British Journal of Medicine & Medical Research
3(4): 1125-1143, 2013

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Evidences for Deleterous Role of Free Radicals in Experimental Varicocele Using Animal Model

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Authors' contributions

This work was carried out in collaboration between all authors. Author LCS designed the study performed the statistical, histological analysis, sperm character determination, wrote part of the draft and undertook in the initial editing of the paper. He also took part in the stereological evaluation. Author GGA wrote the protocol and first draft of the manuscript handled the histological processing of tissue. He also took part in stereological evaluation and undertook the final editing of the paper. Author OSO carried out most of the literature searches determined the enzymatic and non enzymatic antioxidants and took part in spermatozoa analysis. All authors read and approved the final manuscript.

Research Article

Received 20th November 2012
Accepted 17th January 2013
Published 22nd March 2013

ABSTRACT

Aim: It is highly intricate to categorize a solitary or prevailing factor for pathophysiology of varicocele. Herein, the basis of free radicals in the pathogenesis of varicocele was assessed.
Study Design: Experimental using animal models.
Place and Duration of Study: Department of Anatomy, College of Medicine, Lagos State University, Ikeja, Lagos, Nigeria, between April, 2012 and August, 2012.
Methodology: Five (5) groups of rats were used, Group A animals served as the control, while Groups B, C, D and E animals were varicocelized. Groups C, E and E in addition, had intramuscular treatment of 25 mg/kg, 50 mg/kg and 75mg/kg body weight of α-tocopherol respectively. The models were sacrificed on 65th day and Testicular weights and volumes, sperm parameters, histology, morphometry, enzymatic and non enzymatic antioxidants were vastly estimated.
Result: There was a significant ($p<0.05$) increase in activity level of SOD (5.92±4.1), CAT (380.2±7.1) and GPx (0.79±0.8) and a reduced lipid peroxidation evidenced by significant ($p<0.05$) reduction in level of MDA (18.2±6.1) of the varicocelized rat treated with Vitamin E (75mg/kg b.wt.) when compared to the activity of SOD (3.31±4.1), CAT

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(361.2±4.5), GPx (0.36±6.1) and MDA (0.36±6.1) of untreated varicocelized models. The geometric values, sperm characteristics and histological profiles threaded the same pattern as the oxidative status.
Conclusion: These results confirmed and validated the important role of reactive oxygen in the pathogenesis of varicocelized.

Keywords: Varicocele; free radicals; α-tocopherol; pathophysiology; oxidative stress.

1. INTRODUCTION

Personal elucidation to venous anatomical variance between human and animal models has remained the basis for which researchers alike have "*struggled*" in classifying the pathophysiology of varicocele. Although the pathogenesis of varicocele remains uncertain, there is a growing body of data implicating increased testicular and scrotal temperature [1-2] testicular hypoxia by venous stasis and miniature vessel occlusion [3], testicular blood surge [4], dwindle in gonadotrophin and androgen secretion [5], reflux of adrenal toxic metabolites [3,6] toxic substances [3], and reactive oxygen species (ROS) [7].

Even though an equal end between ROS production and scavenging in the seminal plasma has been reported [8], the reverse is the case when varicocele is in the equation.

The principal role of ROS through intracellular signal transduction in optimum functionality of spermatozoa has been acknowledged [4,9] but pathologic circumstances ranging from cryptorchidsm to dilation of veins of the spermatic cord can pave way for excessive production of ROS hence infertility [3,8].

The fact that spermatozoa lack cytoplasmic protection and the presence of polyunsaturated fatty acids in its plasma membrane makes it more sensitive and vulnerable to oxidative injury [10-16].

In 2004, Kamil et al. [17] evaluated the role of ROS and apoptosis in the pathogenesis of varicocele and the efficiency of vitamin E treatment. Chemiluminescence assay (CL) indicated an increase in ROS of varicocelized rat and subsequent decrease of ROS after Vitamin E treatment [17]. Lipid peroxidation appears to be a continuous physiological process. The process, if out of control, can alter essential cell functions and result in subsequent cell death [6].

In both human and animal models, studies have shown a decline in oxidative damage after oral antioxidant treatments [18-21] and dietary antioxidants has help to compliment the human antioxidant defense system which is usually "enough".

Vitamin E (α-tocopherol), a chain-breaking antioxidant has proven to be a key contributor and free radical scavenger against oxidative insult [22-26]. The National Academy of Sciences recommended 50-800 mg of Vitamin E per day for an adult male [27] and numerous studies have reported on its ability to prevent sperm agglutination [12,28-32].

Kessopoulou et al. [33] in a randomized cross-over evaluation reported an improved sperm function as assessed by the zona binding test post oral administration of 600 mg/day of Vitamin E [33] and other reports on its ability in reducing the level of malondialdehyde and lipid

peroxidation have been documented [25,34-39]. These studies have aided in evaluating Vitamin E as a chain breaking antioxidant.

This study aimed to evaluate and elucidate the key role of reactive oxygen species in the pathophysiology of experimental varicocele and the protective efficacy of antioxidant treatment in case of oxidative damage.

2. RESEARCH DESIGN AND METHODOLOGY

2.1 Resources

Vitamin E injection^{R} (Alpha tocopheryl acetate) GB Pharma was obtained from Concord Pharmacy, Bariga, Lagos, Nigeria. Olive oil which was given as vehicle was obtained from Roberts Laboratories Limited, Belton, England.

2.2 Experimental Ligation of Spermatic Vein

Experimental varicocele was induced in the models by anaesthetizing them with intra-abdominal injection of 7 mg/ kg body weight ketemine hydrochloride. A 2 cm median incision was made through the skin, beginning caudal to the prepuce and extending cranially. The right and left spermatic vein were exposed and entirely ligated with a 4-0 nylon suture as described by Sofikitis and Miyagawa [40].

2.3 Animal Grouping and Investigational Procedure

Fifty adult male Wistar rats (11 to 13 weeks old) weighing 250-280g were used for the evaluation. They were randomly divided into five groups of ten rats each and the average weight difference between and within groups did not exceed ± 20% of the average weight of the sample population. The first group (A) served as the control and was treated with 0.5 ml/kg body weight of olive oil as the vehicle for 65 days. Group B, C, D and E rats served as experimental groups in which the animal models were bilaterally varicocelized. Group C, D and E in addition had intramuscular treatment of 25, 50 and 75 mg/kg body weight of α-tocopherol for 65 days respectively, the extent of spermatogenesis in rat being 51.6-56 days [41-42]. The dose selection of *alpha*-tocopherol (25, 50 and 75 mg/kg body weight Vitamin E) was determined according to previous works by Hidiroglou et al. [43] and Patel et al. [44].

The injection of Vitamin E began 24hours post-surgery and was administered to the models once daily by 12 noon for six days (Monday to Saturday) within a week and the study is consistent with the standard of the use of laboratory animals put forward by the American Physiological Society [45].

2.4 Animal Sacrifice and Sample Collection

The rats were first weighed and then anaesthetized by inserting them in a clogged jar which contains chloroform anesthesia. The abdominal cavity was opened up through a midline abdominal incision to expose the reproductive organs. The testes were excised and trimmed of all fat. The testicular weights of each animal were evaluated with an electronic analytical and precision balance (BA 210S, d=0.0001- Sartoriusen GA, Goettingen, Germany). The testes volumes were measured by water displacement method. The two testes of each rat were measured and the average value obtained for each of the two parameters was

regarded as one observation. One of the testes of each animal was fixed in 10% formol-saline for histological and stereological examination. Serum and the remaining testes of each animal were stored at – 25^{o}C for subsequent biochemical assays.

