Pathophysiology of varicocele: evidence for oxidative stress as a mechanism pathway

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SUMMARY

There has never been an unswerving animal model for the study of varicocele; neither has a stable result been obtained. This has been as a result of personal interpretation of venous anatomical differences between human and rat models. Although the pathogenesis of varicocele remains uncertain, there is a growing body of data implicating hyperthermia, venous pressure, testicular blood flow, hormonal imbalance, toxic substances, and reactive oxygen species.

The present study established the role of oxidative stress in the pathogenesis of varicocele using animal models. Four groups of rats were used, the first group served as the control, while the second, third and fourth groups of rats were varicocelized. The third and fourth group, in addition, had intraperitoneal and intramuscular treatment of 20 mg/kg and 25 mg/kg body weight of zinc chloride and alpha-tocopherol respectively. Fifty six days after, testicular weights and volumes, histology, morphometry, enzymatic and non-enzymatic antioxidants were evaluated.

Result showed that the testes of varicocelized models treated with antioxidants had better oxidative status, geometric values and histological profiles compared to the untreated varicocelized models. These results indicated and validated the role of reactive oxygen in the pathogenesis of varicocele.

Key words: Varicocele – Oxidative stress – Alpha-tocopherol – Zinc – Pathophysiology

INTRODUCTION

Through intracellular signal transduction, generation of reactive oxygen species drives the capacitation, acrosomal reaction and attachment of spermatozoa to the oocyte, hence reactive oxygen species (ROS) are paramount for proper sperm function (de Lamirande and Gagnon, 1995). Although equilibrium usually exists between ROS production and removal, pathologic circumstances pave the way for excess production of ROS (Aitken and Clarkson, 1987; de Lamirande and Gagnon, 1995). Such buildup of ROS may result from pathological dilation of the veins of the spermatic cord and subsequently result in testicular toxicity. British surgeon T.B. Curling in 1843 was the first researcher to describe the pathological dilation of veins of the spermatic cord using the term "varicocele" (Eisenberg and Lipshultz, 2011). Varicocele is the most common and treatable cause of male infertility, characterized by abnormal tortuosity of the veins of the pampiniform plexus that drains the testis, (Shiraishi et al., 2009). The mechanism of action by which varicocele causes degeneration of the testis and

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sperm dysfunction has not been clearly indicated (Santoro et al., 2001). However, increased testicular and scrotal temperature, decrease in gonadotrophin and androgen secretion, reflux of adrenal toxic metabolites, testicular hypoxia by venous stasis and small vessel occlusion, leading to Leydig cell and germinal cell dysfunction have all been implicated in its pathophysiology (Marmar, 2001, Koksal et al., 2007; Shiraishi et al., 2009).

There is a balance in the seminal plasma between ROS production and removal, but in varicocele excess production of ROS has been indicated (Aitken and Clarkson, 1987; de Lamirande and Gagnon, 1995).

Although several possible pathophysiological causes of varicocele have been documented (Benoff and Gilbert, 2001; Marmar, 2001; Naughton et al., 2001; Schoor et al., 2001; Benoff et al., 2009), it is highly intricate to categorize a solitary or prevailing factor. It is to be expected that many of these etiological causes contribute to the sterile phenotype seen in medical practice. Current knowledge on the mechanism pathway of varicocele is built upon data extrapolated from the study of both human and animal models.

The important role of Alpha-tocopherol (Vitamin E) in forestalling lipid peroxidation and oxidative damage has been widely reported (Kumar and Derael, 1988). These studies have established Vitamin E as a soluble, chain-breaking and potent antioxidant (Kappus, 1987), and have contributed to the understanding of ROS, redox reaction and their mechanism pathway (Noguchi et al., 1973, Halliwell and Gutteridge, 1989).

On the other hand, avalanches of studies have shown that zinc performs paramount antioxidative functions (Dinsmore et al, 1985; Valberg et al., 1985; Powell, 2000; Zhanxiang et al., 2005). This was first indicated in 1990 as a result of in vitro evidence that paved the way for two distinct mechanisms of action (Mark et al., 2003). Zinc protection of proteins and enzymes against free radical attack was first reported in 1960 when it reversed delayed sexual development and arrested growth (Hong et al., 1984; Skandhan, 1992). It has also been reported that zinc prevents the formation of free radicals by other metals, such as iron and copper, due to its inability to readily undergo oxidation and reduction (Suga et al., 1984; Nordmann, 1994; Parat et al., 1997).

