



**MICROSCOPICAL OBSERVATION OF TESTES FOR
AMELIORATIVE EFFECTS OF VITAMIN - E & C IN
STREPTOZOTOCIN INDUCED DIABETIC RATS**

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Abstract

Oxidative stress induces many serious reproductive tissue damages in male and female gonads and thus poses a serious threat to reproductive health. Lipid peroxidation is an important biological consequence of oxidative cellular damage in patients with DM. Serum lipo-peroxidation products such as malondialdehyde (MDA) reflects oxidative stress. The purpose of this study is to evaluate the effects of Vitamins E and C (VEC) on diabetic rat in prevention of Streptozotocin induced damages on testis. The experiments were performed on twenty four (n = 8) male Albino rats (approx 200-250 g) obtained from Animal house, National Medical College, Birgunj, Parsa, Nepal. The twenty four male albino rats were divided into three groups (n = 8) as 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: (Diabetic + VEC) Albino rats were induced diabetes mellitus with Streptozotocin are evidenced by sustained hyperglycemia and glycosuria after seven days, and then it was treated with VEC supplementation for seven days. The experiment was conducted for a period of five weeks. After the experimental period the serum testosterone and tissue malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH) levels were measured, and testis tissue was examined histopathologically. Elevated malondialdehyde and reduced superoxide dismutase, glutathione, and serum testosterone levels were detected. Histopathological observation detected that marked changes in the testis tissue in all the groups. Our results provide additional evidence for the concept of increase in oxidative stress by diabetes mellitus causes free radical damage in testis tissue. An improvement in this damage can be achieved by vitamin E & C treatment.

Keywords: Diabetes mellitus, Vitamin E & C, Testosterone, Oxidative stress, Testes.

Introduction

Oxidative stress induces many serious reproductive tissue damages in male and female gonads and thus poses a serious threat to reproductive health. Research over recent years has greatly improved the understanding of the important roles that reactive oxygen species (ROS) and oxidative stress

play in mammalian reproduction. Diabetes mellitus (DM) is the most common metabolic disease. It is more likely that long-term, uncontrolled DM with sustained high blood glucose levels is the cause of glucose auto-oxidation with increased oxidative stress.¹ Over-production of reactive oxygen species

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(ROS) through the electron transport chain has been demonstrated in DM. Lipid peroxidation is an important biological consequence of oxidative cellular damage in patients with DM. Serum lipoperoxidation products such as malondialdehyde (MDA) reflect oxidative stress. The increase in ROS causes nonspecific modification of nucleic acids, proteins, and phospholipids leading to DNA, RNA, and protein damage and alterations in antioxidant enzyme levels. All these events result in cellular and tissue damage.

Tissue damage induced by free radicals is thought to be an important factor in the pathogenesis of DM and its complications.² Experimentally, streptozotocin (STZ) induces DM, probably through the generation of ROS, leading to islet cell destruction.³ In accordance with theories on the aging process put forward in recent years, it is well accepted that the balance between antioxidants and prooxidants is one of the important factors in aging. It was also well documented that, as a result of impairment in the mitochondrial respiratory chain, an organism comes under enhanced oxidative stress with age.⁴⁻⁶ The balance between oxidant and antioxidant species has been proposed to have an important role in retarding the aging process and preventing diabetic complications. Dietary antioxidants play a major role in the maintenance of the oxidative balance. Vitamin E, Vitamin C, and other micronutrients protect humans from several diseases, including aging and DM.⁷

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.^{8, 9} The Vitamins-E and C-supplemented diabetic rats that were given the vitamins daily by intraperitoneal injection, 100 mg/kg vitamin E and 200 mg/kg vitamin C.¹⁰

Animals

The experiments were performed on twenty four (n = 8) male Albino rats (approx 200-250 g) obtained from Animal house, National Medical College, Birgunj, Parsa, Nepal. 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.¹¹ No deaths occurred during the study in any of the groups.

Experimental Design

The twenty four male albino rats were divided into three groups (n = 8) 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: (Diabetic + VEC) Albino rats were induced diabetes mellitus with Streptozotocin are evidenced by sustained hyperglycemia and glycosuria after seven days, and then it was treated with VEC supplementation for seven days.

