Original Article

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MICROSCOPIC OBSERVATIONS OF PANCREATIC ISLETS FOR ANTI-DIABETIC EFFECT OF VITAMIN-E&C IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract

Oxidative stress has been suggested to play a key role in the pathogenesis of Diabetic mellitus. Vitamins E and C play important roles in the antioxidant defense system. The purpose of this study was to evaluate the effects of Vitamins E and C (VEC) on diabetic rat in prevention of Streptozotocin induced Type-1 diabetes. Adult albino rats were used in the study, the animals were divided into four groups (n = 6). Group-A: Control group; Group-B: Diabetic group with Streptozotocin (60 mg/kg); Group-C: VEC treatment for seven days followed by Streptozotocin administration and Group-D: Animals treated with Streptozotocin (60 mg/kg), The rats developed diabetes and are evidenced by sustained hyperglycemia and glycosuria after seven days, and then it was treated with VEC for seven days. The observation was made the analysis of blood glucose level and histological architecture of the pancreatic-islet cells. Fasting blood glucose was measured seven days after diabetic induction to determine the severity of blood glucose elevation. Microscopical analysis using Hematoxylin and Eosin (H&E) stain (magnificaionX40) of the VEC administered rats revealed remarkable normal pancreatic-islet cells, unlike the diabetic rats whose Pancreatic-islet cells were necrotic and little in number. VEC effects on serum glucose level was significantly different compared to diabetic control (5.53±0.40 vs. 18.64±3.92 mmol/L, $p<0.0001$), respectively. Through treatment with VEC for one week after induction did not have any significant effect, but has reduced the serum level by about 3 mmol compared before treatment (16.81 ±2.88 vs. 19.64±2.95 mmol/L, $p<0.96$), respectively. Taken together, the result suggests that Vitamin E & C protects against Streptozotocin induced Type-1 diabetes in rats.

Keywords: Streptozotocin, Diabetes, Antioxidant, Vitamin-E, Vitamin-C, Oxidative stress.

Introduction

Free radicals have been implicated in the pathogenesis for many degenerative diseases, including diabetes, atherosclerosis and cancer[1,2]. Diabetes mellitus is a disorder characterized by hyperglycemia.

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It is a heterogeneous primary disorder of carbohydrate metabolism, with varied aetiology culminating in absolute relative insulin deficiency or insulin resistance or both. There is a reservoir of basic information that suggests the involvement of oxidative stress in the pathogenesis of diabetes mellitus. It is now recognized that sustained hyperglycemia in diabetic patients, causes protein glycation and generates free radicals through auto-oxidation and polypol pathways[3,4]. High levels of free radicals with concurrent decline of antioxidant defence

mechanisms may lead to the damage of cellular organelles and enzymes[5]. This can culminate in increased lipid peroxidation and development of insulin resistance, which may consequently promote the development of complications of diabetes mellitus[6].

Antioxidant defence systems are also disturbed in diabetes mellitus[7]. Oxidative stress may cause oxidative damage of cellular membranes and changes in the structural and functional integrity of subcellular organelles and may produce effects that result in various complications in diabetic disease[2, 8, 9, 10].

Streptozotocin (STZ) is an alkylating agent antibiotic that experimentally produces diabetes due to β-cell death by the mechanism of DNA damage in rodent islets[11]. During STZ metabolism, various toxic intermediates are produced, including methyl cations (CH_3^-), methyl radicals ($CH_3^\cdot$), reactive oxygen species (ROS) and nitric oxide (NO)[12,13]. Beta cells are very susceptible to oxidative changes since they possess a low antioxidative capacity [14, 15, 16]. The aim of this study was to evaluate the effects of Vitamins E and C (VEC) on diabetic rat in prevention of Streptozotocin induced Type-1 diabetes.

Materials and Methods

Chemical Agents

Streptozotocin was purchased from Sigma-Aldrich Chemical Company. It was freshly dissolved in ice-cold 0.05 M citrate buffer (pH 4) and given i.p. in a dose of 60mg/kg body weight for 3 consecutive days for induction of diabetes[17, 18].

The Vitamins C and E–supplemented diabetic rats that were given the vitamins daily by intraperitoneal injection, 200 mg/kg vitamin C and 100 mg/kg vitamin E[19].