In animal models, treatments with oral antioxidants have been used effectively in the treatment of oxidative stress-associated injuries (Suziki and Sofikitis, 1999; Saalu et al., 2008; Ozokutan et al., 2000; Akunna et al., 2012). The rationale in the rear of this study is to evaluate and elucidate the key role of reactive oxygen species in the pathophysiology of experimental varicocele, add-

ing to the body of knowledge about the protective efficacy of antioxidant treatment in case of oxidative damage. While the existing studies may have inherent weaknesses, they do provide building blocks for futures studies into the pathogenesis of varicocele.

MATERIALS AND METHODS

Vitamin E injectionR (*Alpha* tocopheryl acetate) GB Pharma was obtained from Tabade Pharmacy, Akoka in Lagos, Nigeria, and Zinc chloride ($ZnCl_2$) was obtained from Merck, Darmstadt, Germany and olive oil was obtained from Roberts Laboratories Limited, Belton, England.

Experimental procedures

Forty adult male Sprague-Dawley rats (10 to 11 weeks old) weighing 200-250g were used for the study. The rats were randomly divided into four groups (A-D) of ten rats each, such that the average weight difference between and within groups did not exceed ± 20% of the average weight of the sample population. Group A served as the control and were treated with 0.5 ml/kg body weight of olive oil as the vehicle for 56 days. Group B, C and D rats served as experimental groups in which the animals were bilaterally varicocelized. Group C and D in addition had intraperitoneal administration of 20 mg/kg body weight of zinc chloride and intramuscular treatment of 25 mg/kg body weight of *Alpha*-tocopherol for 56 days respectively, the extent of spermatogenesis in rat being 51.6-56 days (Heller and Clermont, 1964; Jegou et al., 2002). The vitamin E and Zinc chloride solution was administered once daily by noon during six days (Monday to Saturday) within a week. The study is consistent with the standard of the use of laboratory animals (American Physiological Society, 2002).

Experimental varicocele induction

Experimental varicocele was induced in the animals by anaesthetizing them with intra-abdominal injection of 7 mg/ kg body weight ketamine 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 in 1992.

Animal sacrifice and sample collection

The rats were first weighed and then anaesthetized by inserting them in a clogged jar which contains cotton wool wet with 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 preci-

sion 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°C for subsequent biochemical assays.

Determination of testicular enzymatic antioxidants

Assay of catalase (CAT) activity

Catalase activity was estimated based on the method of Aebi (1983). 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.

Assay of superoxide dismutase (SOD) activity

Superoxide dismutase activity was studied according to the method described by Rukmini et al. (2004). The principle of the assay was based on the ability of SOD to inhibit the reduction of nitroblue 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 560 nm. 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.

Assay of glutathione peroxidase (GPx) activity

Glutathione peroxidase activity was evaluated by the method described by Rotruck et al. (1973). 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. H2O2. 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.

Assay of testicular non-enzymatic antioxidants

Assay of testicular reduced glutathione (GSH) concentration

GSH was determined by the method of Ellman (1959). 1.0 ml of supernatant was treated with 0.5 ml of Ellmans reagent (19.8 mg of 5, 5'-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). 0.4 ml of distilled water was added. The mixture was thoroughly mixed and the absorbance was read at 412 nm, expressed as nmol/mg protein.

Estimation of lipid peroxidation (malondialdehyde)

Lipid peroxidation in the testicular tissue was studied colorimetrically by thiobarbituric acid reactive substances TBARS method of Buege and Aust (1978). 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 535 nm. Concentration was calculated using the molar absorptivity of malondialdehyde which is 1.56×10^5 M^{-1} cm-1 and expressed as nmol/mg protein.

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 (Gundersen and Jenson, 1987; Qin and Lung, 2002).

Diameter (D) of seminiferous tubules

The diameter of seminiferous tubules with profiles that were round or nearly round were estimated for each animal and a mean, *D*, was determined by taking the average of two diameters, *D1* and *D2* (Perpendicular to one another). *D1* and *D2* were taken no more than when *D1/D2 ≥ 0.85*.

