



Effect of Vitamin E on Glucose Levels in Diabetic Mice

Saadia Rasheed^{1*}, Nadia Rashid¹, Sobia Islam¹, Lubna Akhtar¹, Nausheen Jamshed²

¹Department of Anatomy, Foundation university medical college, Foundation University Islamabad, Pakistan,

²Department of Anatomy, Karachi Institute of Medical Sciences, Karachi, Pakistan

ABSTRACT

OBJECTIVE: To evaluate the blood glucose levels in diabetic mice treated with vitamin and those who were untreated.

METHODOLOGY: It was Experimental study. This study was conducted in the Anatomy Department of the Army Medical College, from November 2009 to November 2010 in cooperation with (NIH) National institute of Health, Islamabad. Three groups of thirty mature female BALB/C mice were randomly selected. Group A acted as the study control. Streptozotocin (STZ) was administered intraperitoneally to Group B to cause diabetes. STZ was administered intravenously to Group C, and they had a diet high in Vitamin E (alphatocopherol). All Group's Serum glucose levels were monitored using a glucometer at the beginning of the trial, after 72 hours following STZ injection, and then after 12 weeks.

RESULTS: There is a considerable change in blood glucose levels between the diabetic group (Group B) and diabetic group treated with Vitamin E (Group C) respectively. When compared to experimental Group B, Group C's blood glucose level dropped significantly.

CONCLUSION: Vitamin E treatment for diabetic mice may have significant impact on lowering blood glucose levels through the use of antioxidant therapy.

Key words: Diabetes Mellitus, vitamin E, Streptozocin, Alphatocopherol, Blood glucose

*For Correspondence

Dr. Saadia Rasheed

Professor of Anatomy, Department of Anatomy, Foundation Medical College, Foundation University Islamabad.

Email: saadia.rasheed@hotmail.com

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INTRODUCTION

Diabetes Mellitus is a known metabolic illness due to insulin storage or insulin resistance. It is characterized by elevated blood sugar concentration (Fasting plasma glucose > 125mg/dl or plasma glucose > 180mg/dl, 2hrs postprandial).¹ Due to rise in its incidence and the difficulties that are linked with it globally, its importance has increased. It necessitates ongoing medication therapy, diet modification, and exercise.² Approximately 463 million adults worldwide have diabetes, and 90% of these people suffer from type 2 diabetes mellitus.² According to an article by "The News", Pakistan ranks 3rd in the world in diabetes prevalence after China and India. The prevalence of diabetes in Pakistan in 2016, 2018, and 2019 was 11.77%, 16.98%, and 17.1%, respectively. According to the International Diabetes Federation, in 2022, 26.7% of adults in Pakistan are affected by diabetes making the total number of cases approximately 33,000,000. This number is alarmingly high and is also increasing with each passing year³ and had 43

million people with the disease, and by 2030, 13.8 million people will be affected, placing Pakistan fourth overall.⁴

Increased free radical production and oxidative stress are associated with the pathogenesis of diabetes mellitus and its late complications. They are thought to play a significant role. The plasma membrane's auto-oxidation process between sugar and unsaturated lipids boost the formation of free radicals and could be one of their origins. Compromises in the free radical inhibitory and scavenger systems are the cause of further increase in oxidative stress. Reactive oxygen species are believed to play a role in the development of secondary complications of diabetes include retinopathy, neuropathy, cardiomyopathy, and nephropathy, are produced as a result of hyperglycemia.⁵ Diabetic nephropathy is histologically characterized by tubulointerstitial fibrosis, mesangial enlargement, and glomerular hypertrophy. These findings are directly related to decrease in renal functions.⁶

A decline in oxidative stress is attainable with tight blood glucose management. Antioxidants reduces the oxidative stress

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and hence they act as anti-stress agents.⁷ Role of antioxidants are increasingly investigated now a days for opposing free radical damage. Animal tissue can be protected against oxidative damage such as lipid peroxidation by vitamin E, a naturally occurring antioxidant that is membrane bound and lipidsoluble.¹

METHODOLOGY

This study was carried out at two locations: the Army Medical College in Rawalpindi and National Institute of Health (NIH), Islamabad. 30 mature female BALB/C mice (weighing 25-40 g) were purchased from the NIH's animal house in Islamabad. Mice were fed on a palletted type of laboratory feed prepared at the animal house, water was available at all times, and they were housed in typical laboratory conditions. Three groups were made out of total animals, and in each group were present 10 animals.

