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Histopathological Evaluation of the Effects of Vitamin C on Cisplatin-induced testicular damage in Wistar albino Rats

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ABSTRACT: Cisplatin is a very effective chemotherapeutic agent used in treating various types of cancer, but its application may have damaging effects on testicular morphology. In this study, the effect of vitamin C, a known antioxidant, on cisplatin-induced testicular damage was investigated. Thirty Wistar rats were grouped into six groups (A-F) of five rats per group. Cisplatin was given as a single dose of 8 mg/kg body weight 24 hours prior to daily vitamin C administration at l00 mg and 200 mg respectively, for two weeks in 2 ml distilled water by gastric gavage. The results showed that cisplatin caused reduction in weight which was insignificant ($P>0.05$), but this reduction in weight was excercebated to significant value ($p<0.05$) with vitamin C treatment. The Histology of the testis showed oedema, interstitial vascular congestion and mild hyperplasia of the Leydig cells in rats treated with 8 mg/kg body weight of cisplatin only, while those that received cisplatin and various doses of vitamin C had mild interstitial oedema and vascular congestion. It is noteworthy from the findings that vitamin C on cisplatin-induced testicular damage showed no remarkable protective effects.

Keywords: Histopathological evaluation, Vitamin C, Cisplatin, Testis, Wistar rats.

Introduction:

Vitamin C also referred to as ascorbic acid or ascorbate belongs to water-soluble class of vitamins (Wilson, 2005). It is a water soluble anti-oxidant and enzyme cofactor in plants and animals. Humans are one of the few species who lack the enzyme that catalyzes the final step of the biosynthetic pathway (Linster and Van Schaftingen, 2007). The fact that humans lack the terminal enzyme in biosynthetic pathway of ascorbic acid is because the gene encoding for the enzyme has undergone substantial mutation (Erichsen *et al.,* 2007). The identification of DNA sequence in humans revealed that man had many similarities but not quite the same as the rat gene that codes for the enzyme L-gulono-gamma-lactone oxidase (Inai Ohta and Nishikimi, 2003). Ascorbate is reported to play a role in various hydroxylation reactions and in the redox homeostasis of sub-cellular compactments, including the modulation of pro and anti-carcinogenic mechanisms such as the transport of vitamin C and the anti-oxidant enzyme function (Angulo *et al.,* 2008; Michels *et al,* 2013). Vitamin C (ascorbic acid) has been associated with fertility in primates and it may have evolutionary significance (Zhang *et al.,* 2003). It has also been shown to be important for reproduction in several other mammalian species (Luck *et al.,* 1995; Azari *et al*., 2014). Low or deficient ascorbate levels in humans have been associated with low sperm counts, increased number of abnormal sperm and reduced fertility (Das *et al.,* 2002; Akmal *et al*., 2006).

Studies have shown that antioxidants such as vitamin C, a content of several fruits and vegetables protects spermatozoa from reactive oxygen species (ROS)-induced production of abnormal spermatozoa, stimulate spermatozoa production and prevents agglutination (Fraga *et al.,* 1991; Das *et al.,* 2002; Azu *et al.,* 2011). In men who had decreased sperm count and abnormal motility, the vitamin C level was found to be decreased. This is possible because vitamin C is an important cofactor for hydroxylation of collagen which is an important component of the extracellular matrix and

malfunction of cell-cell interaction of the testicular cell could lead to abnormal spermatogenesis and infertility problem (Yazama *et al.,* 2006). It may therefore benefit fertility in its ability to promote collagen synthesis, hormone production and protection of cells from free radical (Luck *et al.,* 1995; Azari *et al*., 2014).

Vitamin C, a major antioxidant in the testis (Augustiine *et al.,* 2005) neutralizes ROS and enhances hormone production (MSEI- Neweshy and El- Sayed, 2011). It accumulates in both tissues of the ovary and testes and as in males; it has been suggested as a regulator of female fertility (Luck *et al.,* 1995). Although humans lack the enzyme for its final synthesis, It is known that tissue content of vitamin C varies but the highest concentrations occur in the pituitary, adrenal, gonads, leucocytes, eyes and brain but low levels are found in red blood cells and in extracellular fluids such as plasma and saliva (Luck *et al.,* 1995; Jacob and Sotoudeh, 2002). Its supplementation has been reported to cause increase in the epididymal sperm concentration and serum testosterone level, thus improving semen quality (Sonmez *et al,* 2005; Akmal *et al.,* 2006).