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). Diabetic rats that had a fasting glucose greater than 200 mg/dL would be included in the study.¹¹

Serum and Tissue Preparation

Sacrificing procedure; the rats were made unconscious with carbon (IV) chloride before sacrificed by euthanasia. The Testes of the rats

were removed, dissected free of surrounding connective tissue, and rinsed several times in ice-cold 0.9% NaCl then immediately frozen in liquid nitrogen and stored at -20°C until processed for malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD) assays. The dissected testis tissues were fixed with the 10% neutral buffered formalin for microscopical study.

The blood collected via cardiac puncture was allowed to clot, and the serum was obtained by centrifugation at 1500/g for 15 minutes. Aliquots of serum samples were stored at -20°C until analyzed.

Methods of Analysis

The tissues MDA levels were determined using the procedure of Ohkawa et al.¹² GSH levels were measured using the method of Moron et al.¹³ and SOD activity in tissues was determined using the method of Sun et al.¹⁴ Microscopical analysis was done according to Bancroft and Stevens.¹⁵ The serum testosterone levels in male rats were determined by IMMULITE total testosterone

commercial kit (solid-phase, competitive chemiluminescent enzyme immunoassay) with an Immulite One immunoassay analyzer (DPC Cirus Inc., Flanders, NJ).

Statistical Analysis

Statistical analysis of the values in the testis MDA, SOD, GSH, and serum testosterone levels of the groups were compared with each other by Mann-Whitney U-test with a Bonferroni correction for multiple comparisons. If necessary, the results were expressed as median (minimum –maximum) and mean \pm standard deviation. The correlation among total testosterone and MDA, SOD, GSH levels were evaluated by Spearsman's rank test.

Result

Biochemical Analysis

The serum total testosterone level of all the Groups rats are analysed, compared and presented in Table 1. Among the groups, there was statistically less significant difference in the total serum testosterone levels (Mann-Whitney U-test; $P = 0.002$)

Table No. 01: Comparison of Serum total testosterone level

	Group - A	Group - B	Group - C
Testosterone (ng/dL)	273 \pm 67	71 \pm 22 ^a	204 \pm 12

^aGroup - B compared with Group-A (Mann-Whitney U-test; $P = 0.002$)

The serum malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD) are analysed, compared and presented in Table 2. Among the groups, there was statistically less significant difference in the serum malondialdehyde (MDA) levels (Mann-Whitney U-

test; $P = 0.002$). But, there was a significant statistical difference in the serum reduced glutathione (GSH) levels (Mann-Whitney U-test; $P = 0.001$). The serum superoxide dismutase (SOD) levels are shows statistically not significant difference (Mann-Whitney U-test; $P = 0.001$) among the Group-B and Group-A.

Table No. 02: Comparison of Serum MDA, SOD and GSH levels

	Group - A	Group - B	Group - C
MDA (nmol/mg)	92 (58 - 106)	179 (133 - 267) ^a	97 (65 - 118)
SOD (U/g)	46.9 (37 - 76)	25.3 \pm 3.2 (17 - 37) ^b	37.5 (15 - 67)
GSH (nmol/g)	132 (120 - 153)	111 (103 - 119) ^c	132 (102 - 141)

^aGroup- B compared with Group-A (Mann-Whitney U-test; $P = 0.002$)

^bGroup- B compared with Group-A (Mann-Whitney U-test; $P = 0.001$)

^cGroup- B compared with Group-A (Mann-Whitney U-test; $P = 0.005$)

Microscopical Observation

Microscopical features of testis from control group (Group-A) (Figure: 1) shows normal seminiferous tubule lined with Stratified epithelium, composed

of two major cells (Supporting (sertoli) cells & Spermatogenic series of cells). The spermatozoa are arranged in rows between and around the cells of sertoli.