Cross-sectional area (AC) of seminiferous tubules

The cross-sectional areas of the seminiferous tubules was estimated from the formula *AC = πD2/4*, (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)

The number of profiles of seminiferous tubules per unit area was determined using the unbiased counting frame anticipated by Gundersen (1977). Using this frame, in addition to counting profiles completely inside the frame we counted all profiles with any part inside the frame provided they did not intersect the forbidden line.

Numerical density (NV) of seminiferous tubules

This was determined by using the modified Floderus equation: $NV = NA/(D+T)$ (Gilliland et al., 2001) where, NA is the number of profiles per unit area, D is the diameter and T the average thickness of the section. The evaluation of the diameter was done with calibrated eyepiece and stage grids mounted on a light research microscope. Estimation of 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).

Statistical analysis

The data obtained were expressed as mean ± SD of number of experiments (n = 10). The level of homogenecity among the groups was tested using Analysis of Variance (ANOVA) as done by Snedecor and Cochran in 1980. Where heterogenecity 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 (Duncan, 1957).

RESULTS

Gross anatomical changes

Body weight

Figure 1 shows that animals in Group A had a significant (*P<0.05*) enhancement in body weight when compared to rats in other groups. Group B rats lost body weight significantly (*P<0.005*) when compared to their initial weight. Group B and D rats lost body weights when compared with their original weights. However the weight loss experi-

Fig 1. Effect of zinc chloride solution and α-tocopherol (20 mg/kg and 25 mg/kg body weight respectively) on the body weight (grams) of control and experimental model. * P < 0.05; ** P < 0.005 significantly different from control. Values are expressed as mean ± SD for n=10 in each group.
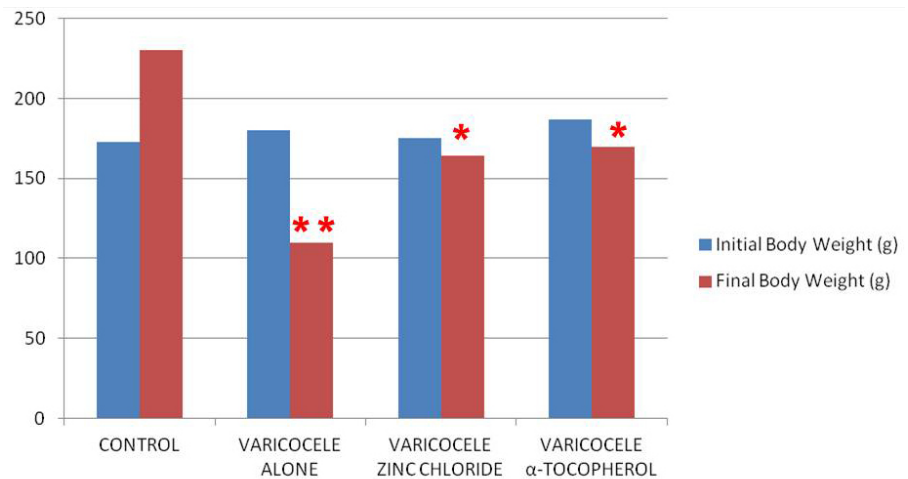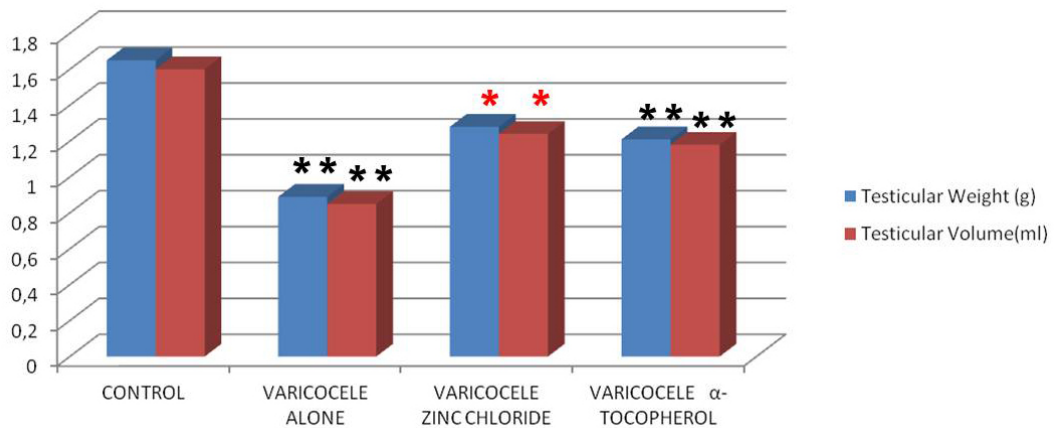