Studies have shown that the weight of the testis and accessory sex organs was significantly decreased by toxic substances such as cisplatin (Atessahin *et al.,* 2006; Cherry *et al.,* 2004), and that administration of vitamin C reversed this reduction (Das *et al.,* 2002). Vitamin C intake was associated with semen quality in human (Eskenazi *et al.,* 2005), in rabbit (Yousef *et al.,* 2003) and in rat (Sonmez *et al.,* 2005).

Cisplatin [Cis-diamminedichloroplatinurn (II) (CDDP] is a chemotherapeutic agent used in treating various types of cancers including sarcomas, some carcinomas (e.g. small cell lung cancer, testicular cancer, ovarian cancer, lymphomas and germ cell tumors (Colpi *et al.,* 2004). This class of drugs reacts *in vivo,* binding to and causing cross linking of DNA, which ultimately triggers apoptosis (Kartalou *et al.,* 2001). Chemotherapy with cisplatin can have profound and long lasting effects on spermatogenesis (Vawda, 1994). Adult rats exposed to cisplatin had a dramatic reduction in serum and intratesticular testosterone seven days after exposure (Maines *et al.,* 1990; Vawda *et al.,* 1994), but cisplatin exposure did not decrease serum luteinizing hormone or follicle stimulating hormone levels. The cisplatin induced changes in testosterone were associated with a decreased number of luteinizing hormone receptors on Leydig cells and with inhibited P_{450} side-chain damage activity (Maines *et al.,* 1990). These cisplatin-induced changes in hormonal regulation of spermatogenesis were interpreted as a primary effect of Leydig cells, with relatively sparring of the hypothalamus and pituitary (Maines *et al.,* 1990).

Changes in Sertoli cell (the seminiferous epithelium of the testis where the germ cells develop) structure and function following cisplatin exposure have indicated that this cell is a target cell for toxicity (Ataman *et al.,* 2015). A decrease in serum and epididymal androgen binding protein levels further indicated an effect of cisplatin exposure on Sertoli cell function (Huang *et al,* 1990). Primary cultures of Sertoli cells exposed to cisplatin had reduced production of transferin androgen binding protein, lactate and extradiol (Huang *et al.,* 1990). Also germ cell apotosis has been reported as another mechanism by which cisplatin causes testicular damage (Zhang *et al.,* 2001; Cherry *et al,* 2004).

The dynamics of cisplatin-induced germ cell apoptosis having been evaluated confirmed that the germ cell death that results from cisplatin exposure due to apoptosis were positive by terminal deoxy-neucleotidyl transferase-mediated nick end labeling staining (Zhang *et al.,* 2001; Seaman *et al.,* 2003).

Redox status within spermatozoa or in semen has been reported to partially account for the aetiology of infertility (Angulo *et al.,* 2011). Treatment involving antioxidants have also been used successfully to decrease oxidative stress related injuries in many organs as well as the testes (Suzuki and Sefikitis, 1999; Saalu *et al,* 2006). Considering that oxygen radical scavengers provide significant restoration of testicular function after testicular vascular disease (Saalu *et al.,* 2006; Sibel *et al.,* 2009) and cisplatin reduces fertility and induces germ cell apoptosis and necrosis (Salem *et al.,* 2012; Ataman *et al.,* 2015)), this study was aimed at evaluating the effects of sole administration of synthetic vitamin C on testicular morphology in rats that have been treated with cisplatin. Humans normally consume vitamin C either in the synthetic form as drug or in fruits and vegetables as natural products. To what extent can the synthetic form of vitamin C, administered solely rather than as a component of plant extract with other phytochemical components remedy testicular tissue from cisplatin- induced damage is the focus of this study.

Methodology:

The two drugs (cisplatin and vitamin C) used were bought from Monic-Tee Pharmacy Ltd No. 47, Ugbowo-Lagos Road, Opposite UBTH, Benin City, Edo State.

Animals and Grouping: Thirty (30) adult Wistar albino rats weighing between 200-280 g were used in this study. The rats were purchased from the animal holding of Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Edo State, Nigeria where they were housed and maintained in wooden cages with proper ventilation. The rats were allowed access to water and fed with finishers mash produced by Edo feeds and flour mill limited, Ewu, Edo State. After three week period of acclimatization, they were weighed and categorized into six groups of five animals per group.

