

L-Norvaline and Alpha-tocopherol Treatment Protect Against Diabetes-Induced Oxidative Stress in Testes of Male Rats

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Abstract

The present study aimed to evaluate the effect of L-Norvaline and alpha-tocopherol in protection and combating the oxidative stress of testes in hyperglycemic male rats. In our study single dose of streptozotocin was used for induction of diabetes and fasting serum glucose level was checked and those animals showing FSG above 250 mg/dL were selected. The diabetic animals were randomly divided into seven groups comprising six animals in each group and one group of normal non diabetic animals received the vehicle 0.1 M citrate buffer pH 4.5. L-Norvaline was administered at higher (10 mg/kg) and lower (8 mg/kg) dose intraperitoneally to the different groups of diabetic animals along with alpha-tocopherol (900 mg/kg) orally for a period of 30 days. Sildenafil at an oral dose of 5 mg/kg was used as reference drug. After that period, testis was isolated and homogenized. The homogenized tissue was centrifuged, separating the supernatant and preserved it for assessing the oxidative stress parameters. The level of various antioxidant enzymes were assessed such as superoxide dismutase, reduced glutathione, glutathione peroxidase and glutathione reductase. Assessment of lipid peroxidation was also done in treated groups. The statistical analyses were carried out using Graph Pad Prism 5.0 software. All values were presented as Mean ± S.E.M. The level of superoxide dismutase, reduced glutathione, glutathione peroxidase and glutathione reductase were highly diminished in the diabetic rats compared with normal group. L-Norvaline alone showed no significant improvement in those enzymes parameters except in reduced glutathione ($P < 0.05$). L-Norvaline both at higher dose and lower dose along with alpha-tocopherol showed significant improvement in the level of antioxidant enzymes as compared with diabetic group ($P < 0.05$, $P < 0.01$, $P < 0.001$). Treated groups also showed a significant decrease in the level of malondialdehyde as compared to diabetic group ($P < 0.01$, $P < 0.001$). Standard drug, Sildenafil showed no improvement in any parameters after 30 days. The present can be concluded by establishing the fact that L-Norvaline and Alpha-tocopherol could be a good combination in scavenging free radicals in testes due to streptozotocin induced hyperglycemia in male Wistar rats.

Keywords: Alpha-tocopherol; Glutathione peroxidase; Glutathione reductase; L-Norvaline; Malondialdehyde; Superoxide dismutase

Introduction

Diabetes Mellitus is a metabolic degenerative disorder with alterations in carbohydrate metabolism and insulin homeostasis. Majorly there are two types of diabetes: Type I occur due to autoimmune destruction of pancreatic β cells and Type II occurs due to insulin resistance or desensitization of gluco-receptors [1]. Among various factors, The Massachusetts Male Aging Study considered diabetes and aging as the most prominent initiator of erectile dysfunction [2]. Presently the incidence of erectile dysfunction is increased by three folds in diabetic male populations rather than in normal population [3]. About 35% to 78% of diabetic males suffer from sexual dysfunction depending upon the status of metabolic condition and age [4]. Diabetic patients with improper glycemic status, chronically affects the testicular architecture and penile smooth muscle due to disruption in endothelial architecture and formation of excessive free radicals [5]. The net effect is the sexual impairment. The conflict arises in the fact that which type of diabetes is actually responsible for this testicular impairment. Moreover the loss of control

over nervous and endocrine function affects the steroidogenesis and penile erection in males.

However, the mechanisms related to altered endothelium-dependent vascular relaxation in diabetes mellitus was proposed to be multifactorial which seems to be dependent on the lifespan of hyperglycaemic state and vascular bed. Indeed, enhancement in ROS sensitivity of the contractile elements to Ca^{2+} [6] and facilitate mobilization of cytosolic Ca^{2+} in the vascular smooth muscles. The excess generation of oxygen in diabetic vessels has been contributed to increased activity of several oxygen generating enzymes including NOSs. Indeed, it is suggested that NOSs are capable to generate O_2^- in Ca^{2+} dependent manner particularly in case of absence of substrate, L-arginine and cofactor H4B, both of which being associated with the diabetes [7]. Generally, while the endothelial and vascular oxidases appear to be active constantly generating low levels of ROS and using NADH as a cofactor, phagocytic oxidase activated in response to stimulation, generating high levels of ROS and preferentially uses of NADPH as a cofactor (so the term NAD(P)H oxidase/ NADH/ NADPH oxidase) [8]. Vascular smooth muscle cell-related reactive oxygen species also derived mainly from an NAD(P)H oxidase [9]. NAD(P)H oxidase is a major source of O_2^- in the endothelial as well as in the vascular smooth muscle cells [10].