Fig 2. Effect of zinc chloride solution and α-tocopherol (20 mg/kg and 25 mg/kg body weight respectively) on the testicular weight (g) and volume (ml) of control and experimental groups. P < 0.05; ** P < 0.005 significantly different from control. Values are expressed as mean ± SD for n=10 in each group.

enced was not as significant as that of the un-treated varicocelized group.

Mean weights and volume of testes

The testicular weights and volumes of Group B rats 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 and zinc chloride solution. However, the testicular weights and volume of the control rats were fairly the same compared with their initial values (Fig. 2).

Testicular oxidative stress

Activities of testicular enzymes SOD, CAT and GPx

Experimental varicocele in Group B rats caused a statistically significant (*P<0.005*) decrease in SOD activity compared to control animals. Varicocelized rats that had zinc chloride solution and α-tocopherol showed a significantly (*P<0.05*) increased testicular SOD activity compared to those in Group B. As shown in Table 1, the testicular activities of CAT after zinc chloride solution and α-tocopherol treatment were similar to that of the control values. Group B rats, however, had a significant (*P<0.05*) reduction in testicular CAT activity compared to control rats. Concomitant treatment of varicocelized rats with zinc chloride solution and α-tocopherol caused a significantly (*P<0.05*) increased testicular CAT activity when compared to that of rats in Group B. The activities of CAT in Group C and D rats were in this roughly similar to that of the control values. The GPx activity of varicocelized rats treated with zinc chloride solution and α-tocopherol administration 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.

Testicular content of glutathione (GSH) and malondialdehyde (MDA)

A remarkable decrease in GSH content was observed in varicocelized rats that were not treated when compared to the control animals. Treatment of Group C and D rats with both zinc chloride solution and α-tocopherol significantly elevated the testicular content of GSH compared to animals that were only varicocelized.

As shown in Table 1, Group B rats had a significantly (*P<0.005*) elevated testicular MDA as compared to the control value. Co-administration of zinc chloride solution and α-tocopherol exhibited caused a remarkable reduction (*P<0.05*) in the testicular MDA level compared to rats in Group B.

Testis geometry

As shown in Table 2, the mean seminiferous tubular diameters of untreated varicocelized rats were significantly reduced (100.2±4.1 μm) as compared to that of the control groups (162.2±3.1 μm). However, there was a significant ($P<0.05$) increase in the tubular diameter of animals treated with 20 mg/kg and 25 mg/kg body weight of zinc chloride solution and α-tocopherol for 56 days respectively (139.2±5.1 and 152.1±2.3 μm respectively) as compared to tubular diameter of

Table 1. Effect of zinc chloride solution and alpha-tocopherol (20 mg/kg and 25 mg/kg body weight, respectively) on testicular enzymatic antioxidants (SOD, CAT and GPx) and testicular non-enzymatic antioxidants (MDA and GSH).

Parameters	Control	Varicocele-alone	Varicocele-zinc	Varicocele – vitamin E
SOD *(u/mg protein)*	7.31±1.4	3.83±2.1**	6.51±1.1*	5.11±1.7*
CAT *(u/mg protein)*	378.22±1.8	353.4±9.1*	380.01±2.1	375.1±0.1
MDA *(nmol/mg protein)*	22.71±4.3	49.03±9.1**	21.2± 1.1	29.1±3.0*
GSH *(nmol/mg protein)*	8.92±5.1	6.7±1.1*	9.01±1.9	9.3±7.1
GPx *(nmol/mg protein)*	0.95±2.2	0.35±3.21**	0.9±2.2	0.7±1.3*

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

Table 2. Effect of zinc chloride solution and alpha-tocopherol (20 mg/kg and 25 mg/kg body weight, respectively) on seminiferous tubular diameter (μm), cross sectional area (μm^2), numerical density of seminiferous tubules (μm^{-3}) and number of profiles per unit area (μm^{-2}).