Animals

The experiments were performed on Albino rats (approx 200 - 250 g) obtained from Animal House, SRM University, Tamilnadu, India. All aspects of animal care complied with the ethical guidelines and technical requirements approved by the Institutional Animal Ethics Committee. Animals were housed individually in cages in an environmentally controlled animal facility (room temperature, 12 h light: 12 h dark cycle) with free access to a standard commercial diet and water ad libitum. The experiment was conducted for a period of five weeks. All animals were fed on normal diet for seven days of acclimatization. Diabetes was induced by an intraperitoneal (IP) injection of freshly prepared dissolved mixture of Streptozotocin with ice-cold 0.05 M citrate buffer (pH 4) and given in a dose of 60mg/kg body weight for three consecutive days.

Blood glucose levels were measured daily three days prior and seven days after Streptozotocin administration. Development of diabetes mellitus was proven by sustained hyperglycemia and glycosuria. Diabetic rats that had a fasting glucose greater than 200 mg/dL would be included in the study[20].

Experimental Design

The animals were randomly divided into four groups (n = 6) as follows:

Group-A: Control group with normal diet (Saline solution, i.p.);

Group-B: Diabetic group with Streptozotocin;

The rats developed diabetes after injection was evidenced by sustained hyperglycemia and glycosuria seven days after the induction;

Group-C: VEC supplemented diet for seven days followed by Streptozotocin administration; and

Group-D: Animals treated with Streptozotocin, the rats developed diabetes and are evidenced by sustained hyperglycemia and glycosuria after seven days, and then it was treated with VEC supplementation for seven days.

Methods of Analysis

Animals were starved for 16 h before blood collection. Fasting blood glucose was estimated by glucose oxidase method according manufacturer's procedure (Randox laboratories Ltd. Ardmore, United Kingdom). Urine was collected in cage urine separator bottle containing 1 mL of 10% thymol and glucose determined using Combosik (DFI Co. Ltd., Gimhae,

Gyuang-Nam, Korea). Histochemical analysis was done according to Bancroft and Stevens[21]. Sacrificing procedure, the rats were made unconscious with carbon (IV) chloride before sacrificed by euthanasia.

Statistical Analysis

Two-Sample Independent student t-test as well ANOVA used in the comparison of group means to determine the significance of the result.

Results

Table-1 shows the fasting blood glucose one week before and one week after induction of diabetic mellitus. Blood glucose level of VEC group was compared with diabetic control. The effect of VEC on glucose level in the serum showed significant difference compared to diabetic control ($p<0.0001$). Histochemical analysis suggested that VEC protected the integrity of pancreas tissue against Streptozotocin induced damage. Glucosuria increased significantly in diabetic animals compared with VEC treated group where there was no glucosuria. Though treatment with VEC one week after Streptozotocin induced diabetes did not have any significant effect compared with result before treatment with VEC (16.81 ±2.88 vs. 19.64±2.95 mmol/L, $p<0.96$, respectively), it however reduced the level of glucose by 3 mmol/L, suggesting that the normal level of serum glucose seem in VEC treated group before induction was achieved by the protection of the pancreatic tissue against Streptozotocin induced damage rather than reversing the effect by VEC (Table-1).

Table-1: Fasting blood sugar level (m mol/L)

GROUPS	BEFORE INDUCTION OF DIABETES	SEVEN DAYS AFTER INDUCTION OF DIABETES	SEVEN DAYS AFTER TREATMENT WITH VEC
A - Non -Diabetic control	5.12 ±0.29	5.62 ±0.22	-
B - Diabetic control	5.02 ±0.88	18.64 ±3.92[a]	-
C – VEC	5.05 ±0.79	5.53 ±0.40[b]	-
D – VEC after induction	4.99 ±1.02	19.64 ±2.95[c]	16.81 ±2.88

Result : Mean ± SD for n = 6, [a]p <0.0001 for comparing diabetic control and non diabetic control after induction of diabetes [b]p<0.001 for comparing diabetic control (Group-B) and VEC (Group-C) after Streptozotocin treatment, [c]p<0.0001 for comparing non-diabetic control and group (Group-D) before VEC supplementation.