Group A served as the main control group.
Group B rats were given 8 mg/kg body weight of cisplatin in 2 ml distilled water intraperitoneally, once.
Group C rats were given 100 mg/ kg body weight of vitamin C in 2 ml distilled water once daily for two weeks prior to intraperitoneal injection of 8 mg/kg body weight of cisplatin.
Group D rats were administered cisplatin at 8 mg/kg body weight and 24 hours later, they were given 100 mg/kg body weight of vitamin C once daily, for 2 weeks.
Group E rats were administered 200 mg/kg body weight for two weeks before, and after intraperitoneal injection of cisplatin at 8 mg/kg body weight.
Group F rats received 100 mg/kg body weight of vitamin C daily, dissolved in 2 ml distilled water for two weeks without cisplatin treatment.
At the end of the experiment, the animals were weighed and recorded.
Histopathology: On the 14th day of Vitamin C and cisplatin administration to the Wistar rats, they were sacrificed by cervical dislocation. The organs were harvested and fixed in Bouin's fluid for 24 hours, after which it was processed (Drury *et al.,* 1980) using the automated processor and stained with haematoxylin and eosin. The prepared tissues were viewed on the binocular light microscope at magnification x100 and x400 for histological features.

Statistical Analysis: Data were expressed as Mean ± standard error of the mean (SEM). Analysis was done using one-way ANOVA at $p \leq 0.05$ level of significance. Separation of means was done using Duncan multiple range test (Duncan, 1957).

Results

The bodyweight of the rats before experiment was compared with the weight after the experiment and it was observed that there was reduction in weight of the rats in all the groups except group (A) which is the control group. The highest reduction in weight was observed in the rats in group E which were administered with cisplatin and higher dose of Vitamin C (200mg). Group F, the group administered with the diluent or conveying vehicle had the least weight loss.

Thus, rats of the control group A changed from an initial mean weight value of 215 ± 5.00 to final mean weight value of 248 ± 2.55. The rats in group B which received 8 mg/kg of cisplatin experienced insignificant ($p>0.05$) weight loss from initial mean weight value of 240 ± 6.89 to a final mean weight of 230 ± 9.30. Group C rats which received l00 mg/kg body weight of vitamin C experienced insignificant ($p>0.05$) weight loss from 261 ± 7.48 to final mean weight value of 251 ± 8.72. Group D rats experienced significant ($p<0.05$) loss in weight from initial mean weight of 260 ± 1.87 to final mean weight of 217 ± 3.74, while Group E rats also experienced significant ($p<0.05$) weight loss from initial mean weight value of 263 ± 3.74 to final mean weight of 209 ± 5.10. These two groups, D and E were both administered with 8 mg/kg body weight of cisplatin but the difference was that group D received l00 mg/kg body weight of vitamin C while group E received 200 mg/kg body weight of vitamin C. Group F rats that received 2 ml of distilled water experienced insignificant ($p>0.05$) loss in weight from an initial mean value of 258 ± 5.83 to 251 ± 5.79 as final mean value.

Thus the difference in the mean weight values in groups D and E animals before and after experiment was found to be significant statistically compared to control ($P < 0.05$). Conversely, those of groups B, C and F experienced weight loss that was however not statistically significant ($P>0.05$).

Histopathology: The histological report on the testis of the rats in group A (control) showed normal features. The section revealed the seminiferous tubule, and the Leydig cells found in the interstitial spaces (plate 1). The micrograph of the testis of rats in group B that received cisplatin at 8mg/kg body weight and sacrificed 24 hours thereafter, revealed areas of mild interstitial congestion (A) and oedema (B) (plate 2). The ones sacrificed 72 hours post-cisplatin administration had mild interstitial congestion (A) and oedema (B) (plate 3), and the ones sacrificed two weeks post-cisplatin showed mild interstitial vascular congestion and hypertrophy (A), mild oedema (B) and mild hyperplasia of Leydig cells (C) (plate 4).

The micrograph of testis of the rats in group C that received l00 mg/kg body weight of vitamin C prior to cisplatin showed the testes had mild interstitial congestion and dilatation (A) (plate 5). The micrograph of testis of rats in group D that received 8mg/kg of cisplatin 24 hours after l00 mg/kg body weight of vitamin C showed mild interstitial vascular congestion (A) and oedema (B) (plate 6). The micrograph of testis of rats in group E that received 8mg/kg of cisplatin and a higher dose of vitamin C (200 mg) showed mild interstitial oedema (A) and vascular congestion (B) (plate 7). The micrograph of the testis of rats in group F that received 2 ml of distilled water showed unremarkable testicular architecture, implying that the diluent had no detectable effect on the testis, compared to control (plate 8).

Table 1: Variations in the mean weight value between the groups of experimental animals and their standard errors before and after the experiment.

Groups	Mean of initial weight and standard error of mean	Mean of the final weight and standard error of mean
A	215 ±5.00	248 ±2.55[a]
B	240 ± 6.89	230 ±9.30[a]
C	261 ±7.48	251 ±8.72[a]
D	266 ±1.87	217 ±3.74[b]
E	263 ±3. 74	209 ±5.10[b]
F	258 ±5.83	251 ±5.79[a]

Mean±SEM (n=5).
Mean with different alphabetic remark are significantly different from control ($p \leq 0.05$).