Free radicals generate in diabetes by non-enzymatic glycosylation of proteins, gluco-oxidation and enhanced malondialdehyde formation (lipid peroxidation). Combinely they impair vascular machinery and increased insulin resistance. Moreover activation of polyol pathway diminishes the production of cellular protective NADPH which may involve in the production of glutathione. Lipid is also the apolipoprotein component of LDL that forms insoluble complexes oxidatively due to the hydroxyl radical- induced cross-linkage in apo-B monomers that is also responsible for oxidative injury in diabetic complications [11]. Mammalian sperm cells composed of a specific lipid component especially with a high content of polyunsaturated fatty acids, cholesterol, sphingomyelins and plasmalogens. Hence the presence of lipids in spermatozoa will be the main substrates for lipid peroxidation.

L Norvaline, also known as 5-[(aminoiminomethyl)amino]- (mol formula- C₅H₁₁NO₂, mol Weight-117.15) is an amino acid and constitutional isomer with valine. This amino acid is often made synthetically. It is a white crystalline powder with melting point 303°C to 307°C and well soluble in water (10.5 g/100 mL), very soluble in hot water and dilute hydrochloric acid. De and his co-workers had established the fact that L-Norvaline is a well established arginase inhibitor when administered in diabetic male rats [12].

Moreover, alpha-tocopherol acts as a peroxy radical scavenger, preventing the propagation of the free radicals in tissues, by reacting with them to form tocopheryl radical, that will then be reduced by hydrogen donor. Since an enzymatic activity regulator, for instance, protein kinase C (PKC), which plays a role in smooth muscle growth, can be inhibited by α -tocopherol. α -Tocopherol has a stimulatory effect on the dephosphorylation enzyme, protein phosphatase 2A, which in turn, cleaves phosphate groups from PKC, leading to its deactivation, bringing the smooth muscle growth to a halt [11].

Our present study aimed to find out the role of L-Norvaline alone and its combination with alpha-tocopherol in combating oxidative stress and improving the testicular status in streptozotocin induced diabetic male rats.

Methodology

Animals

Adult twelve-week-old inbred male albino rats of Wistar strain (body weights ranging 225-250 g) were used for the present study. The animals were housed singly in standard polypropylene cages and maintained under controlled room temperature (24-28°C) and relative humidity (60-70%) with 12:12 hour light and dark cycle.

All the animals were provided with commercially available rat normal pellet diet and de-mineralized drinking water ad libitum throughout the study. All the animals were allowed to acclimatize for a week with the laboratory environment before the beginning of the experiment.

The guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) of the Govt. of India were strictly followed and prior permission was granted from the Institutional Animal Ethics Committee (Reg. No. IAEC/273/CPCSEA/SBS/01/2013-2014) for conducting the experimental studies [12].

Reagents

L-Norvaline, Apha-tocopherol, Streptozotocin, Superoxide dismutase standard, Epinephrine and Reduced glutathione standard were supplied from Sigma-Aldrich. Sodium Carbonate, Sodium biocarbonate, EDTA, Trichloroacetic Acid (TCA), Sodium Citrate, Sodium hydroxide, Potassium dihydrogen orthophosphate, 5,5 dithiobis 2- nitrobenzoic acid and Thiobarbituric Acid (TBA) were provided from HiMedia (India).

Induction of Diabetes Mellitus

Diabetes Mellitus was induced in 16 hour fasted adult male rats by intraperitoneal (i.p) injection of single dose of streptozotocin (65 mg/kg) in 0.1 M Citrate buffer pH 4.5 [13]. The streptozotocin induced rats were given 5% glucose solution on the next day and continued for 24 hour to protect animals from initial hypoglycemic mortality. After 72 hour blood was withdrawn from the animals. Fasting serum glucose (FSG) level was checked by glucose oxidase-peroxidase method using Seimen Diagnostic Kits. Animals showing FSG level above 250 mg/dL were considered as diabetic and included for the study [12].