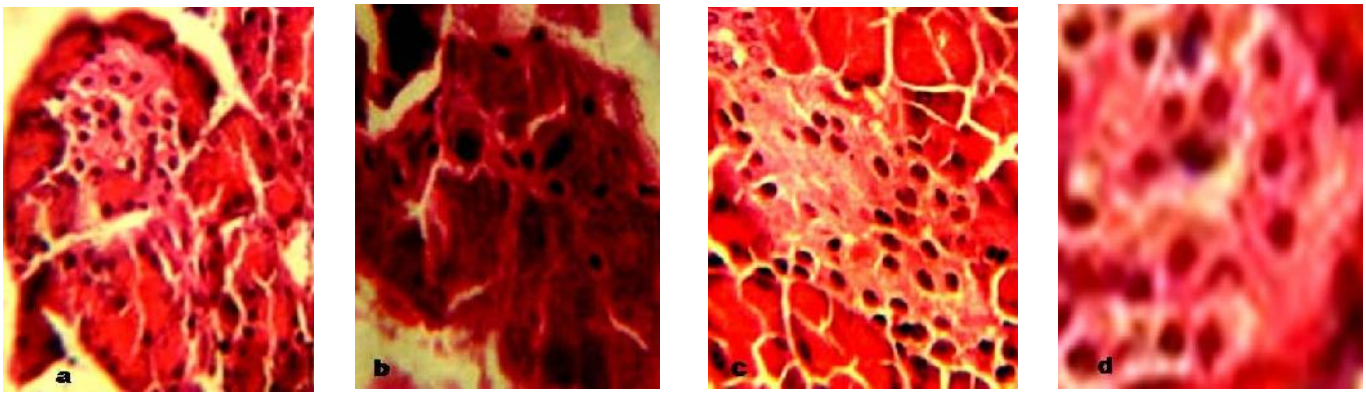

Fig. 1: Histochemical structure of Pancreatic Islet cells of albino rats stained with Hematoxylin & Eosin

(a) Normal control group (Group A), (b) Diabetic control group (Group B), (c) VEC treated group (Group C), (d) VEC after induction group (Group D).

Histochemical study of pancreatic-islet cells are shown in fig.1a-d. VEC groups (Fig. 1c-d) were compared to both controls (diabetic and normal control).The normal control group showed normal Pancreatic Islets architecture (Fig. 1a).The diabetic control Group B showed necrotic Islet cells, suggesting that the integrity of pancreatic tissue was compromised following diabetes induction (Fig. 1b).VEC group (Group-C) revealed normal and remarkable pancreatic islet cells, indicating that the integrity of pancreatic tissues was protected by VEC against Streptozotocin induced damage (Fig. 1c).VEC after induction group (Group-D) showed slight or no protective effect against the Streptozotocin (Fig. 1d).

Discussion

Antioxidants are frequently used for diabetes and its complications. Plasma Vitamin C and E concentrations are reduced in diabetes [22, 23, 24, 25, 26]. A positive relation has been demonstrated between high plasma Vitamin C level and reduction in complications of diabetes[27]. Vitamin C plays a central role in the antioxidant protective system, protecting all lipids undergoing oxidation and diminishing the number of apoptotic cells [28, 29, 30].

Vitamin C regenerates the oxidized Vitamin E [31]. Vitamin E, on the other hand, acts as a non-enzymatic antioxidant and reduces lipid peroxidation and glutathione [26, 32, 33]. Vitamin E is very effective in glycemic control, lowering the HbA1c levels [34] and preventing the hypertrophic effects of hyperglycemia [35]. However, this is in contrast to the results of some studies, which showed that vitamin E was not beneficial in glycemic control and lipid metabolism in diabetes [36]. Streptozotocin induced diabetes in addition to Vitamins C and E, it was observed that lipid peroxidation was significantly reduced, glutathione peroxidase (GSH-Px) was increased and reduced glutathione (GSH) level was decreased [37]. Since the understanding that the selective destruction by Streptozotocin of Pancreatic Islet cells is mediated via generation of oxidative stress [38], interest has been stimulated in the use of antioxidant to prevent chemically induced damage of pancreatic islet cells. Toxicity of Streptozotocin is elicited through its reduction by glutathione to dialuric acid, in which redox recycling process generates ROS that damages the Pancreatic Islet cells [39]. Furthermore, transition metals such as iron and copper, which are potentially involved in the generation of hydroxyl free radical, are also involved in Streptozotocin mediated killing of beta cells [39, 40, 41]. It has been known that vitamin E prevented Streptozotocin induced diabetes in rats[42]. This study suggested that Vitamin E & C consumption has beneficial effect and dose used in this study was effectively potent to inhibit the toxicity of Streptozotocin on Pancreatic islet cells.

Conclusion

In conclusion, this work provided insight as to the potential of Vitamin E & C dietary supplement -ation at human RDA in the prevention of chemically induced Diabetes mellitus. Vitamin E & C has been demonstrated to protect against chemically induced destruction of pancreatic islet cells. In this study, we were able to show that Vitamin E & C supplementation at daily recommended dose given to human can protect against Streptozotocin induced Type-1 diabetes.

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