2.5 Sperm Parameters

The testes from each rat were carefully exposed and removed. They were trimmed free of the epididymides and adjoining tissues. From each separated epididymis, the caudal part was removed and placed in a beaker containing 1mL physiological saline solution. Each section was quickly macerated with a pair of sharp scissors and left for a few minutes to liberate its spermatozoa into the saline solution. Sperm motility, concentration and progressive motility and morphology were determined as earlier described Saalu *et al*. (19) and Akunna et al [19,21]. Semen drops were placed on the slide and two drops of warm 2.9% sodium citrate were added. The slide was covered with a cover slip and examined under the microscope using X40 objective for sperm motility. Sperm count was done under the microscope using improved Neubauer haemocytometer.

2.6 Determination of Testicular Enzymatic Antioxidants

2.6.1 Assay of catalase (CAT) activity

Catalase activity was estimated based on the method of Aebi [46]. 0.1 ml of the testicular homogenate (supernatant) was pipetted into cuvette containing 1.9 ml of 50 mM phosphate buffer, pH 7.0. Reaction was started by the addition of 1.0 ml of freshly prepared 30% (v/v) hydrogen peroxide (H_2O_2). The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of enzyme was expressed as units /mg protein.

2.6.2 Assay of superoxide dismutase (SOD) activity

Superoxide dismutase activity was studied according to the method described by Rukmini et al. [47]. The principle of the assay was based on the ability of SOD to inhibit the reduction of nitro-blue tetrazolium (NBT). The reaction mixture contained 2.7 ml of 0.067M phosphate buffer, pH 7.8, 0.05 ml of 0.12mM riboflavin, 0.1 ml of 1.5mM NBT, 0.05 ml 0f 0.01M methionine and 0.1 ml of enzyme samples. Uniform illumination of the tubes was ensured by placing it in air aluminum foil in a box with a 15W fluorescent lamp for 10 minutes. Control without the enzyme source was included. The absorbance was measured at 560nm. One unit of SOD was defined as the amount of enzyme required to inhibit the reduction of NBT by 50% under the specific conditions. It was expressed as u/mg protein.

2.6.3 Assay of glutathione peroxidase (GPx) activity

Glutathione peroxidase activity was evaluated by the method described by Rotruck et al. [48]. The reaction mixture contained 2.0 ml of 0.4M Tris- HCl buffer, pH 7.0, 0.01 ml of 10mM sodium azide, 0.2 ml of enzyme. 0.2 ml of 10mM glutathione and 0.5 ml of 0.2mM. H_2O_2. The contents were incubated at 370C for 10 minutes followed by the termination of the reaction by the addition of 0.4 ml 10% (v/v) TCA, centrifuged at 5000 rpm for 5 minutes. The absorbance of the product was read at 430nm and expressed as nmol/mg protein.

2.7 Assay of Testicular Non-Enzymatic Antioxidants

2.7.1 Assessment of lipid peroxidation (Malondialdehyde)

Lipid peroxidation in the testicular tissue was studied colorimetrically by thiobarbituric acid reactive substances TBARS method of Buege and Aust [49]. A principle component of TBARS being malondialdehyde (MDA), a product of lipid peroxidation. In brief, 0.1 ml of tissue homogenate (Tris-Hcl buffer, pH 7.5) was treated with 2 ml of (1:1:1 ratio) TBA-TCA-HCl reagent (thiobarbituric acid 0.37%, 0.25 N HCl and 15% TCA) and placed in water bath for 15 min, cooled. The absorbance of clear supernatant was measured against reference blank at 535nm. Concentration was calculated using the molar absorptivity of malondialdehyde which is 1.56 x105 M-1 cm-1 and expressed as nmol/mg protein.

2.7.2 Assessment of plasma levels of testosterone

Plasma testosterone concentrations were estimated using the Enzyme Immunology Assay (EIA) method as earlier described by Saalu et al. [50]. Plasma samples were collected were stored at –20ºC while assayed. The EIA kits used were obtained from Immunometrics (London U. K) and contained testosterone EIA substrate reagents and EIA quality control samples. A quality control sample was run for the hormone at the beginning and at the end of the assay variation. The EIA kit used had a sensitivity level of 0.3 nmol/L (0.1 ng /mL). The intra and inter assay variations were 11.00 and 10.10% respectively.

2.7.3 Histological analysis

This was done as described by Akpantah et al. [51]. The organs were cut in slabs of about 0.5 cm thick and fixed in Bouin's fluid for a day after which it was transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and chloroform for different durations before they were transferred into two changes of molten paraffin wax for 20 min each in an oven at 57ºC. Serial sections of 5 µ m thick were obtained from a solid block of tissue and were stained with haematoxylin and eosin stains, after which they were passed through a mixture of equal concentration of xylene and alcohol. Following clearance in xylene, the tissues were oven–dried. Light microscopy was used for the evaluations.

2.8 Determination of Stereological Parameters

Histological slides were prepared from the formol-saline fixed testes. However, before embedding, it was ensured that the sections were placed perpendicular to their long axes, and chosen as "vertical sections". For each testis, five vertical sections from the polar and the equatorial regions were sampled and an unbiased numerical estimation of the following morphometric parameters was estimated using a systematic random scheme [52-53].

Seminiferous Tubular Diameter (D) for each animal was estimated with calibrated eyepiece and stage grids mounted on a light research microscope for profiles that were round or nearly round. A mean diameter, *D*, was determined by taking the average of two parameters, *D1* and *D2* (Perpendicular to one another). *D1* and *D2* were taken no more than when $D1/D2 \geq 0.85$.

Tubular Cross-Sectional area (AC) was estimated from the formula $AC = \pi D2/4$, (where π is equivalent to 3.142 and *D* the mean diameter of the seminiferous tubules.

Number of profiles of seminiferous tubules in a unit area of testis (NA) was determined using the unbiased counting frame projected by *Gundersen* [54]. Using this frame, in addition to counting profiles completely inside the frame we counted all profiles with any part inside the frame provided they do not intersect the forbidden line.

Numerical Density (NV) of seminiferous tubules was determined by using the modified Floderus equation: *NV =NA/ (D +T)* where, *NA* is the number of profiles per unit area, *D* is the diameter and *T* the average thickness of the section.

The volume density of testicular components and number of seminiferous tubules were done on a computer monitor onto whom a graph sheet was superimposed and on which slides were projected from a research light microscope (Model N -400ME, CEL-TECH Diagnostics, Hamburg, Germany).

2.9 Statistical Analysis

The obtained data were expressed as mean ± SD of number of experiments (n = 10). A homogenic level among the groups was tested using Analysis of Variance (ANOVA) (*55*). Where heterogeneity occurred, the groups were separated using Duncan Multiple Range Test (DMRT). A value of $p < 0.05$ and $p<0.005$ was considered to indicate a significant difference between groups.