Treatment groups

The diabetic animals were randomly divided into seven groups comprising six animals in each group and one group of normal non diabetic animals received the vehicle 0.1 M citrate buffer pH 4.5. L-Norvaline at a dose of 10 mg/kg and 8 mg/kg was administered i.p to the diabetic animals for a period of 30 days at different dose levels. Alpha tocopherol at a dose of 900 mg/kg was administered orally as an adjuvant to the L-Norvaline treated groups. Oral dose of sildenafil citrate suspension at a dose of 5 mg/kg was used as reference drug respectively [14].

Isolation of testes

After 30 days lower abdominal part was dissected and each testis was isolated from the sacrificed animals, homogenized in Tris buffer pH 7.5 in homogenizer at a speed of 2500 rpm. The homogenate was centrifuged in the centrifuge machine (Remi- motors Ltd., Mumbai) and the supernatant was taken and evaluated the following oxidative stress parameters.

Analysis of superoxide dismutase (SOD)

SOD was analyzed spectrophotometrically using the supernatant of testicular tissue [15]. In this method, SOD has the ability to inhibit auto-oxidation of epinephrine to adrenochrome at pH 10.2. This inhibition can be measured colorimetrically at 480 nm. 0.5 ml of homogenate was diluted with 0.5 mL of distilled water. All reagents should be in cold condition. The mixture was incubated with 0.25 mL ethanol and 0.15 mL chloroform. Then it was shaken and centrifuged at 2000 rpm for 10 min. The supernatant was separated and Epinephrine was added just before taking OD. The initial absorbance at zero minute was noted and taken for 3 min with 30 sec interval at 480 nm using spectrophotometer. Its activity was expresses as EU/dL.

Analysis of Reduced Glutathione

Reduced glutathione was analyzed spectrophotometrically using the supernatant of testicular tissue [16]. DTNB, a disulphide compound, was readily attacked, by the sulphhydryl group and forms a yellow colored anion which was measured colorimetrically at 412 nm. Tissue

supernatant will be mixed with TCA and centrifuged. The supernatant will be mixed with DTNB and phosphate buffer and estimated at 412 nm. Its activity was expressed as μg of GSH/mg of tissue.

Analysis of Glutathione peroxidase

Glutathione peroxidase (G-Px) activity was determined spectrophotometrically [17]. In this method, GPx catalyses the oxidation of glutathione in the presence of tert-butyl hydroperoxide (t-BHP). Oxidized glutathione is converted to the reduced form in the presence of glutathione reductase and NADPH, while NADPH is oxidized to NADP^+ . Testicular supernatant (30 mL) was incubated for 5 min, in the dark, with 100 mL of phosphate buffer (containing 0.25 mM KH_2PO_4 , 0.25 mM K_2HPO_4 and 500 mM EDTA, pH 7), 100 mL GSH (freshly made and protected from light), 100 mL glutathione reductase (1 unit), and 460 mL H_2O . The quantification occurred after the addition of 100 mL of NADPH (2.5 mM) and 100 mL of t-BHP, at 30°C with continuous magnetic stirring. The measurements were made against blanks prepared in the absence of NADPH. Glutathione peroxidase activity was calculated by the absorbance change per minute of NADPH at 340 nm, using a molar extinction coefficient of, $6220 \text{ cm}^2 \text{ mol}^{-1}$, and its activity was expressed as nmol NADPH/min/mg protein.

Analysis of Glutathione reductase

Glutathione reductase (G-Red) activity was determined according to a modified method [18]. G-Red catalyses the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) in the presence of NADPH. The testicular supernatant (100 mL) was incubated with 500 mL of phosphate buffer (containing 0.2 M of K_2HPO_4 and 2 mM EDTA, pH 7.0) supplemented with 50 mL of 2 mM NADPH and 350 mL H_2O . The measurements were initiated with the addition of 50 mL GSSG (20 mM), for 400 s, at 30°C with continuous magnetic stirring, against blanks prepared in absence of GSSG. Glutathione reductase activity was calculated by the absorbance change per minute of

NADPH at 340 nm, using a molar extinction coefficient of $6220 \text{ cm}^2 \text{ mol}^{-1}$, and its activity was expressed as nmol NADPH/min/mg protein.