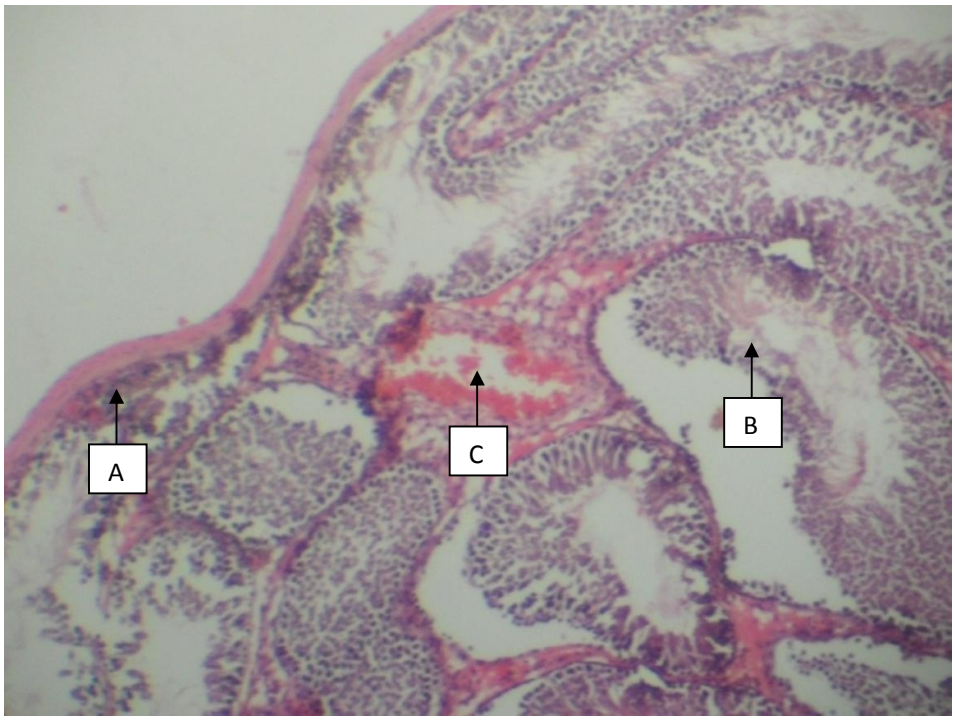

Fig 1: Control Testis showing tunica albuginea A, seminiferous tubules B and vascular interstitial spaces C (H&E x 100)

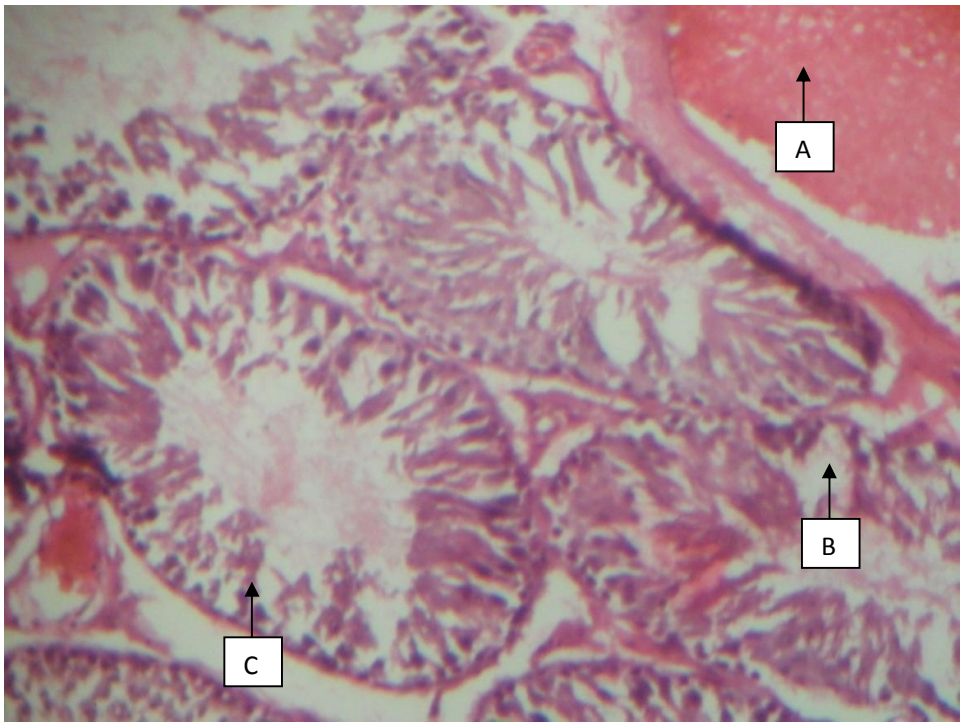

Fig 2: Rat Testis treated with 8mg/kg body weight of cisplatin and sacrificed after 24 hours showing mild interstitial congestion A, oedema B and distorted seminiferous epithelium C (H&E x 400)