3. RESULTS

3.1 Gross Anatomical Changes

3.1.1 Body weight

As shown in Fig. 1, models in Group A had a significant (*P<0.05*) improvement in body weight compared to animals in other groups. Group B rats loss body weight significantly (*P<0.005*) compared to their initial weight. Group C, D and E rats lost body weights when compared with their original weights. However the weight loss experienced was not as significant as that of the untreated varicocelized group (B).

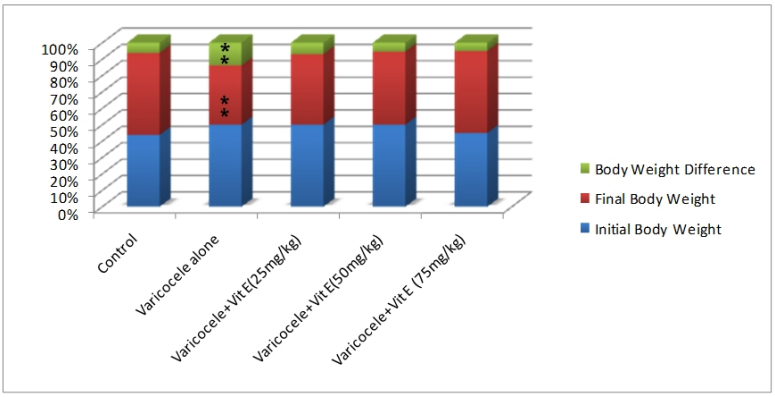

Fig. 1. Showing the Weight Differences pre and post treatment with *Alpha* tocopherol
** P < 0.05; ** P < 0.005 significantly different from control. Values are expressed as mean ± SD for n=10 in each group.*

3.1.2 Weights and volume of testes mean

It was shown in Fig. 2 that the testicular weights and volumes of models in Group B were the least, being significantly lower (*P<0.005*) compared to the mean testicular weights and volumes of the varicocelized rats that in addition were treated with Vitamin E. However, the testicular weights and volume of the control rats were fairly the same compared with their initial values.

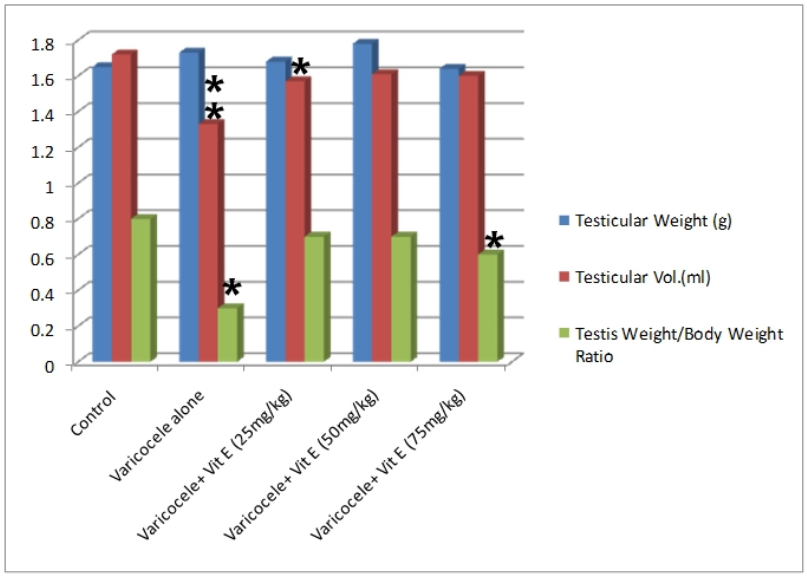

Fig. 2. Showing the Testicular Weight, Volume and Testis Weight/body weight Differences.

** P < 0.05; ** P < 0.005 significantly different from control. Values are expressed as mean ± SD for n=10 in each group.*

3.1.3 Epididymal sperm characteristics

As shown in Table 1, the varicocelized alone group had marked oligospermia (57.3±4.1) with their sperm concentration being significantly lower (*P*<0.005) compared to the control models. The varicocelized models treated with Vitamin E however, showed moderate (*P*<0.05) oligospermia which are comparable to that of the control (125.8±3.1).

3.1.4 Spermatozoa motility

The percentage sperm motility of the treated varicocelized models were significantly (*P*<0.005) higher compared to the untreated model group (31.2±4.5) and comparable with that of the control value (98.2±8.2).

3.1.5 Sperm progressivity and sperm morphology

The results of the sperm progressivity and morphology followed that of the sperm count and motility. However, the group that had 25 mg/kg body weight of Vitamin E had slow progressive motility similar to the untreated varicocelized group. Also the varicocelized

models treated with 75 mg/kg body weight of Vitamin E had a significant (P<0.005) decrease in abnormal sperm similar to that of the control value (Table 1).

Table 1. Showing the sperm Characteristics of the Models

Treatment Groups	Sperm count ($X10^6$/ml)	Sperm motility (%)	Progressivity	Morphology	
				% Normal	%Abnormal
Control	125.8±3.1	98.2±8.2	a_1	95.3±2.1	9.5±2.1
Varicocele-Alone	57.3±4.1**	31.2±4.5**	b_1	36.5±6.1**	50.4±2.1**
Varicocele/Vitamin E (25mg/kg)	127.1±5.1*	80.1±5.5*	b_1	77.2±0.1*	21.5±2.1*
Varicocele/Vitamin E (50mg/kg)	120.4±7.1*	75.8±9.1*	a_1*	89.4±0.4*	22.3±2.1*
Varicocele/Vitamin E (75mg/kg)	110.9±4.1*	80.2±7.1*	a_1**	79.6±0.8*	10.2±2.1**

**, ** represent significant decreases and increases at P< 0.05 and P<0.005 respectively when compared to the control values. Values are means ± S.E.M. n = 10 in each group.In this study, a spermatozoon was considered abnormal morphologically if it had one or more of the following features: rudimentary tail, round head and detached head. a1 = rapid linear progressive motility, b1 = show sluggish linear or non-linear motility.*

3.2 Testicular Oxidative Stress

3.2.1 Activities of testicular enzymes SOD, CAT and GPx

As shown in Table 2, untreated varicocelized model (Group B) had a significant decrease in SOD activity when compared to control counterpart. Varicocelized rats that had Vitamin E treatments showed a significantly increased testicular SOD activity which is comparable to control values. The testicular activities of CAT after antioxidant treatment were similar to that of the control values. Group B rats, however, had a significant reduction in testicular CAT activity compared to control rats. The activities of CAT in Group C, D and E rats were roughly similar to that of the control values.

Table 2. Showing results for antioxidative evaluation

Treatment Groups	SOD (u/mg protein)	CAT (u/mg protein)	MDA (nmol/mg protein)	GPx (nmol/mg protein)
Control	6.11±3.1	382.21±8.2	26.7±5.3	0.95±2.1
Varicocele-Alone	3.31±4.1*	361.2±4.5*	59.3±4.2*	0.36±6.1**
Varicocele/Vitamin E (25mg/kg)	5.13±5.1*	380.1±5.5*	29.2±8.1*	0.77±0.1*
Varicocele/vitamin E (50mg/kg)	5.41±7.1*	375.8±9.1*	30.3±7.1*	0.89±0.4*
Varicocele/vitamin E (75mg/kg)	5.92±4.1*	380.2±7.1*	18.2±6.1**	0.79±0.8*

**, ** represent significant decreases and increases at P<0.05 and P<0.005 respectively when compared to the control values. Values are means ± S.E.M. n = 10 in each group.*

The GPx activity of varicocelized rats treated with ∝-tocopherol approximated (*P<0.05*) that of the control groups of animals. Group B rats, however, had a markedly decreased GPx activity compared to that of control values.