Analysis of lipid peroxidation

Lipid peroxidation or level of malondialdehyde (MDA) was determined spectrophotometrically. The method estimates Malondialdehyde, a product of lipid peroxidation process. One molecule of MDA reacts with two molecules of TBA under mildly acidic condition to form a pink colored chromogen, whose intensity is measured colorimetrically at 535 nm. The testicular supernatant was incubated with 2 mL TCA. The mixture was cooled for 15 min and centrifuged separating the supernatant. The supernatant (2 mL) was then incubated with TBA by keeping it in boiling water for 10 min and cooled before measurements were done. Extent of lipid peroxidation was assessed by reading the absorbance of the test against blank at 535 nm using spectrophotometer.

Statistical analysis

The statistical analyses were carried out using Graph Pad Prism 5.0 software. All values were presented as Mean \pm S.E.M. Comparison between two groups performed using Student t-test. Multiple comparisons between different groups was performed using Analysis of Variance (ANOVA) followed by Dunetts Multiple comparison Test. Difference level at $P < 0.05$ was considered as statistically significant [12].

Results

Effect of L-Norvaline and Alpha-tocopherol on the level of SOD

As indicated in Table 1 and Figure 1, the level of superoxide dismutase (SOD) was highly decreased in the diabetic rats.

Group	Treatment	Superoxide dismutase (EU/dL)
I	Normal Control (0.1 M Citrate buffer pH 4.5)	24.28 \pm 0.90
II	Untreated Diabetic (STZ 65 mg/kg i.p)	12.68 \pm 0.38
III	L-Norvaline alone (10 mg/kg i.p)	14.99 \pm 1.52
IV	L-Norvaline (10 mg/kg i.p) and Alpha-tocopherol (900 mg/kg p.o)	19.48 \pm 1.48 ^c
V	L-Norvaline (8 mg/kg i.p) and Alpha-tocopherol (900 mg/kg p.o)	17.81 \pm 0.42 ^b
VI	Alpha-tocopherol (900 mg/kg p.o) alone	16.70 \pm 0.92 ^b
VII	Sildenafil (5 mg/kg p.o)	11.61 \pm 1.32

The statistical significance of difference between means was calculated by ANOVA followed by t-test for unpaired comparison. N=6
Values are expressed as Mean \pm S.E.M, b $P < 0.01$, c $P < 0.001$.

Table 1: Effect of L-Norvaline and Alpha-tocopherol on the level of superoxide dismutase.

SOD level was significantly increased ($P < 0.01$) in the diabetic rats treated with combination of L-Norvaline at lower dose along with alpha-tocopherol and alpha-tocopherol alone as compared to diabetic animals.

Diabetic rats treated with combination of L-Norvaline at higher dose along with alpha-tocopherol showed more significant increase ($P < 0.001$) in SOD level than L-Norvaline at lower dose along with alpha-tocopherol. Diabetic animals treated with L-Norvaline alone and standard drug Sildenafil showed no significant increase in SOD level as compared with diabetic animals.