Treatment groups	D (μm)	A_c (×10^{3}μm^2)	N_A (×10^{-8}μm^{-2})	N_v (×10^{-10}μm^{-3})
Control	162.2±3.1	28.3±4.2	26.21±4.1	11.1±4.5
Varicocele-alone	100.2±4.1	14.2±6.1	12.34±6.2	6.2±8.1
Varicocele/zinc	139.2±5.1	20.1±1.1	19.1±4.1	8.1±3.3
Varicocele/vitamin E	152.1±2.3	19.2±3.12	29.4±8.1	9.8±2.1

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

the control groups. The disparity in the cross-sectional area of the tubules, the number of 13 tubular profiles per unit area and the mean numerical density of seminiferous tubules of the Group B (14.2±6.1, 12.34±6.2 and 6.2±8.1 respectively), C (20.1±1.1, 19.1±4.1 and 8.1±3.3 respectively) and D (19.2±3.12, 29.4±8.1 and 9.8±2.1 respectively) treaded a similar pattern as the tubular diameter (Table 2).

Testis histological profile

Figure 3 shows the representative sections of the seminiferous tubules of control animals were oval in outline with normal epithelium and intact interstitium. The histological profiles of the testes of varicocelized rats treated with zinc chloride solution and α-tocopherol were largely similar to those of the control counterparts with attenuated seminiferous epithelium and interstitium and mild degenerative changes (Figs. 5 and 6). There were evidences of degenerative changes in the

seminiferous epithelium characterized by interstitial oedema and vacuolization of the interstitium of rats in which experimental varicocele was induced without treatment (Fig. 4).

DISCUSSION

Depending on an individual point of view, varicocele has been considered the most indicated and the most "*correctable*" cause of infertility. These have resulted from a need for clear understanding of the implication of varicocele on spermatogenesis, spermatozoa maturation, motility, morphology and fertilizing capacity (Koksal et al., 2007; Shiraishi et al., 2009).

Consequently, there is a dearth of information on the pathophysiology of varicocele on testicular damage (Santoro et al., 2001; Saalu et al., 2008). It has been indicated that infertility in the case of varicocele, could be as a result of excess production of ROS resulting in testicular oxidative insult
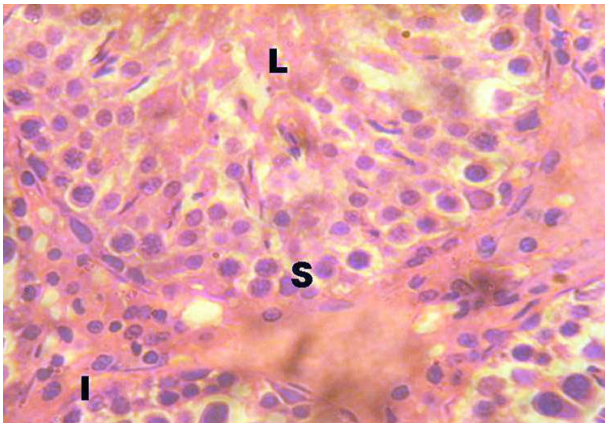

Fig 3. Control rat (group A) testes stained with H&E. L, lumen; S, seminiferous epithelium; I, interstitium. x 400.
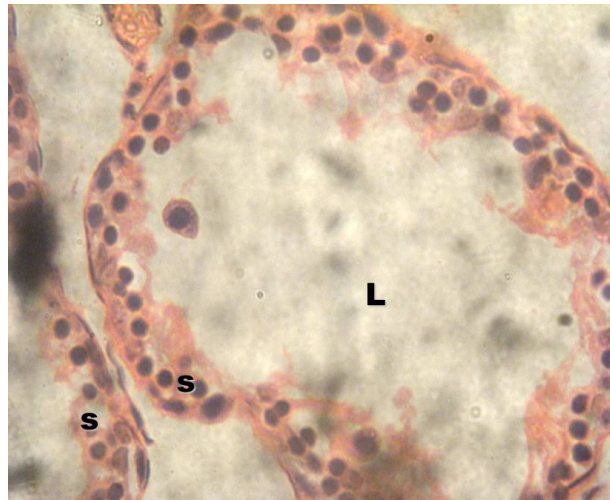