3.2.2 Testicular content of malondialdehyde (MDA)

Group B rats had a significantly (*P<0.005*) elevated testicular MDA as compared to the control value. Administration of ∝-tocopherol caused a remarkable reduction in the testicular MDA level compared to rats in Group B.

3.2.3 Serum testosterone levels

As shown in Fig. 3, there was **nonsignificant** difference in the serum basal testosterone levels between the untreated varicocelized models and the control values. The testosterone levels between the treated groups did not also significantly differ from one another.

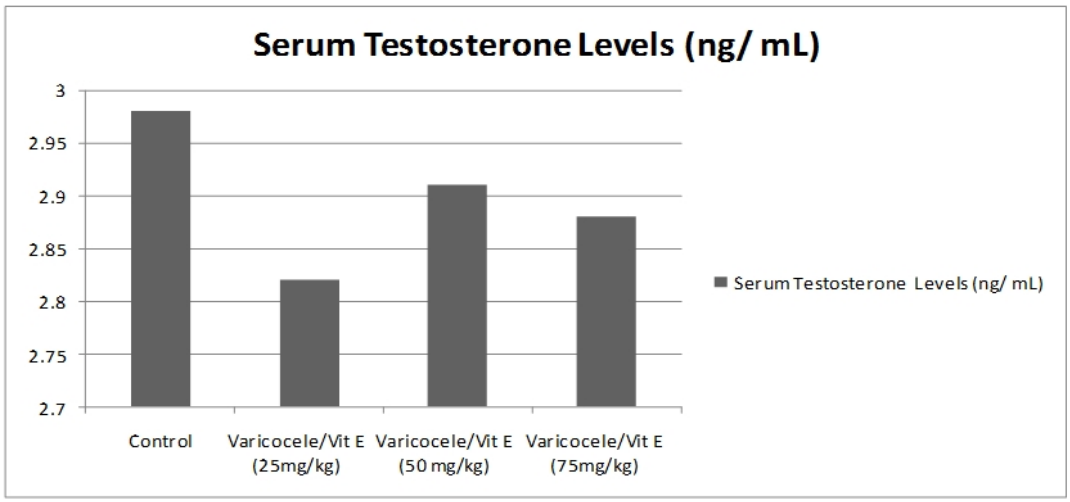

Fig. 3. Level of Serum Testosterone (ng/ mL).

3.2.4 Testis morphology

As shown in Figs. 4-8. The seminiferous tubules of the control rats were completely differentiated with numerous spermatozoa, had oval outline with normal epithelium and intact interstitium. Testes of untreated varicocelized group showed evidences of atrophied seminiferous tubules, interstitial oedema, degenerated and vacuolated germinal epithelium and absence of late stage germ cells. The histological profiles of the testes of varicocelized rats treated with ∝-tocopherol were largely similar to those of the control counterparts with attenuated seminiferous epithelium and interstitium and mild degenerative changes.

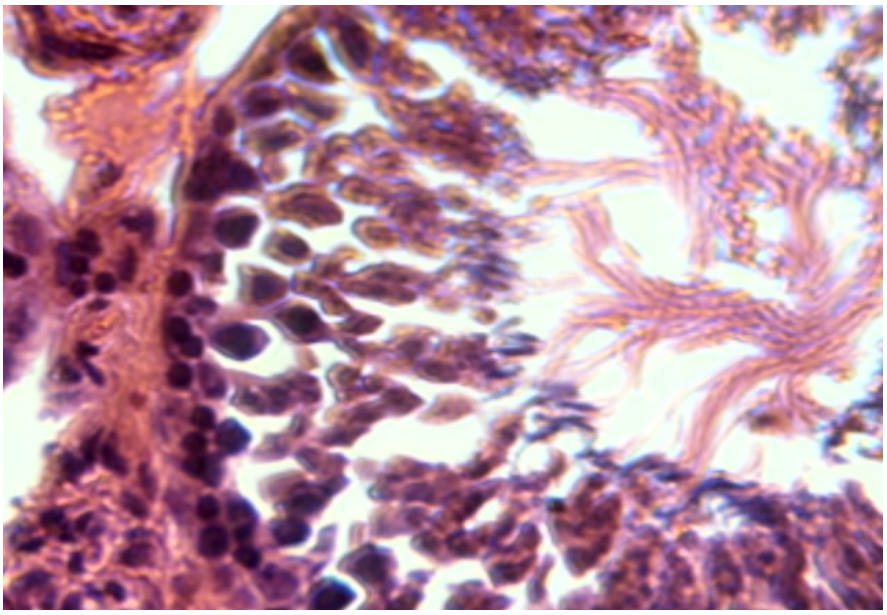

Fig. 4. The testis of control rats. The seminiferous tubules are completely differentiated, spermatozoa shown in some of the tubules H & E (X400)

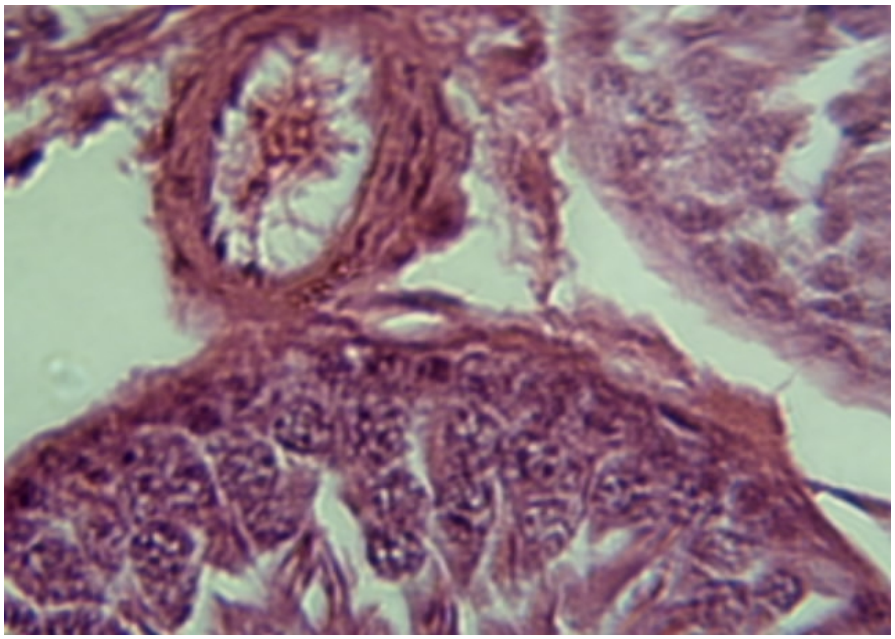

Fig. 5. Testis of varicocelized rats without Vitamin E treatment. Marked degeneration of the germinal epithelium and absence of late stage germ cells. H & E (X400)