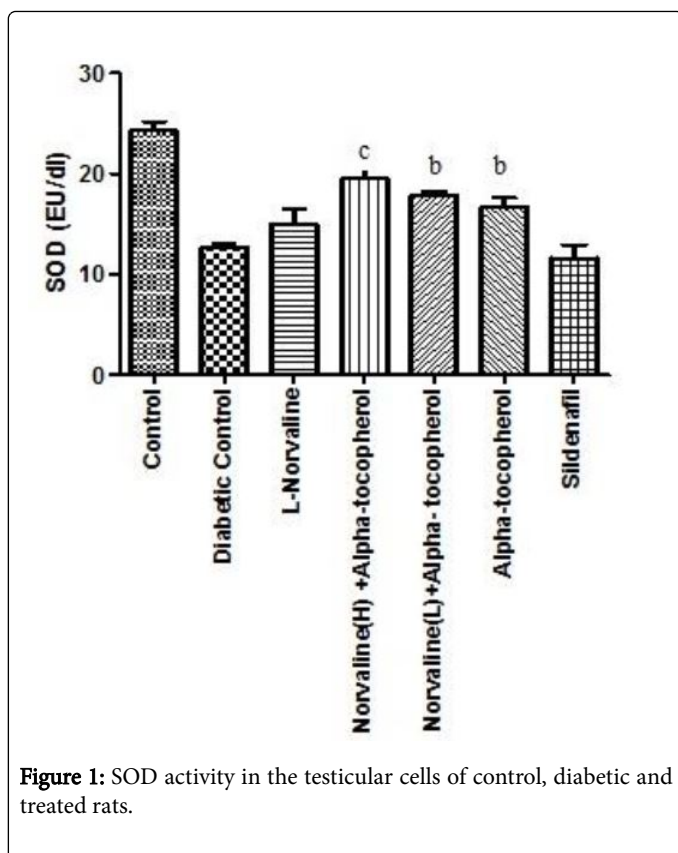


Figure 1: SOD activity in the testicular cells of control, diabetic and treated rats.

Effect of L-Norvaline and Alpha-tocopherol on the level of Reduced Glutathione

As indicated in Table 2 and Figure 2, administration of streptozotocin caused significant decrease in the level of glutathione in diabetic rats.

Diabetic rats treated with L-Norvaline alone and Alpha-tocopherol alone showed significant increase ($P < 0.05$, $P < 0.01$) in the glutathione level as compared to diabetic rats.

However, glutathione level was more significantly increased ($P < 0.001$) in the diabetic rats treated with L-Norvaline both at higher and lower dose along its combination with Alpha-tocopherol as compared to diabetic rats.

Diabetic rats treated with the standard drug Sildenafil did not produce significant rise in the level of glutathione as compared to diabetic rats.

Effect of L-Norvaline and Alpha-tocopherol on the level of Glutathione Peroxidase

As indicated in Table 2, administration of streptozotocin caused significant decrease in the level of glutathione peroxidase in diabetic rats.

Diabetic rats treated with L-Norvaline alone and Alpha-tocopherol alone showed significant increase ($P < 0.01$) in the glutathione peroxidase level as compared to diabetic rats.

However, glutathione peroxidase level was significantly increased ($P < 0.05$, $P < 0.01$) in the diabetic rats treated with L-Norvaline both at higher and lower dose along its combination with Alpha-tocopherol as compared to diabetic rats.

Diabetic rats treated with the standard drug Sildenafil did not produce significant rise in the level of glutathione peroxidase as compared to diabetic rats.