Fig 4. Varicocele-alone rat (group B) testes stained with H&E. L, lumen; S, seminiferous epithelium. x 400.
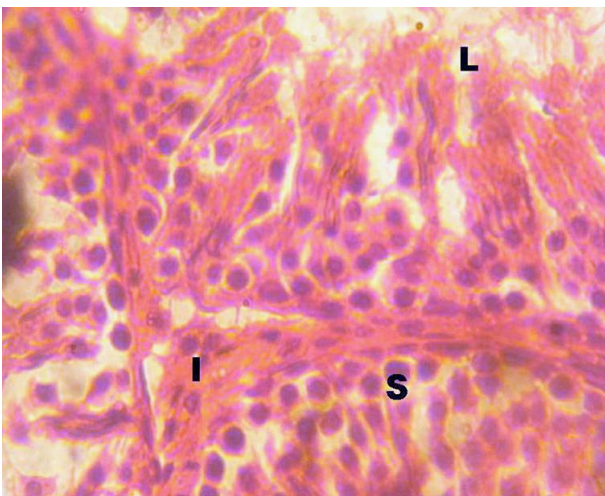

Fig 5. Zinc chloride (20 mg/kg) treated rat testis (group C). Testes were stained with H&E. L, lumen; S, seminiferous epithelium; I, interstitium. x 400.
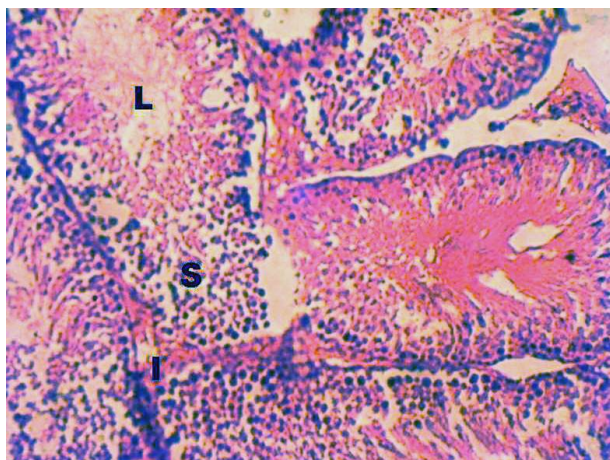

Fig. 6. Rat treated with 25mg/kg Vitamin E (group D). Testes was stained with H&E. L, lumen; S, seminiferous epithelium. x 250.

(Aitken and Clarkson, 1987; Saalu et al., 2008; Akunna et al., 2012). The present study was designed to evaluate the principal role of oxidative stress in the pathophysiology of varicocele using alpha-tocopherol and zinc, which have both been proven as potent antioxidants (Dinsmore et al., 1985; Kumar and Derael, 1988).

Our results herein indicates that varicocele caused a significant (P<0.005) body weight loss, as well as decrease in both testicular weights and testicular volumes in the animal models that were used. These findings are in conformity with previous reports, which indicated considerable evidences of decrease in testicular weight due to various oxidative derangements (Suziki and Sofikitis, 1999; Ozdamar et al., 2004; Saalu et al., 2011).

In 2003, Semercioz et al. reported a loss in testicular weight and volume in varicocelized animal models as a result of degenerative changes caused by heat from venous stasis in the seminiferous epithelium. The observed body weight loss might be due to reduction in the food intake by the varicocelized rats. Also, the process for achieving experimental varicocele confers a conspicuous level of stress on the rats and this could have affected their metabolic process, hence loss of body weight (Saalu et al., 2008; Akunna et al., 2012). The control group of animal had a significant (P<0.005) increase in body weight, which could mean that they were still in their active growth phase during the study (Saalu et al, 2008, 2011).

On the other hand, varicocelized models that were treated with with 20 mg/kg and 25 mg/kg body weight of zinc chloride solution and α-tocopherol for 56 days respectively demonstrated largely preserved body weight, testis weights and testis volumes which are indications of the attenuating role of antioxidant treatment.

Biomarkers such as malondialdehyde, superoxide dismutase, catalase, reduced glutathione and glutathione peroxidise are valuable when investigating oxidative stress in animal models (Halliwell and Gutteridge, 1998; Carlsson et al., 1995; Brigelius-Flohe, 1999; Harrison, 2002; Akunna et al., 2012).