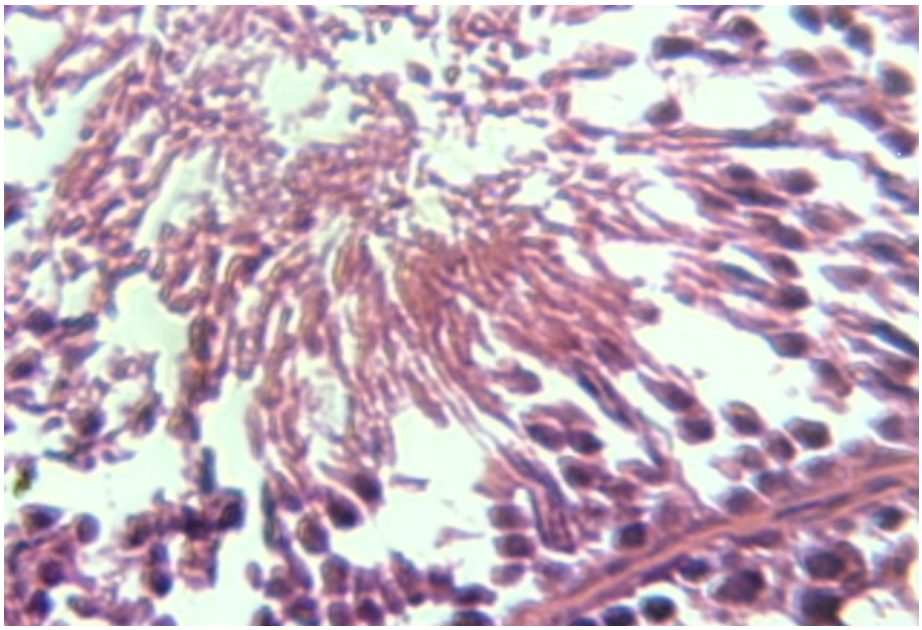

Fig. 6. Testis of varicocelized rats treated with 25 mg/kg body weight treatment of Vitamin E. Atrophied seminiferous tubules and presence of some late stage germ cells in some tubules. Left H & E (X400)

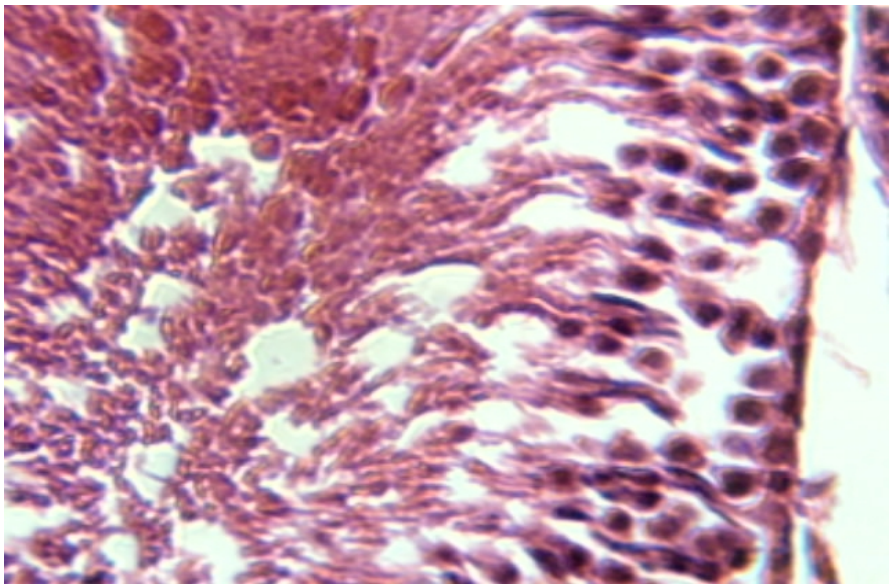

Fig. 7. Testis of varicocelized rats treated with 50 mg/kg body weight treatment of Vitamin E. Moderate seminiferous tubules atrophy and presence of some late stage germ cells in some tubules. Left H & E (X400)

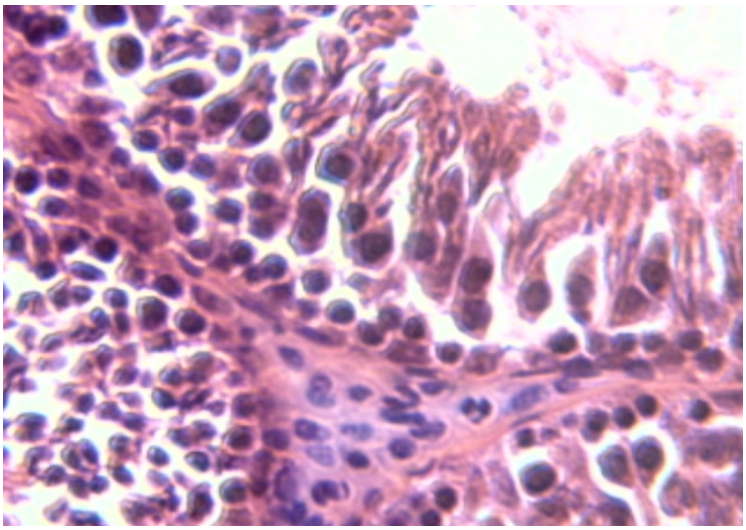

Fig. 8. Section through testis of varicocelized rats treated with 75 mg/kg body weight treatment of Vitamin E. Moderate seminiferous tubules atrophy and presence of some late stage germ cells in some tubules. Left H & E (X100)

3.3 Testicular Geometry

Fig. 9 showed a significant (*P*<0.005) reduction in the mean seminiferous tubular diameters of untreated varicocelized model compared to the control groups. However, there was a significant (*P<0.005*) increase in the tubular diameter of animals treated with ∝-tocopherol as compared to tubular diameter of the control groups. There were significant (*P*<0.005) increase in the cross-sectional area of the tubules, the number of tubular profiles per unit area and the mean numerical density of seminiferous tubules of the models treated with vitamin E which are comparable with that of the control value.

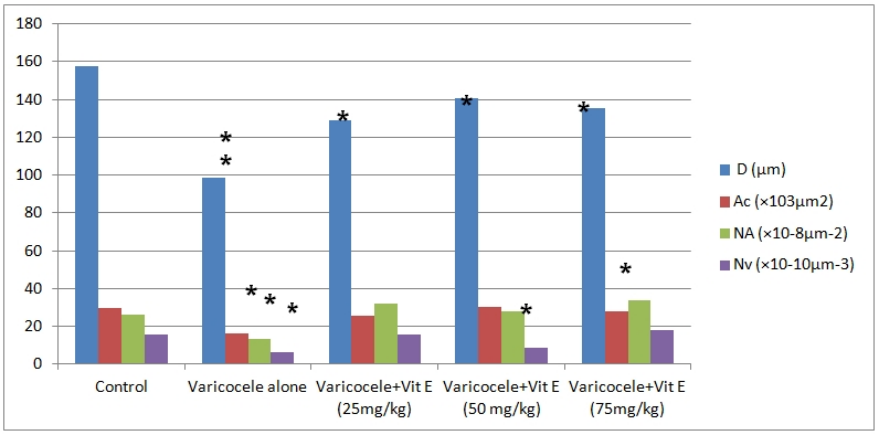

Fig. 9. Showing seminiferous tubular diameter (µm), cross sectional area A_c ($\times 10^3 \mu m^2$), numerical densities of seminiferous tubules N_A ($\times 10^{-8} \mu m^{-2}$) and number of profiles per unit area N_v ($\times 10^{-10} \mu m^{-3}$) in experimental and control models

** P < 0.05; ** P < 0.005 significantly different from control. Values are expressed as mean ± SD for n=10 in each group.*

4. DISCUSSION

Varicocele has been considered the most indicated and the most "*correctable*" cause of infertility. It must be clearly stated that studies has reported the consequence of varicocele on male fertility [3,19] but it's possible mechanism of damage remain speculative [3]. Among these reports are the cases that implicate excess production of ROS resulting in testicular oxidative insult [8,19,21,56-57]

Our result indicated a significant loss in body weight; testicular weights and testicular volumes in untreated varicocelized model. In agreement with preceding reports which reported considerable evidences of decrease in testicular weight due to various oxidative derangements [40,48,58].