Negative control group (Group A), for 12 weeks, was kept on a typical NIH diet. Group B (Positive control group) was given a single intraperitoneal injection of STZ at a dose of 55 mg/kg body weight per animal to induce diabetes to prepare the disease control. They were kept on a standard NIH diet for a period of 12 weeks. Group C was the last group and was given a single injection of STZ and was fed the standard NIH diet for 12 weeks while the diet was fortified with Vitamin E (500mg/kg of the diet). STZ (obtained from Bio-world Research Chemicals) was injected intraperitoneally into mice to cause diabetes (Fig 1). STZ was administered at a dose of 55 mg/kg body weight in 0.05mm/L citrate buffer (pH 4.5) to mice. Diabetes was defined as having a blood glucose level more than 250mg/dl in mice.

One touch blood glucose meter (Model no. AW 063-555-01B, USA) was used for measuring blood glucose level. Blood sampling was done by using the mouse tail as a venepuncture needle (Fig 2a). Serum glucose levels were measured at the beginning of the trial, after 72 hours (Fig. 2b) and then after 12 weeks of STZ injection. Mice are nocturnal creatures feeding at night times, so an overnight fast before checking blood sugar usually corresponds to a fast that last for about 24 hours. As a result, fasting should begin in the early morning, on the day of blood sample collection. In order to conduct the study, a standard NIH protocol was devised, that includes a fast from 6 a.m. to 12 p.m. Utilizing SPSS (Statistical Package for Social Sciences) Version 16, data were entered into the database. Using the paired student t test, the statistical significance of the difference in blood glucose levels between the experimental and control groups was ascertained. If the difference had a p-value of 0.05 or below, it was considered statistically significant.



Fig. 1: Photograph demonstrating intraperitoneal injection of STZ into mouse

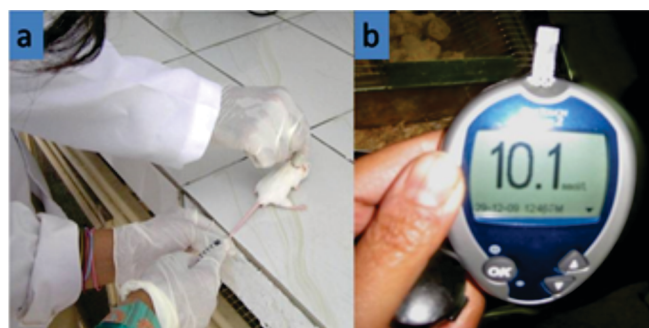


Fig. 2: Photograph demonstrating blood sampling and glucose measurement by using Glucometer

RESULTS

The mean fasting blood glucose level at the beginning of experiment, in Group A (Negative control group), Group B (Positive control group), and Group C (experimental) were 11.3 ± 0.648 , 11.59 ± 0.433 and 11.06 ± 0.864 respectively (Fig. 3). At the beginning of the trial, no discernible difference was there in the fasting blood glucose of these groups ($p > 0.05$) (Table 1). After 72 hours of the STZ injection, The fasting blood glucose for group A, B, and C were 11.45 ± 0.926 , 18.5 ± 4.249 and 16.7 ± 2.406 respectively after 72hrs of injecting the streptozotacin injection (Fig. 3). In the control groups A and B there was statistically significant variations between the mean blood glucose levels. However, there was no difference between the experimental groups B and C (Table 1). It was observed that blood levels of glucose (fasting) after 12 weeks of the beginning of the study, Group A, Group B, and Group C, the mean fasting blood glucose readings were 11.78 ± 0.898 , 21.17 ± 3.289 and 11.96 ± 0.766 respectively (Fig. 3). There was a statistically significant difference between Group A and Group B. Similarly, there was no statistically