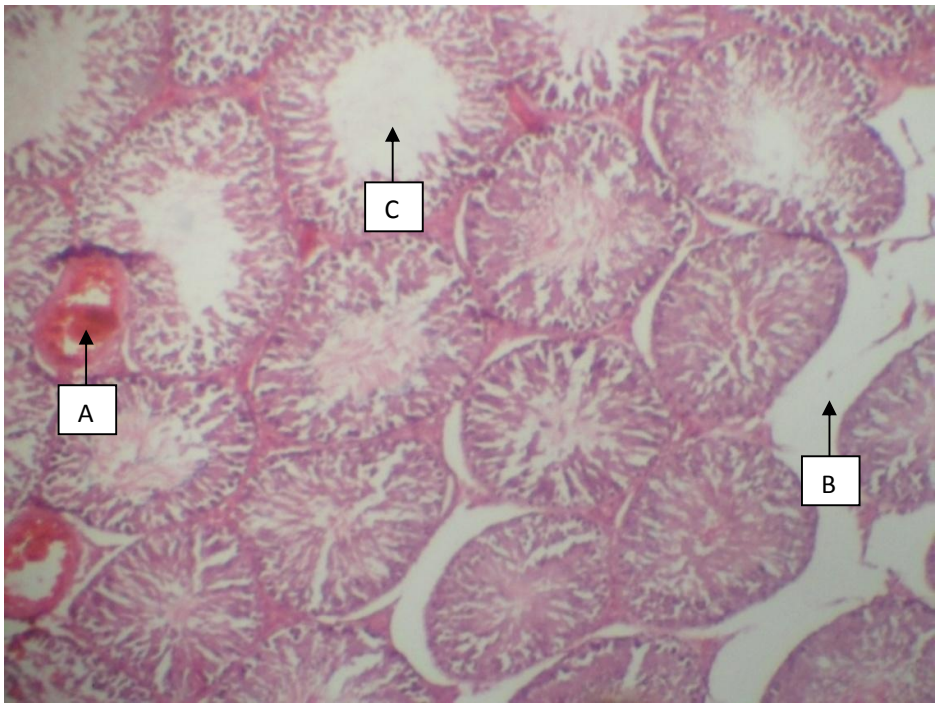

Fig 3: Rat Testis treated with 8mg/kg body weight of cisplatin and sacrificed after 72 hours showing mild interstitial congestion A, oedema B and luminal depletion of spermatozoa C (H&E x 100)

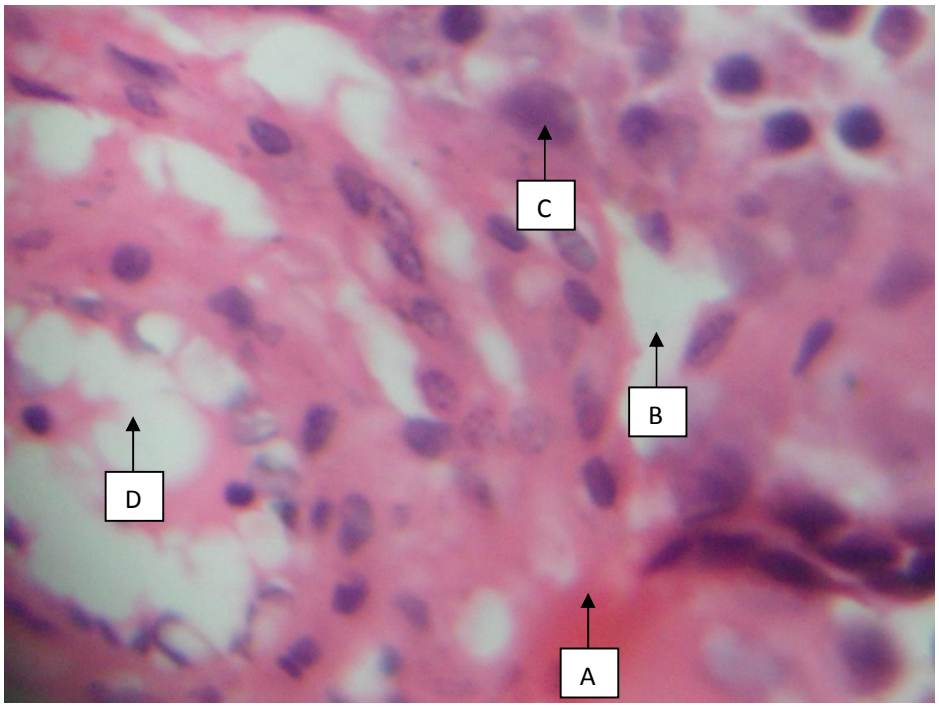

Fig 4: Rat Testis treated with 8mg/kg body weight of cisplatin and sacrificed after 2 weeks showing mild interstitial vascular congestion and hypertrophy A, mild oedema B, mild hyperplasia of the Leydig cells C and distorted seminiferous epithelium D (H&E x 400)

