data entry was done on MS Excel and "SPSS version 17" software was used for data analysis. One-way ANOVA test was used to compare the effect of the drugs on different groups. Tukey's honestly significant difference test was used for post hoc analysis of significant overall differences.

RESULTS

From first week to last week the mean fasting blood glucose value among non-diabetic group rats was stable. In diabetic control group the animals became more hyperglycaemic in subsequent weeks. In non-diabetic rats there was no significant change in blood sugar level.

There was gradual reduction of fasting blood glucose in group four (diabetic with thiamine group) throughout the study period and at the end of 6th week fasting blood glucose level became near normal. Changes in fasting blood glucose level in different weeks have been shown in table 1.

Table 1: Showing changes in FBS in all groups on 0, 7th, 14th, 21st, 28th, 35th & 42nd day. All the values are expressed in mean ± standard deviation

| Fasting Blood Glucose	NORMAL CONTROL (Group 1)	DIABETIC CONTROL (Group 2)	NON DIABETIC WITH THIAMINE (Group 3)	DIABETIC WITH THIAMINE (Group 4)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Day 0	82.00 ± 2.61	215.33 ± 8.48	84.33 ± 4.64	213.50 ± 7.99
Day 7	80.83 ± 4.02	227.00 ± 7.62	83.33 ± 4.37	192.33 ± 6.98
Day 14	80.33 ± 2.42	236.67 ± 7.23	82.50 ± 4.04	172.83 ± 4.26
Day 21	83.33 ± 4.89	249.83 ± 7.57	82.33 ± 2.25	154.83 ± 4.02
Day 28	83.33 ± 3.93	259.83 ± 7.89	82.33 ± 2.73	136.67 ± 4.50
Day 35	82.17 ± 2.79	272.33 ± 8.14	82.83 ± 2.99	110.33 ± 3.72
Day 42	80.83 ± 4.49	284.17 ± 7.08	83.00 ± 2.61	87.67 ± 2.25
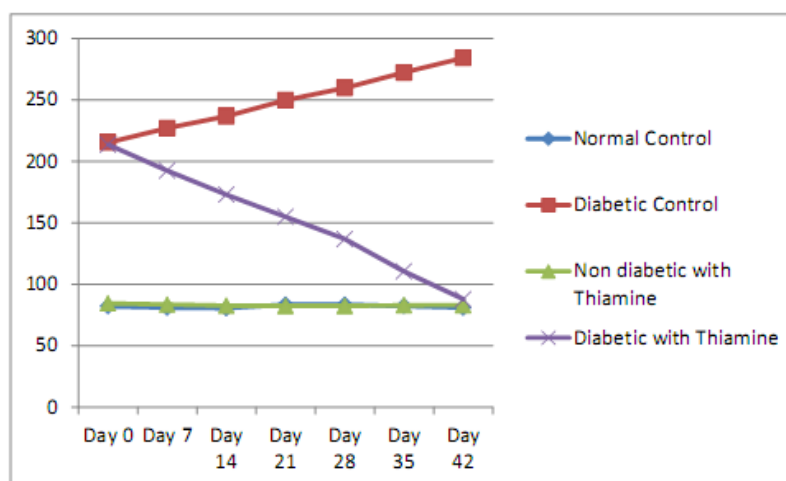
Fig-1: Showing changes in FBS in all groups on 0, 7th, 14th, 21st, 28th, 35th & 42nd day

DISCUSSION

In this study a novel model of type 2 diabetes was produced by streptozotocin and nicotinamide. Thiamine was administered in non-diabetic as well as diabetic group to see the effect on fasting blood glucose. The results are showing that thiamine has no effect on fasting blood glucose of non-diabetic albino rats. On the other hand the blood glucose level gradually decreased in the diabetic group and at the end of 6th week, there was no significant mean difference with the normal control group. It indicates that thiamine has antihyperglycemic effect. Accumulation of triosephosphates arising from high cytosolic glucose concentrations in hyperglycaemia is one likely or potential trigger for biochemical dysfunction implicated in signalling for polyol, hexosamine, protein kinase C and AGE pathway by the liver leading to insulin resistance and development of diabetic complications [6]. This may be prevented by disposal of excess triosephosphates via the reductive pentose phosphate pathway [7]. Thiamine is the co-factor of transketolase in pentose phosphate pathway and in high doses it increases the activity of this enzyme leading to disposal of excess cytosolic glucose [16]. The products of pentose phosphate pathway are not harmful to cell. On the other hand the decreased flux through hexosamine and protein kinase C pathway decreases glycosylation of glucose transporters. This may lead to enhanced insulin action and may decrease blood glucose [17]. Sadek KM *et al* [14] with his group showed that high dose thiamine (50mg/kg) had no effect on fasting blood glucose of non-diabetic albino rats but revert fasting hyperglycaemia of diabetic albino rats. Pandhiani *et al* [18] also showed that thiamine decreased post prandial blood glucose in diabetic rats but had no effect on non-diabetic albino rats. Gonzalez-Ortiz *et al* [19] showed that high dose thiamine therapy (600mg/day) for one month significantly decreased fasting blood glucose in diabetic individuals. So, these findings and our study show that thiamine in high doses does not cause hypoglycemia in non-diabetic group but decreases blood glucose in diabetic group.

CONCLUSION

Thiamine has significant glucose lowering effect on streptozotocin induced diabetic albino rats. It does not affect the blood glucose level in normal rats. Further study on its different aspects is necessary to focus it as a novel anti hyperglycaemic agent.

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