In the present study a significant reduction in the activity level of antioxidant enzymes and testicular content of SOD (P<0.005), CAT (P<0.05) and GPx (P<0.005) were observed in varicocelized rats that were not treated compared to that of the control animals. There was also a significant elevation in the level of MDA (P<0.005), signifying high lipid peroxidation as a result of experimental varicocele. There was also a slight change in the level of GSH which was not significant when compared to that of the control. Our findings are in accordance with several other reports (Faizi et al., 1994; Saalu et al., 2008; Akunna et al., 2012).

Decrease in activity level of CAT might have allowed more peroxide conversion to toxic hydroxyl radicals which could have contributed to severe oxidative damage in varicocelized animals (Aitken, 1995; Hendin et al., 1999; Saalu et al., 2008). Although CAT has been 16 established as a powerful antioxidant and its localization restricted to the peroxisome, they do not donate electron, and for this reason its ability to detoxify hydrogen peroxide completely is aided other antioxidants. The reduction in activity level of GPx could be as a result of epididymal degeneration caused by varicocele. Although GPx is found and secreted in the caput and cauda epididymides, it is located exclusively in the epididymis (Hall et al., 1998; Rejraji et al., 2002).

It constitutes 6 % of the secretory epididymal proteins (Fouchecourt et al., 2000), thus, the protection of the testes and sperm membrane against oxidative insult is a possible function of this epididymis-specific isoform (Vernet et al., 1999).

Remarkably, varicocelized rats treated with 20 mg/kg and 25 mg/kg body weight of zinc chloride solution and α-tocopherol for 56 days respectively, had a significantly increased activity level of GPx (P<0.05 and P<0.005 respectively) and reduced lipid peroxidation which is evidenced by significant (P<0.05) reduction in level of MDA.

Faizi et al. in 1994 indicated that enhancing the antioxidant system levels can favour reproductive potentials since the sperm cytoplasm contained very low concentrations of scavenging enzymes.

The findings in our study indicated the role of reactive oxygen species in the pathogenesis of varicocele. This is in accordance with previous reports (Alvarez et al., 1987; Weese et al., 1993; Mazzilli et al., 1994; Cocuzza et al., 2008; Saalu et al., 2008). Our findings also showed that histological profile of the testes of varicocelized rats treated with 20 mg/kg and 25 mg/kg body weight of zinc chloride solution and α-tocopherol respectively, were largely similar to those of the control counterparts with attenuated seminiferous epithelium, interstitium and mild degenerative changes. However, there were evidences of degenerative changes in the seminiferous epithelium characterized by interstitial oedema and vacuolization of the interstitium of rats in which experimental varicocele was induced without treatment of any sort. Our findings are in agreement with our previous report implicating varicocele in testicular degeneration (Saalu et al., 2008; Akunna et al., 2012). Describing histological sections with stereological

methods helps to unravel some essential issues allied with qualitative microscopic investigation.

Our results showed a significant (P<0.05) reduction in the mean seminiferous tubular diameters of untreated varicocelized rats as compared to that of the control groups. However, there was a significant (P<0.05) increase in the tubular diameter of animals treated with antioxidants as compared to tubular diameter of the control groups. There was also significant differences 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 (P<0.005), Group C (P<0.05) and Group D (P<0.05) which followed the same pattern as that of seminiferous tubular diameter. Our findings indicated the role of antioxidants in ameliorating the testicular toxicity as a result of varicocele. And this is in conformity with previous report (Faizi et al., 1994; Jorge et al., 2005; Cocuzza et al., 2008; Saalu et al., 2011). Three dimensional deductions obtained from the present study provide quantitative evidence that zinc and Alpha-tocopherol attenuated testicular insult induced by experimental varicocele (Saalu et al., 2008).

Conclusion

The increased oxidative stress resulting from varicocele in testicular tissue might be accountable, at least in part, for the histopathological changes evidenced in our study. 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. Nevertheless, the three dimensional evaluations obtained in this study are a sound conclusion of the histo-morphometric characteristics of experimental varicocele in animal models.

The study, however, did implicate oxidative stress as a principal player in its pathogenesis, which was indicated by the attenuating efficacy of zinc and alpha-tocopherol.

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