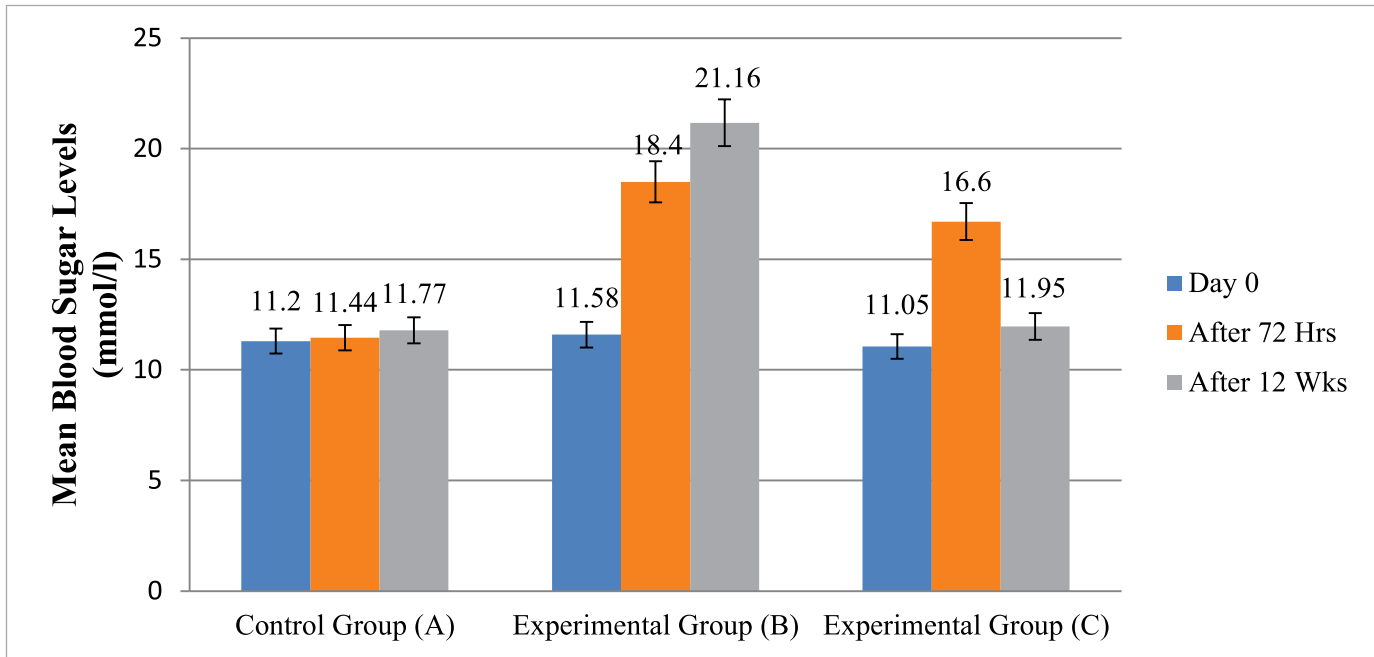


Fig. 3: A histogram comparing fasting blood sugar levels between the control and experimental group at various study stages

significant difference between the experimental groups B and C (Table 1).

	Group A (Control)	Group B (Experimental)	Group C (Experimental)	P value between group A & B	P value between group A & C	P value between group B & C
Day 0	11.3±0.65	11.59±0.4	11.06±0.86	0.320	0.399	0.121
72 Hrs after STZ induction	11.45±0.93	18.5±4.3	16.7±2.41	0.001*	0.000*	0.302
12 Wks. after STZ induction	11.78±0.9	21.17±3.3	11.96±0.77	0.000*	0.540	0.000*

Table 1: Blood Sugar Level values of animals during the experiment

*Statistical difference is significant, P value < 0.05 is statistically significant

DISCUSSION

In a recent experiment, vitamin E was utilized as a potent antioxidant and it reduced blood glucose levels of STZ treated mice. Tan GCJ et al.¹¹ studied the effects different vitamin E doses on kidney biochemical markers in the normal group and the Streptozotocin-induced diabetic rats group. It was noticed that creatinine levels and blood urea nitrogen (BUN) had somewhat decreased following vitamin E administration orally.

In the current experiment, it was found that experimental groups B and C had significantly different blood glucose levels.