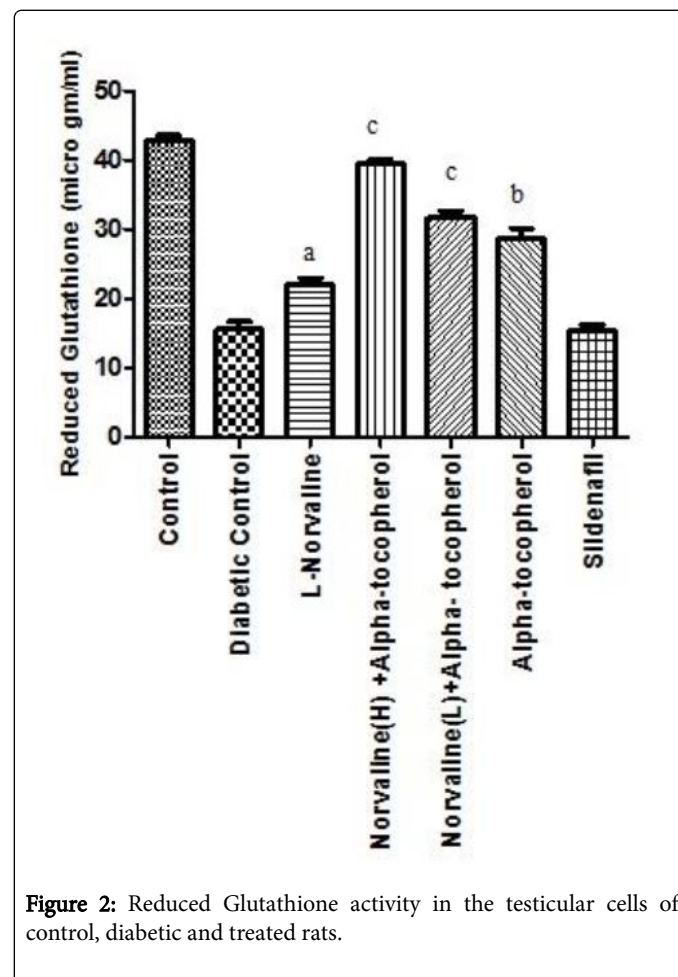


Figure 2: Reduced Glutathione activity in the testicular cells of control, diabetic and treated rats.

Effect of L-Norvaline and Alpha-tocopherol on the level of Glutathione Reductase

As indicated in Table 2, administration of streptozotocin caused significant decrease in the level of glutathione reductase in diabetic rats.

Diabetic rats treated with L-Norvaline alone and Alpha-tocopherol alone showed significant increase ($P < 0.05$) in the glutathione reductase level as compared to diabetic rats.

However, glutathione reductase level was significantly increased ($P < 0.01$) in the diabetic rats treated with L-Norvaline both at higher and lower dose along its combination with Alpha-tocopherol as compared to diabetic rats.

Diabetic rats treated with the standard drug Sildenafil did not produce significant rise in the level of glutathione peroxidase as compared to diabetic rats.

Group	Treatment	Reduced Glutathione (µg/mL)	Glutathione Peroxidase (nmol NADPH/min/mg protein)	Glutathione Reductase (nmol NADPH/min/mg protein)
I	Normal Control (0.1 M Citrate buffer pH 4.5)	42.85 ± 0.77	0.038 ± 0.015	20.02 ± 0.056
II	Untreated Diabetic (STZ 65 mg/kg i.p)	15.56 ± 1.12	0.015 ± 0.027	11.5 ± 0.067
III	L-Norvaline alone (10 mg/kg i.p)	22.11 ± 0.91 ^a	0.018 ± 0.036	13.72 ± 0.036
IV	L-Norvaline (10 mg/kg i.p) and Alpha-tocopherol (900 mg/kg p.o)	39.54 ± 0.63 ^c	0.029 ± 0.016 ^b	17.76 ± 0.027 ^b
V	L-Norvaline (8 mg/kg i.p) and Alpha-tocopherol (900 mg/kg p.o)	31.76 ± 0.92 ^c	0.025 ± 0.049 ^a	16.36 ± 0.016 ^b
VI	Alpha-tocopherol (900 mg/kg p.o) alone	28.73 ± 1.4b	0.027 ± 0.026b	16.90 ± 0.075 ^b
VII	Sildenafil (5 mg/kg p.o)	15.30 ± 0.93	0.015 ± 0.032	11.9 ± 0.029

The statistical significance of difference between means was calculated by ANOVA followed by t-test for unpaired comparison. N=6
 Values are expressed as Mean ± S.E.M, a P< 0.05, b P< 0.01, c P< 0.001.

Table 2: Effect of L-Norvaline and Alpha-tocopherol on the level of Reduced Glutathione, Glutathione Peroxidase and Glutathione Reductase.

Effect of L-Norvaline and Alpha-tocopherol on the level of malondialdehyde or lipid peroxidation

As indicated in Table 3 and Figure 3, malondialdehyde level was highly increased in the diabetic rats. Diabetic rats treated with Alpha-tocopherol alone showed significant decrease (P<0.01) in the malondialdehyde level as compared to diabetic rats. However,

malondialdehyde level was more significantly decreased (P<0.001) in the diabetic rats treated with L-Norvaline both at higher and lower dose along its combination with Alpha-tocopherol as compared to diabetic rats. Diabetic rats treated with L-Norvaline alone and the standard drug Sildenafil did not produce significant reduction in the level of malondialdehyde as compared to diabetic rats.