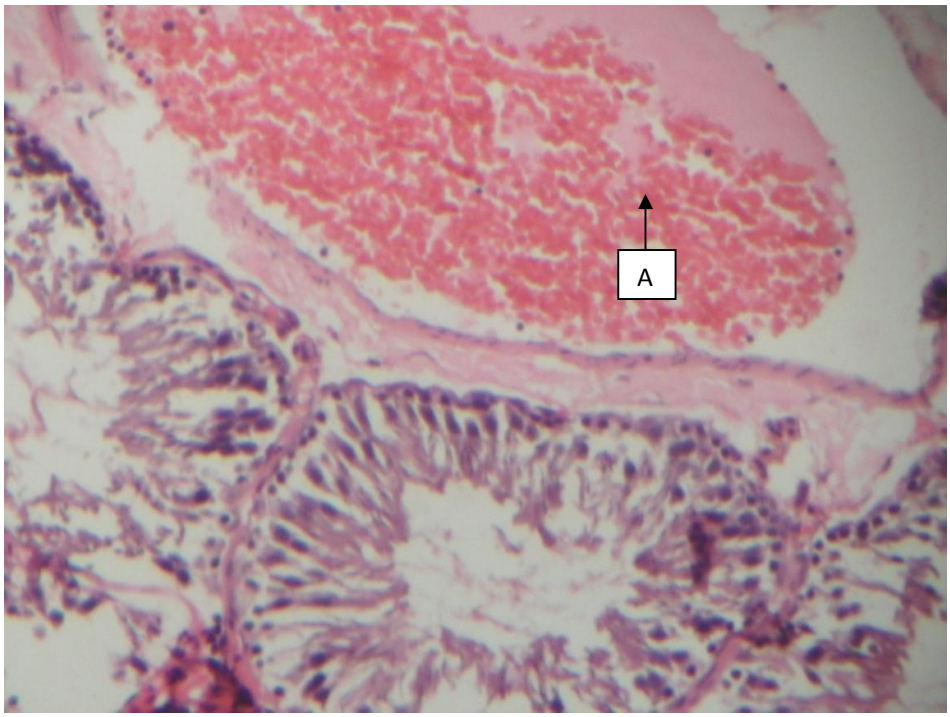

Fig 5: Rat Testis treated with 100 mg/kg body weight of vitamin C prior to cisplatin and sacrificed after 2 weeks showing mild interstitial congestion and dilatation A (H&E x 400)