In a previous report by *Semercioz* et al. [59], a decrease in testicular weight and volume in varicocelized animal were indicated and this reduction was as a result of degenerative damage caused by heat from venous stasis in the seminiferous epithelium [60].

Successfully achieving experimental varicocele confers a conspicuous level of stress on the rats and this could have affected, in part the metabolic process, hence loss of body weight experienced by the varicocelized models [19,21,61].

The control models having a significant enhancement in body weight could mean that they were still in their active growth phase during the study [19,48].

Varicocelized models that were treated with ∝-tocopherol on the other hand, had a largely preserved body weight, testis weights and testis volumes which are indications of the salutary role of Vitamin E [48,62].

Infertility as a result of varicocele was confirmed in the varicocelized alone group which had marked oligospermia compared to the control models, low sperm motility and progressivity compared to that of the control. High percentage of abnormal sperm was also observed indicating the deleterious effect of varicocele. The varicocelized models treated with Vitamin E however, had moderate oligospermia which are comparable to that of the control. In conformity with that of Fazeli-Matin et al. (60), Jungwirth et al. [61] and Testini et al. [63]. Numerous studies has confirmed malondialdehyde, superoxide dismutase, catalase, reduced glutathione and glutathione peroxidise as a viable means of evaluating oxidative damage in animal model [21,25,64-66].

We hereby report in this study, a significant decrease in the activity level of antioxidant enzymes and testicular content of SOD, CAT and GPx of untreated varicocelized models compared to that of the control animals. Also there was a significant elevation in the level of MDA, signifying high lipid peroxidation as a result of experimental varicocele. In accordance with several reports [19,21,67]. Reduction in the activity level of superoxide dismutase might have allowed more peroxide conversion to toxic hydroxyl radicals causing serious oxidative damage in varicocelized animals [19,68-70]. Superoxide dismutase has been indicated as a potent antioxidant. On its inability to donate electron which tents to increase its ability to detoxify hydrogen peroxide. The epididymis secretes glutathione peroxidase [71-72]. Epididymal degeneration caused by varicocele could explain the reason for the reduction in activity level of GPx in our study. The epididymis constitutes six (6) percent of the secretory

epididymal proteins, thus, testicular protection against oxidative insult is a possible function of this epididymis-specific isoform [73-74].

Remarkably, varicocelized rats treated with Vitamin E had a significantly increased activity level of SOD, CAT and GPx and a reduced lipid peroxidation which is evidenced by significant reduction in level of MDA of the varicocele-alone group.

In 1998, Bartfay et al. [75] reported the ability of glutathione peroxidase in acting synergistically with vitamin E hence a necessary component for the synthesis of glutathione peroxidase. This could explain the changes in activity level of GPx pre and post treatment with Vitamin E evidenced in our study.

In 1994, Faizi et al. [66] reported that enhancing the antioxidant system levels can favour reproductive potentials. The findings in our study confirmed the role of reactive oxygen species in the pathogenesis of varicocele being in accordance with previous reports [17,19,76-78].

Although varicocele had significant effect on testicular antioxidative enzymes, there was no significant difference in the serum basal testosterone levels between the untreated varicocelized models and the control values.

Our findings showed evidences of atrophied seminiferous tubules, interstitial oedema, degenerated and vacuolated germinal epithelium and absence of late stage germ cells in untreated varicocelized models. On the other hand, the histological profiles of the treated varicocelized rats fairly similar to those of the control models that had completely differentiated seminiferous tubules with numerous spermatozoa and normal and intact epithelium. Our findings are in accordance with our previous report implicating varicocele in testicular degeneration [17,19,21].

Although the histological profile of the treated varicocelized models were similar to that of the control, there have been no studies that clearly demonstrate that testicular histology can predict success after varicocele treatment [79]. However, to evaluate essential issues that have to do with qualitative microscopic investigation, it helps to describe histological sections stereologically.

Our findings showed a significant reduction in the mean seminiferous tubular diameters of untreated varicocelized model when compared to that of the control groups. However, there was a significant increase in the tubular diameter of animals treated with Vitamin E as compared to tubular diameter of the control groups. The disparity in the cross-sectional area of the tubules, the number of tubular profiles per unit area and the mean numerical density of seminiferous tubules of the Group B, C and D treaded a similar pattern as the tubular diameter.

Our end result indicated the role of antioxidants in ameliorating testicular toxicity as a result of varicocele. In conformity with previous report [48,64,76,80] Three dimensional deductions obtained from the present study provided quantitative evidence that Vitamin E attenuated testicular insult induced by experimental varicocele.

Although the testes can be affected greatly by ROS, there are sufficient inbuilt mechanisms of antioxidant protections capable of decreasing the progressive damage. Various measures

such as oral antioxidant treatment can be employed when there is imbalance between ROS generation and the natural antioxidant defenses as in the case of varicocele.

Due to the quantity of spermatogenic cells in the basal layer and the Sertoli-Sertoli cell barrier which determines the number of cells in the adluminal compartment, we would be unable to conclude based on the histo-morphometric alterations in the present study. However, the oxidative evaluation in our study is a sound indication of the characteristics of experimental varicocele in animal models with and without treatment with Vitamin E.

5. CONCLUSION

Oxidative stress is a principal player in the pathogenesis of Varicocele and this was indicated by the attenuating efficacy of α-tocopherol.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”.

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hong CY, Chiang BN, Turner P. Calcium ion is the key regulator of human sperm function. Lancet, 1984;2:1449-1451.
2. Marmar JL. The pathophysiology of varicoceles in the light of current molecular and genetic information. Hum Reprod Update. 2000;7:461-72.
3. Santoro G, Romeo C, Impellizzeri P, Cutroneo G, Trimarchi F. Nitricoxide synthase patterns in normal and varicocele testis in adolescents. BJU Int. 2000;88:967-73.
4. de Lamirande E, Gagnon C. Impact of reactive oxygen species on spermatozoa: a balancing act between beneficial and detrimental effects. Hum Reprod. 1995;10:15-21.
5. Eisenberg ML, Lipshultz LI. Varicocele-induced infertility: Newer insights into its pathophysiology. Indian J Urol. 2011;27:58-64.
6. Shiraishi K, Takihara H, Matsuyama H. Elevated scrotal temperature, but not varicocele grade, reflects testicular oxidative stress-mediated apoptosis. World J Urol; 2009.