Group	Treatment	Malondialdehyde (nmol/L)
I	Normal Control (0.1 M Citrate buffer pH 4.5)	49.96 ± 1.07
II	Untreated Diabetic (STZ 65 mg/kg i.p)	77.49 ± 3.04
III	L-Norvaline alone (10 mg/kg i.p)	66.04 ± 4.69
IV	L-Norvaline (10 mg/kg i.p) and α-tocopherol (900 mg/kg p.o)	52.65 ± 2.81 ^c
V	L-Norvaline (8 mg/kg i.p) and α-tocopherol (900 mg/kg p.o)	59.96 ± 1.08 ^c
VI	α-tocopherol (900 mg/kg p.o) alone	58.90 ± 0.76 ^b
VII	Sildenafil (5 mg/kg p.o)	75.70 ± 5.39

The statistical significance of difference between means was calculated by ANOVA followed by t-test for unpaired comparison. N=6
 Values are expressed as Mean ± S.E.M, b P< 0.01, c P< 0.001.

Table 3: Effect of L-Norvaline and Alpha-tocopherol on the level of malondialdehyde.

Discussion

Diabetes Mellitus is a complicated metabolic disorder associated with various microvascular and macrovascular alterations. As reported earlier, intraperitoneal administration of single dose of streptozotocin significantly enhanced the serum glucose level in fasted male rats [12, 19, 20]. High diabetic condition had empowered the level of not only glucose but also the production of free radicals that induced testicular damage and vascular impairment [21, 22].

High glucose level activates protein kinase C that directly activates NADPH oxidase. This enzyme in turn accelerates the Rho activation and ultimately over expression of oxygen free radicals. Peroxynitrates may be generated due to administration of L-Norvaline which is indirectly a nitric oxide donor [12]. Prolonged administration of this drug may cause accumulation of higher concentration of nitric oxide and accelerates the cavernosal smooth muscle proliferation. Hence the administration of natural antioxidant, alpha-tocopherol was introduced with L-Norvaline to counteract this effect. Moreover it was reported that the level of SOD and glutathione peroxidase was highly

diminished in diabetic condition [23]. Treatment with a tocopherol alone showed the most promising effect on the SOD and reduced glutathione than the combination with L-Norvaline.

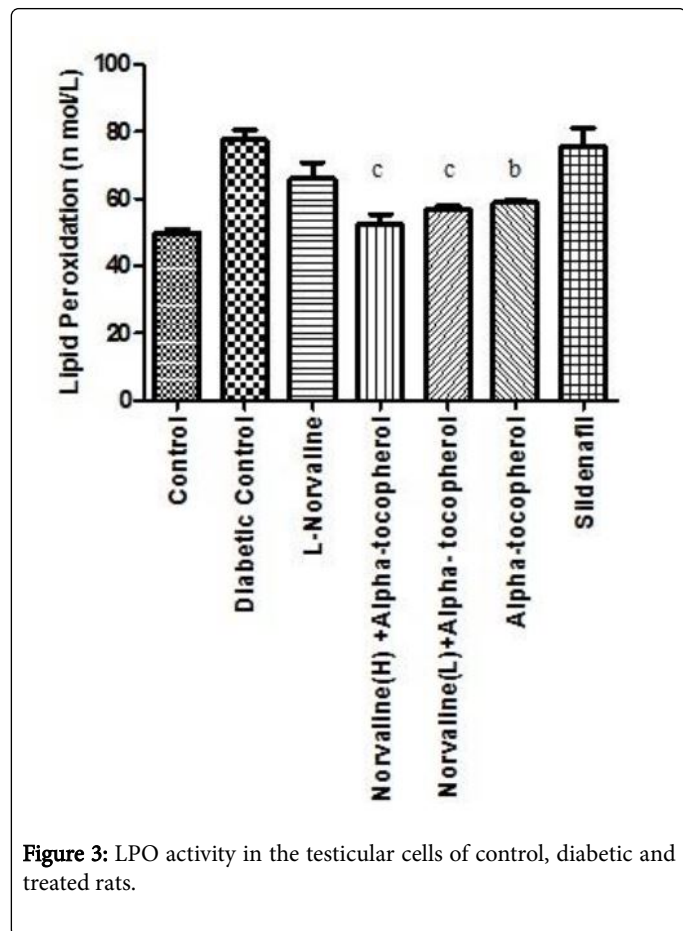


Figure 3: LPO activity in the testicular cells of control, diabetic and treated rats.