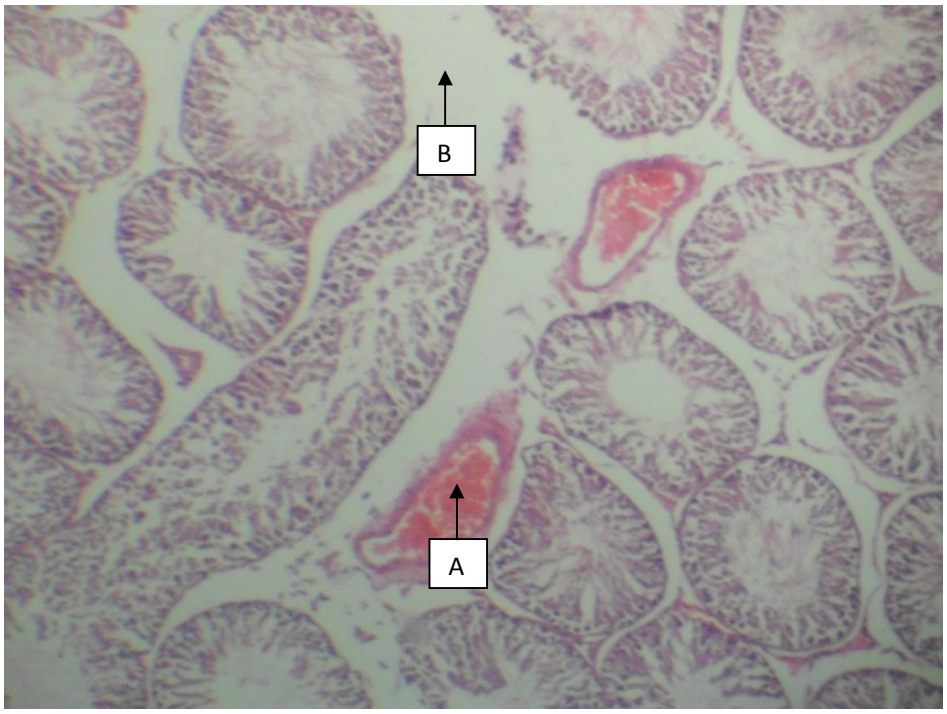

Fig 6: Rat Testis treated with 8mg/kg body weight of cisplatin and 100 mg/kg body weight of vitamin C and sacrificed after two weeks showing mild interstitial vascular congestion A and oedema B (H&E x 100)

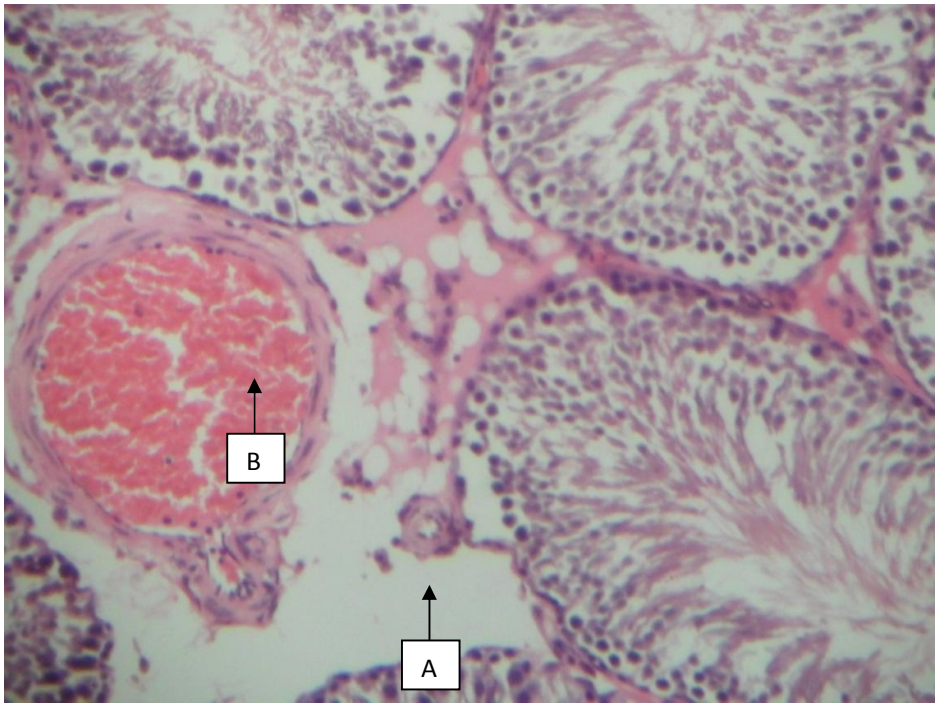

Fig 7: Rat Testis treated with 8 mg/kg body weight of cisplatin and 200 mg/kg body weight of vitamin C and sacrificed after two weeks showing mild interstitial oedema A and vascular congestion B (H&E x 400)