7. Koksal IT, Ishak Y, Usta M, Danisman A, Guntekin E, Bassorgun IC, et al. Varicocele-induced testicular dysfunction may be associated with disruption of blood-testis barrier. Arch Androl. 2007;53:43-48.
8. Aitken RJ. Clarkson JS. Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. J Reprod Fertil, 1987;81:459-69.
9. Davies KJ. Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. IUBMB Life. 2000;50:279 -289.
10. Donnelly ET, McClure N, Lewis SE. Antioxidant supplementation in vitro does not improve human sperm motility. Fertil Steril. 1999;72:484-495.
11. Saleh RA, Agarwal A. Oxidative stress and male infertility: from research bench to clinical practice. J Androl. 2002;23:737-752.
12. Agarwal A, Nallella KP, Allamaneni SS, Said TM. Role of antioxidants in treatment of male infertility: an overview of the literature. Reprod Biomed Online. 2004;8:616-627.
13. Kobayashi H, Gil-Guzman E, Mahran AM, Rakesh, Nelson DR, Thomas AJ Jr, Agarwal A. Quality control of reactive oxygen species measurement by luminol-dependent chemiluminescence assay. J Androl. 2001;22:568-574.
14. Zalata AA, Ahmed AH, Allamaneni SS, Comhaire FH, Agarwal A. Relationship between acrosin activity of human spermatozoa and oxidative stress. Asian J Androl. 2004;6:313-318
15. Moustafa MH, Sharma RK, Thornton J, Mascha E, Abdel-Hafez MA, Thomas AJ Jr, Agarwal A. Relationship between ROS production, apoptosis and DNA denaturation in spermatozoa from patients examined for infertility. Hum Reprod. 2004;19:129-138.
16. Tominaga H, Kodama S, Matsuda N, Suzuki K, Watanabe M. Involvement of reactive oxygen species (ROS) in the induction of genetic instability by radiation. J Radiat Res (Tokyo). 2004;45:181-188
17. In 2004, Kamil et al. evaluated the role of ROS and apoptosis in the pathogenesis of varicocele and the efficiency of vitamin E treatment. Chemiluminescence assay (CL) indicated an increase in ROS of varicocelized rat and subsequent decrease of ROS after.
18. Suziki N, Sofikitis N. Protective effects of antioxidants on testicular fractions of varicocelized rats. Yonago Acta Medica, 1999;42:87-94.
19. Saalu LC, Udeh R, Oluyemi KA, Jewo PI, Fadeyibi LO. Efecto de aminoramiento del extracto de semilla de pomelo en la morfologia y funcion testicular de ratas varicocelizadas. Int. J. Morphol. 2008;26(4):1059-1064.
20. Ozokutan BH, Kucukaydin M, Muhtaroglu S, Tekin Y. The role of nitricoxide in testicular ischemiareperfusion injury. J. Pediatr. Surg. 2000;35:101-3.
21. Akunna GG, Ogunmodede OS, Saalu CL, Ogunlade B, Akunna GG, Bello AJ. Laurus nobilis Extract Preserves Testicular Functions in Cryptorchid Rat. World Journal of Life Sciences and Medical Research. 2012;2(2):91-9.
22. Dinsmore W, Callender ME, McMaster D, Todd SJ, Love AH. Zinc absorption in alcoholics using zinc-65. Digestion. 1985;32:238-242.
23. Valberg LS, Flanagan PR, Ghent CN, Chamberlain MJ. Zinc absorption and leukocyte in zincalcoholic and non alcoholic cirrhosis. Dig Dis Sci.185;30:329-333.
24. Kappus H. Oxidative stress in chemical toxicity. Arch. Tocicol. 1987;60:144-149.
25. Halliwell B, Gutteridge JMC, editors. Free Radicals in Biology and Medicine, 3rd ed. Oxford; 1998, Oxford Science.
26. Powell SR. The antioxidant properties of zinc. J Nutr. 2000;130:1447S-1454S.
27. National Academy of Sciences. Recommended Dietary Allowances. 10th ed.;1989, National Academy Press.

28. Sies H, Stahl W, Sundquist AR. Antioxidant functions of vitamins. Vitamins E and C, beta-carotene, and other carotenoids. Ann N Y Acad Sci. 1992;669:7-20.
29. Lewis SE, Sterling ES, Young IS, Thompson W. Comparison of individual antioxidants of sperm and seminal plasma in fertile and infertile men. Fertil Steril. 1997;67:142-147
30. Dawson EB, Harris WA, Rankin WE, Charpentier LA, McGanity WJ. Effect of ascorbic acid on male fertility. Ann N Y Acad Sci. 1987;498:312-323.
31. Mathiesen L, Wang S, Halvorsen B, Malterud KE, Sund RB. Inhibition of lipid peroxidation in low-density lipoprotein by the flavonoid myrigalone B and ascorbic acid. Biochem Pharmacol. 1995;51:1719-1725.
32. de Whalley CV, Rankin SM, Hoult JR, Jessup W, Leake DS. Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. Biochem Pharmacol. 1990;39:1743-1750.
33. Kessopoulou E, Powers HJ, Sharma KK, Pearson MJ, Russell JM, Cooke ID, Barratt CL. A double-blind randomized placebo cross-over controlled trial using the antioxidant vitamin E to treat reactive oxygen species associated male infertility. Fertil Steril. 1995;64:825-831.
34. Suga T, Watanabe T, Matsumoto, Hortes Y. Effects of long term Vit E deficiency and Restoration of rat hepatic peroxisones. Biochem. Biol. Phys. Acta. 1984;794:218–24.
35. Kumar DA, Derael R. Physiological antioxidants and antioxidative enzymes in Vitamin E – deficient rats. Toxicol. Lett. 1988;44:47-54.
36. Noguchi T, Cantor A, Scott MC. Mode of action of Selenium and Vitamin E. in prevention of oxidative stress in chicks. J. Nutr. 1973;103:1502-11.
37. Nordmann R. Alcohol and antioxidant systems. Alcohol Alcohol. 1994;29:513-522.
38. Parat MO, Richard MJ, Beani JC, Favier A. Involvement of zinc in intracellular oxidant/antioxidant balance. Bio Trace Elem Res.1997;60:187-204. (Pub Med)
39. Skandhan KP. Review on copper in male reproduction and contraception. Rev. Fr. Gynecol. Obstet. 1992;87:594-598.
40. Sofikitis N, Miyagawa I. Experimental models for the study of varicocele: A selected review. Jpn. J. Fertil. Steril.1992;38:168-77.
41. Heller CG, Clermont Y. Spermatogenesis in man: An estimate of its duration. Science. 1964;140:184-186.
42. Jegou B, Pineau C, Toppari J. Spermatogenesis in vitro in mammals. In: de Jonge, C.J., and Barratt, C.L.R., (Editors), Assisted Reproductive Technology: Accomplishments and New Horizons. 1st Edition. Cambridge: Cambridge University Press. 2002;3-60.
43. Hidiroglou N, Gilani GS, Long L, Zhoa X, Madere R, Cockell K, Belonge B, Ratnayake WM, Peace R. The influence of dietry vitamin E, fat, and methionine on blood cholesterol profile, homocysteine levels, and oxidizability of low density lipoprotein in gerbil. 2004;15(12):730-40.
44. Patel Manali B, Deshpande S, Shah G. Evaluation of efficacy of Vitamin E and N-acetyl cystein in gentamicin-induced nephrotoxicity in rats. Renal Fail. 2011;33(3):341-7.
45. American Physiological Society. Guiding principles for research involving animals and human beings. Am J Physiol Regul Integr Comp Physiol. 2002;283:281-283.
46. Aebi H. Catalase. In methods of enzymatic Analysis, Bergmeyer, H.Ed. Verlag Chemical, Weinheim. 1983;3:273.
47. Rukmini MS, Benedict I, Vivian D. Superoxide dismutase and catalase activities and their correlation with malondialdehyde in Scizophrenic patients. Ind. J. Clin. Biochem. 2004;19(2):114-118.

48. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. Sci. 1973;179:588-590.
49. Buege JA, Aust SD. Microsomal Lipid Peroxidation. In: Fleischer, S., Packer L., (Editors), Methods in Enzymology. New York: Academic Press. 1978;302-330.
50. Saalu LC, Osinubi AA, Akinbami AA, Yama OE, Oyewopo AO, Enaibe BU. Moringa oleifera Lamarck (drumstick) Leaf Extract Modulates the Evidences of Hydroxyurea – Induced Testicular Derangement International Journal of Applied Research in Natural Products. 2011;4(2):32-45.
51. Akpantah AO, Oremosu AA, Ajala MO, Noronha CC, Okanlawon AO. The effect of crude extract of Garcinia kola seed on the histology and hormonal milieu of male Sprague-Dawley rats' reproductive organs. Nig. J. Health and Biomed. Sci. 2003;2(1):40-46.
52. Gundersen HJG, Jenson EB. The efficiency of systematic sampling in stereology and its prediction. J Microsc. 1987;147:229-263.
53. Qin D, Lung MA. Morphometric study on Leydig cells in capsulotomized testis of rats. Asian J Androl. 2002;4:49-53. Available: www.asiaandro.com/1008-682X/4/49.pdf
54. Gundersen HJG. Notes on the edge of the numerical density of arbitrary profiles: the edge effect. J Microsc. 1977;111:219-223.
55. Snedecor GW, Cochran WG. Statistical Method, 7th edn. Iowa: Iowa State University Press, 215. Tindal HD. Commercial vegetable growing. Oxford Press. London. 1968;69.
56. Hendin BN Kolettis PN, Sharma RK, Thomas A. Varicocele is associated with elevated spermatozoa reactive oxygen species production and diminished seminal plasma antioxidant capacity. J. Urol. 1999;161:1831- 4.
57. Weese DL, Peaster ML, Himsl KK, Leach GE, Lad PM, Zimmern PE. Stimulated reactive oxygen species generation in the spermatozoa of infertile men. J Urol.1993;149:64-7.
58. Ozdamar AS, Soylu AG, Culha M, Ozden M, Gokalp A. Testicular oxidative stress effects of experimental varicocele in adolescent rats. Urol. Int. 2004;73:343-7.
59. Semercioz A, Onur R, Ogras S, Orhan I. Effect of melatonin on testicular tissue nitricoxide level and antioxidant enzyme activities in experimentally induced left varicocele. Neuro Endocrinol. Lett. 2003;24(1/2):86-90.
60. Fazeli-Matin S, Morisson G, Goldstein M. What is the pregnancy rate in vasovasostomy and varicocelectomy patients who are "lost to follow-up"? J Urol. 1994;151:303. Abstract 301.
61. Turner TT. The study of varicocele through the use of animal model. Human Reproduction Update. 2001;7(1):78-84.
62. Jungwirth A, Gogus C, Hauser G, et al. Clinical outcome of microsurgical subinguinal varicocelectomy in infertile men. Andrologia. 2001;33:71-4.
63. Testini M, Miniello S, Piccinni G, et al. Microsurgical treatment of varicocele in outpatients using the subinguinal approach. Minerva Chir. 2001;56:655-9.
64. Carlsson LM, Jonsson J, Edlund T, Marklund SL. Mice lacking extracellular superoxide dismutase are more sensitive to hyperoxia. Proc Natl Acad Sci USA. 1995;92:6264-8.
65. Brigelius-Flohe R. Tissue-specific functions of individual glutathione peroxidases. Free Radic Biol Med. 1999;27:951-65.
66. Harrison R. Structure and function of xanthine oxidoreductase: where are we now? Free Radic Biol Med. 2002;33:774-97.
67. Faizi S, Siddiqui BS, Saleem R, Siddiqui S, Aftab K , Gilani AH. Isolation and structure elucidation of new nitrile, mustard oil glycosides from *Moringa oleifera* and their effect on blood pressure. J. Nat. Prod. 1994;57:1256-1261.

68. Aitken RJ. Free radicals, lipid peroxidation and sperm function. Reprod. Fertil. Dev. 1995;7:659- 68.
69. Hendin BN, Kolettis PN, Sharma RK, Thomas A. Varicocele is associated with elevated spermatozoa reactive oxygen species production and diminished seminal plasma antioxidant capacity. J. Urol. 1999;161:1831- 4.
70. Hall L, Williams K, Perry AC, Frayne J, Jury JA. The majority of human glutathione peroxidase type 5 (GPX5) transcripts are incorrectly spliced: implications for the role of GPX5 in the male reproductive tract. Biochem. J. 1998;333:5-9.
71. Rejraji H, Vernet P, Drevet JR. GPX5 is present in the mouse caput and cauda epididymidis lumen at three different locations. Mol Reprod Dev. 2002;63:96-103.
72. Vernet P, Rock E, Mazur A, Rayssiguier Y, Dufaure JP, Drevet JR. Selenium-independent epididymis-restricted glutathione peroxidise 5 protein (GPX5) can back up failing Se-dependent GPXs in mice subjected to selenium deficiency. Mol Reprod Dev. 1999;54:362-70.
73. Fouchecourt S, Metayer S, Locatelli A, Dacheux F, Dacheux JL. Stallion epididymal fluid proteome: qualitative and quantitative characterization; secretion and dynamic changes of major proteins. Biol Reprod. 2000;62:1790-803.
74. Bartfay WJ, Hou D, Brittenham GM, Bartfay E, Sole MJ, Lehotay D, Liu PP. The synergistic effects of vitamin E and selenium in iron-overloaded mouse hearts. Can J Cardiol. 1998;14:937-941.
75. Alvarez JG, Touchstone JC, Blasco L, Storey BT. Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa. Superoxide dismutase as major enzyme protectant against oxygen toxicity. J Androl. 1987;8:338-48.
76. Weese DL, Peaster ML, Himsl KK, Leach GE, Lad PM, Zimmern PE. Stimulated reactive oxygen species generation in the spermatozoa of infertile men. J Urol. 1993;149:64-7
77. Mazzilli F, Rossi T, Marchesini M, Ronconi C, Dondero F. Superoxide anion in human semen related to seminal parameters and clinical aspects. Fertil Steril. 1994;62:862-68.
78. Cocuzza M, Athayde KS, Agarwal A, Pagani R, Sikka SC, Lucon AM et al. Impact of clinical varicocele and testis size on seminal reactive oxygen species levels in a fertile population: a prospective controlled study. Fertil Steril. 2008;90:1103-1108.
79. Pasqualotto FF, Lucon AM, de Goes PM et al. Testicular growth, sperm concentration, percent motility, and pregnancy outcome after varicocelectomy based on testicular histology. Fertil Steril. 2005;83:362-6.
80. Jorge KA, Sanchez AA, Gallegoss DG. The fluidity of erythrocyte membrane in the presence of phenolic compounds. Chinese Journal of magnetic resonance. 2005;22(4):349-356.

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