Alpha-tocopherol, a potent protein kinase C inhibitor inhibits the activation of Rho A and scavenges the oxygen radicals. In the polyol pathway the affinity of aldose reductase for excess glucose increases and utilization of NADPH to NADP⁺ also rises due to which the availability of required NADPH for generation of glutathione and other cellular protection mechanism diminishes. The drug and its combination with antioxidant may restore the glutathione level probably by maintaining the level of cellular NADPH in sorbitol pathway.

After streptozotocin injection, malondialdehyde level has been reported to increase in diabetes. Treatment of diabetic rats with alpha-tocopherol alone and its combination with L-Norvaline showed significant reduction in malondialdehyde level. As previously explained that, exposure of spermatozoa to ROS induces a loss of sperm motility that can be directly correlated with the level of malondialdehyde experienced by the spermatozoa [24].

More over De and his co-workers have shown an improvement in sperm parameters in L-Norvaline treated male rats [12]. The underlying mechanisms behind the loss of motility by lipid peroxidation are probably changes in the integrity and fluidity of the plasma membrane and failure of the flagellar movement.

In addition, lipid peroxidation also disturbs other membrane dependent sperm functions such as sperm-oocyte interaction and the

ability for physiological acrosome reaction [25,26]. Level of malondialdehyde was restored to normal level after treatment with L-Norvaline and combination with alpha-tocopherol suggesting the antioxidant activity of the drug.

The findings are confirmed by the results of previous literature indicating useful effect of alpha-tocopherol in sexual dysfunction. Due to this improvement in these oxidative stress parameters, it can be hypothesized that not only L-Norvaline but its combination with antioxidant might show a positive role in treating diabetes induced testicular impairment.

Diabetic oxidative stress can lead to overexpression of arginase which is responsible for testicular damage. L-Norvaline was previously explained as arginase inhibitor that inhibit arginase enzyme and the expression of eNOS was increased [27,28]. So its combination with alpha-tocopherol acts at a different site by inhibiting protein kinase C. The mechanistic approach has been indicated in Figure 4.

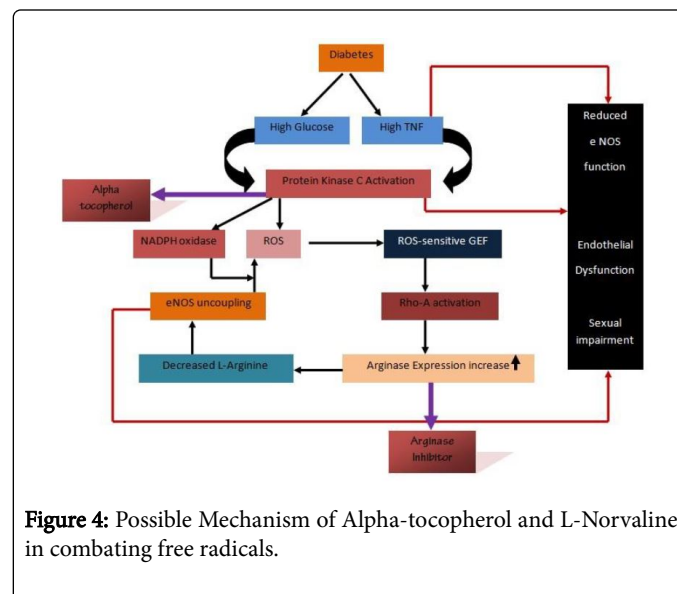


Figure 4: Possible Mechanism of Alpha-tocopherol and L-Norvaline in combating free radicals.

Conclusion

It was already explained that testicular impairment is directly related to sexual impairment [12]. But no data was available upon the effect of L-Norvaline in combating the hyperglycemia induced oxidative stress in rat testes. However, the mechanism is not clear but after compiling all the results, it can be concluded that L-Norvaline when combined with alpha-tocopherol shows a positive effect in scavenging free radicals by improving the level of antioxidant enzymes. Still the combination can be assessed completely to find out the effect on diabetes induced other complications.

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Conflict of Interest

The author declares no conflict of interest.

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