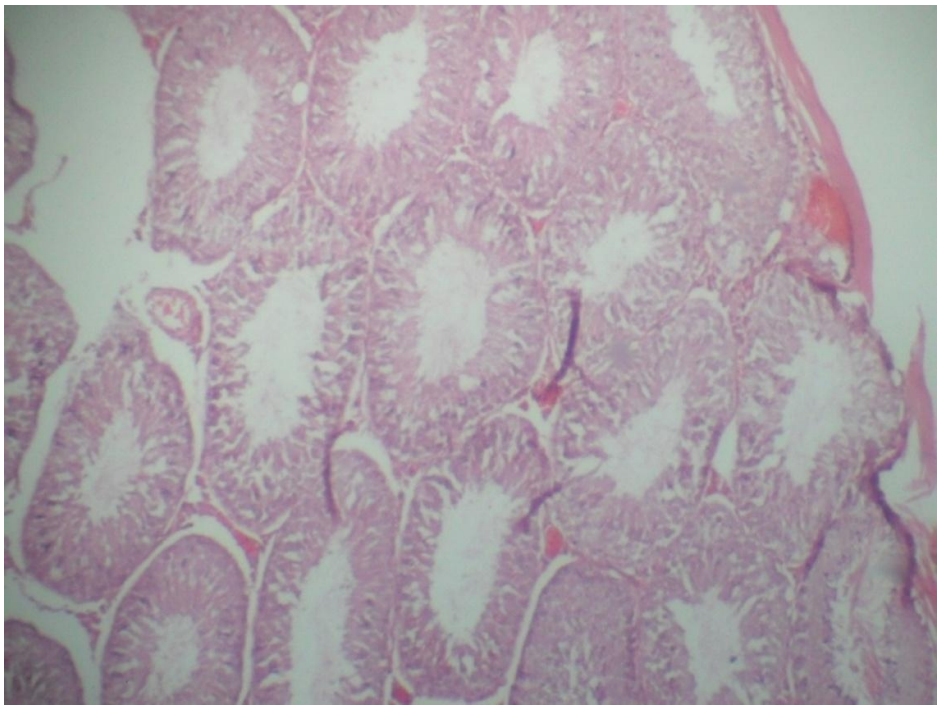

Fig 8: Rat Testis given 100 mg of vitamin C in 2 ml of distilled water and sacrificed after 2 weeks showing no remarkable distortion of testicular architecture (H&E x 100)

Discussion

Vitamin C has been associated for a long time with fertility (Luck *et al,* 1995; Das *et al.,* 2002) and being an antioxidant, scavenges free radical activities which suggests that it may modulate DNA damage in mammalian cells (Erichsen *et al.,* 2007; MSEI-Neweshy and El- Sayed, 2011). The effect of vitamin C treatment on testicular damage with cisplatin in this study resulted in significant change ($P < 0.05$) in the mean bodyweight values of groups D and E animals compared to control. The Sertoli cells of Leydig which forms the seminiferous epithelium of the testis provides nutritional support to the developing germ cells (Augustine *et al.,* 2005). Any distortion to the cytoarchitectural frame work of the germinal epithelium affects the nourishment of the testis and could cause weight reduction (Ataman *et al,* 2015). The reduction in weight in this study, might be attributed to poor delivery of vitamin C due to damaged Sertoli cells (Angulo *et al.,* 2011), because its deficiency has been reported to cause decrease in the weight of testis (Cherry *et al.,* 2004; Atessahin *et al.,* 2006). Conversely, those of groups B, C and F experienced weight loss that was however not significant ($P>0.05$) compared to control. This finding of weight reduction on treatment with cisplatin is consistent with previous reports (IIbey *et al,* 2009; Salem *et al.,* 2015).

Treatment with cisplatin also brought about changes in the histology of the testes such as mild interstitial congestion, oedema, mild interstitial vascular congestion, hypertrophy, and mild hyperplasia of the Leydig cells which was also noted by (Maines *et al.,* 1990; IIbey *et al.,* 2009). Oxidative stress might have contributed to this damage (Agarwal *et al.,* 2008). These effects were however not ameliorated with vitamin C treatment. This finding was in contrast with Appenroth's conclusion that vitamin C plays a role in protecting against cisplatin toxicity and damage in mammalian system (Appenroth *et al.,* 1997). Sertoli cells have been reported to express functionally active vitamin C transporters and may thus control the amount of vitamin C in the adluminal compactment as well as regulate the amount of this metabolite through one of the spermatogenesis process (Wilson, 2005; Angulo *et al.,* 2011). With the damage observed to the cyto-architecture of the testis in this study, it can be inferred that defective delivery of functionally active vitamin C may have contributed to the decreased size and the non-recovery of the normal testis. It should be noted also, that what was administered was the synthetic form of vitamin C and not its form in bio-active samples such as plant extracts which might have the complementary action of other antioxidants (Sibel *et al.,* 2009).

That the result showed that vitamin C did not have any significant protective effect on cisplatin-induced-testicular damage is not particularly strange with an earlier report (Narayana *et al.,* 2009) on vitamin C partial protection in cancer chemotherapy. This is further corroborated by earlier submissions (Weijl *et al.,* 1997) that administration of

antioxidants although may not compromise the antitumor effect of chemotherapeutic drugs, may not cause any remarkable protective effect. What is however yet to be conclusive on this study is the comparative role of vitamin C obtained from natural products under similar conditions, as well as the role vitamin C might play under prolonged therapy well beyond the spermatogenic cycle; an issue for further studies.

Conclusion

Cisplatin, although an effective chemotherapeutic agent, caused damage on the testes when given to rats at 8mg/kg body weight. Sole treatment with synthetic vitamin C formulation did not cause any significant reduction on the damaged testes. It is therefore not suggestive from this report to administer only vitamin C tablets on short-term as adjuvant in cisplatin chemotherapy, but perhaps on long-term and in combination with other anti-oxidants to minimize or prevent testicular damage.

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