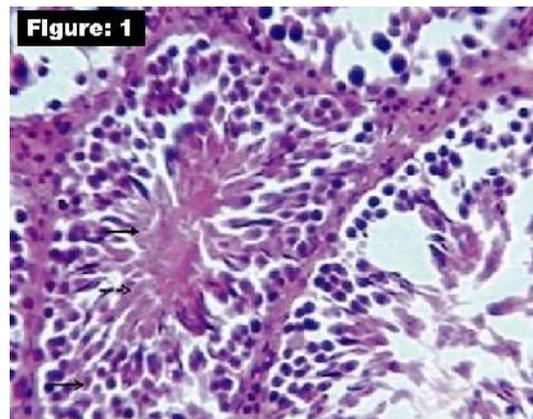


Fig. No. 01: Microscopical features of testis from control group (Group-A)

Microscopical features of testis from diabetic group (Group-B) (Figure: 2) shows degeneration of the

Spermatogenic cells, occlusion of the lumen and hypertrophied seminiferous tubules.

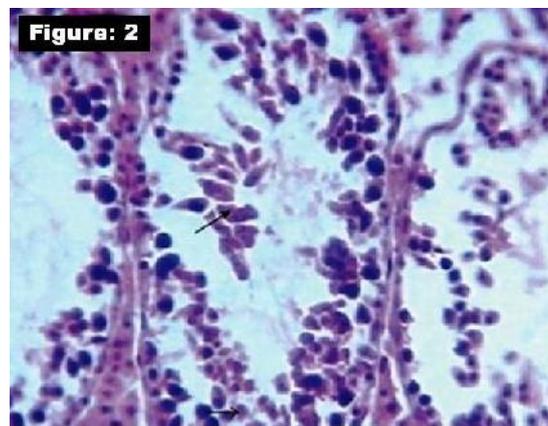


Fig. No. 02: Microscopical features of testis from diabetic group (Group-B)

Microscopical features of testis from diabetic + VEC group (Group-C) (Figure: 3) shows increase in the number of Spermatogenic series cells also

showing matured spermatozoa in the seminiferous tubules.

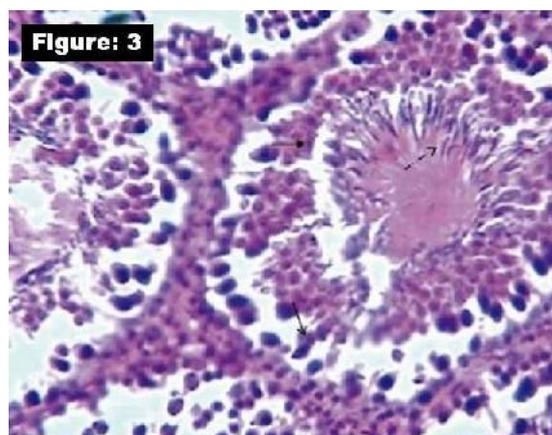


Fig. No. 03: Microscopical features of testis from diabetic + VEC group (Group-C)

Discussion

Our study demonstrated that DM caused oxidative imbalance resulting in an impairment of the

steroidogenic function of the testis. The results also suggest that Vitamin E & C prevents the free radical damage in DM resulting in an improvement

in the steroidogenic function of the testis. The function and life cycle of the cells can be impaired by ROS. Living organisms possess antioxidant defense systems against ROS. These defence systems include endogenous antioxidants, which can be classified as enzymatic (SOD, GSH) and non-enzymatic (vitamin E, uric acid, bilirubin) defense systems. In the reproductive system, there is a balance among ROS production and antioxidant defense systems. This balance minimizes the ROS levels, which is required to maintain the regulation of normal sperm function.¹⁶ Increased ROS production or a decreased antioxidant defense system is associated with lipid peroxidation in the sperm plasma membrane.¹⁷ Studies have detected increased semen ROS levels in 25% to 40% of infertile men.^{18, 19, 20}

In our study, the antioxidant enzymes MDA and SOD levels are not statistically significant in between Diabetic (Group-B) and Control group (Group-A). The results of this study reveal microscopical changes in the testis tissues in all of the groups. However, in diabetic groups the elevated testis MDA levels correlates with reduced serum testosterone levels; after antioxidant administration, the serum testosterone levels increased to control levels. These observations can be attributed to a relationship between steroidogenesis and ROS in testis of diabetic rat. Several previous studies support our results.^{21, 22}

Conclusion

Our results provide additional evidence for the concept of increase in oxidative stress by diabetes mellitus causes free radical damage in testis tissue. An improvement in this damage can be achieved by vitamin E & C treatment. However, the increased oxidative stress in DM possibly inhibits steroidogenesis with marked changes in the microscopical features.

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