After 12 weeks of testing, the significance of Blood Sugar fasting (BSF) in experimental group C was nearly equal to that of control group A. These findings show that vitamin E may play a substantial role in the prevention of hyperglycaemia. Additionally, vitamin E was said to lower plasma glucose levels and may help to moderate insulin activity.¹² According to the data and studies given, vitamin E may modify glucose metabolism. Previous research has demonstrated that oral vitamin E administration is associated with a reduction in oxidative stress, which is associated with secondary expansion of in the physiochemical integrity of the plasma membrane as measured by improving its micro-viscosity.¹³ This final phase would aid insulin in facilitating glucose passage through the membrane of skeletal muscle cells, which is linked to an increase in insulin mediated glucose excretion. This final phase would aid insulin in facilitating glucose passage through the membrane of skeletal muscle cells, which is linked to an increase in insulin-mediated glucose excretion. This result was in contrast to a 2-week-long trial done on rats. In the trial, the participants were split into control and diabetic rats (40 mg/kg body weight) with or without vitamin E. Despite the fact that diabetic rats' blood glucose levels were significantly higher than control rats' treatment with vitamin E had no effect on body weight, kidney weight, or blood glucose levels in either control or diabetic rats. Rats receiving STZ had their pancreatic beta-cells damaged, which prevented insulin secretion and raised plasma glucose levels. Additionally, it was suggested that improvements in glucose metabolism might be related to improved glycemic status. Supplementing with vitamin E may also increase the

production of insulin, increases its availability, and protects against cell destruction caused by lipid peroxidation thanks to vitamin E's antioxidant characteristics. Chronic hyperglycemia was first caused by the destruction of cells, but vitamin E may have prevented more damage through an antioxidant mechanism.¹⁴ The results of the current study are consistent with those of 2009 study that looked at the impact of antioxidants on blood glucose levels. Animals were treated with vitamin E (40 mg/kg/day i.p.) until the 35th day after STZ was used to make them diabetic. According to the findings, vitamin E supplementation significantly reduced the blood glucose increase caused by STZ.¹⁵ Thus, it is possible to hypothesize that vitamin E's antioxidant effects and reduction of oxidative stress in beta-cells are what cause the drop in blood glucose levels.¹⁶

In contrast to the parenteral approach employed in the aforementioned investigation, Vitamin E was administered orally in the current study. According to Wong et al., diabetic rats had increased levels of plasma glucose, glycosylated haemoglobin, and protein or lipid peroxidation. The protective benefits of alphatocopherol in the kidney were connected to its anti-inflammatory and antioxidant properties¹⁷, which supported our results. Additionally, several studies showed that diabetic mice receiving vitamin E supplements showed significantly increased glomerulosclerosis and tubule-interstitial lesions compared to untreated diabetic mice.¹⁸ Balbi et al. discovered in their research that proteins with type II diabetes mellitus who supplemented with specific vitamins, particularly vitamin E, saw significant improvements in their antioxidant status and glycemic control.¹⁹ Antioxidant groupings may be a useful strategy for the management of diabetic patients, according to a further study.²⁰ A three-month investigation on the effects of vitamin C and E supplements revealed that patient blood sugar levels fell while their levels of glutathione (GSH) and superoxide dismutase (SOD) rose.²¹

Limitation of study: Limitation of study was that the present study should have been conducted on large sample size and histological findings showing any damage on other organs should also have been included.

Small sample size and the histological findings in different organs due to increased blood glucose level were the major limitations in this study.

CONCLUSION

Overall, our findings show that vitamin E's antioxidant properties have the ability to reduce blood glucose levels. Therefore, diabetics should consume foods which are rich in Vitamin E i.e. sunflower seeds, Almonds, and breakfast cereals. Tomato sauce and wheat germ oil are also excellent source.

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CONFLICT OF INTEREST

Author declared no conflict of interest

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AUTHORS CONTRIBUTIONS

SR: Conception, Design of the work, Data collection, and Drafting, Reviewed, Final approval, Agreement to be accountable.

NR: Conception, Design of the work, Acquisition, Data Analysis, and Drafting, Reviewed, Final approval, Agreement to be accountable.

SI: Conception, Design of the work, Interpretation of data for the work, and Drafting, Reviewed, Final approval, Agreement to be accountable.

LA: Conception, Design of the work, Data collection, and Drafting, Reviewed, Final approval, Agreement to be accountable.

NJ: Conception, Design of the work, Data analysis, and Drafting, Reviewed, Final approval, Agreement to be accountable



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