



# Protective effect of vitamin C on chronic carbamazepine-induced reproductive toxicity in male wistar rats

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## ABSTRACT

The aim of this study was to evaluate the protective effect of vitamin C on chronic carbamazepine-induced reproductive toxicity in male Wistar rats. Four groups of 10 rats were respectively exposed to distilled water (2 ml/kg), vitamin C (100 mg/kg), carbamazepine (20 mg/kg) and vitamin C followed by CBZ, after 30 min.. The regimens were given by gavage once daily for 15 weeks. The pituitary glands and testicular tissues were assayed for oxidative stress parameters, sperm characteristics, relative weight and histological changes. Sera samples were also assayed for concentration of sex hormones. The results showed that treatment with vitamin C protected against the alteration in parameters measuring oxidative changes, sex hormones, sperm characteristics, relative pituitary and testicular weight and histological changes. The study concluded that protection against CBZ-induced alteration in reproductive parameters by vitamin C was partly due to its antioxidant effect.

## 1. Introduction

Carbamazepine is one of the major medications used for the treatment of epilepsy and neuropathic pain [1]. Its mode of action includes stabilization of the inactivated stage of the voltage-gated sodium channels, potentiating gamma aminobutyric acid (GABA) receptors as a GABA agonist, and serotonin releasing effect [2,3]. CBZ is metabolized in the liver, primarily by CYP450 3A4. CBZ and its metabolite (carbamazepine-10, 11-epoxide) are known to induce oxidative stress [4,5], by overwhelming the cellular antioxidant systems, resulting in cellular damage.

One of the systems mostly affected by carbamazepine toxicity is the reproductive system. CBZ administration causes reduced testicular weight, sperm concentration, testosterone, follicle stimulating hormone, and luteinizing hormone [6]. CBZ adversely alters semen quality in epilepsy diagnosed men [7]. Chronic exposure to CBZ causes increased level of plasma prolactin, which could lead to degeneration of the testicular tissue [6]. Indeed, environmentally relevant concentration of CBZ has been shown to have endocrine disrupting effect [8].

Oxidative stress seen in reproductive disorders [9] has been linked to CBZ-induced reproductive toxicity [8]. Oxidative stress evoked by chronic CBZ administration causes increased serum concentration of sex hormone-binding globulin in men [10]. The increased serum concentration of sex hormone binding globulin causes reduced bioactivity of serum androgens, and this, may be seen as reduced sexual

performance [11]. Severe oxidative challenges as seen in CBZ toxicity overwhelms the body's innate antioxidant mechanisms thereby resulting in oxidative injury. In this situation, supplementation with antioxidant molecules such as vitamin C becomes imperative [12]. Vitamin C, a water-soluble chain-breaking antioxidant, alleviate oxidative challenges evoked by chemical and environmental challenges [12]. It reduces free radical generation and maintain thiols of proteins, that built up antioxidant compounds [13,14]. Vitamin C is a powerful antioxidant both in plasma and intracellularly, inhibit lipoperoxidation, reduce endothelial dysfunction and regenerates vitamin E in lipoproteins and membranes [15]. Vitamin C protects spermatogenesis and plays a vital role in semen integrity and fertility in man and animals, increases serum testosterone [16,17] and prevents sperm agglutination [18].

The objective of this study was to evaluate the protective effect of vitamin C on biochemical, hormonal, sperm characteristics, pituitary and testicular pathological changes evoked by prolonged carbamazepine administration in male Wistar rats.

## 2. Materials and methods

### 2.1. Ethical approval

The study was in accordance with the specification of Ahmadu Bello University Committees on Animal Use and Care with the approval no:

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ABUCAUC/2016/040 and compliance with the National Research Council on Guide for Care and Use of Laboratory Animals (2011).

## 2.2. Experimental animals

The Forty (40) adult male Wistar rats (150–250 g), used for this studies were obtained from Animal House of the Department of Veterinary Pharmacology and Toxicology, University of Ilorin, Nigeria. They were housed in plastic cages and fed with commercial grower's feed (Top Feed®, Nigeria). They were provided access to water ad libitum. The rats were allowed to acclimatize for two weeks before the experiment commenced.

## 2.3. Drug acquisition and preparation

Carbamazepine (200 mg) (Tegretol®, Novartis Farma, Italy) and vitamin C tablets (100 mg, Biopharma, Nigeria) were purchased in a reputable pharmaceutical outlet in Ilorin, Nigeria. These were dissolved in appropriate volume of distilled water to make solution before use.

All other chemicals were of analytical grade and were obtained from a reputable chemical stores in Ilorin, Nigeria.

## 2.4. Dosing protocols

Forty male Wistar rats were randomly divided into four groups, with each group contain 10 animals and were exposed to the following regimens: Group I (DW) was given distilled water (2 ml/kg); Group II (VC) was administered vitamin C at 100 mg/kg [19]; Group III (CBZ) was exposed to carbamazepine at 20 mg/kg [20], while Group IV (VC + CBZ) was treated with vitamin C (100 mg/kg) then followed by carbamazepine (20 mg/kg) after 30 min. The regimens were given once in a day for 15 weeks through gavages. At the end of the treatment period, the rats were weighed using digital weighing balance (Sensor Disc Exclusive Tech, USA), then sacrificed by jugular venesection after light chloroform anaesthesia. Blood was collected into vacuum containers, containing the anticoagulant, potassium ethylenediaminetetraacetic acid (EDTA) and into plain containers without anticoagulant for serum. Tissue samples (pituitary gland and testis) were also collected for analysis.

## 2.5. Evaluation of sperm characteristics

### 2.5.1. Epididymal sperm count

One epididymis from each of the rats in each group was carefully removed and carefully evaluated as described by Verma and Chinoy [25].

### 2.5.2. Sperm motility

Sperm motility was evaluated using the method described by Sonmez et al. [17].

### 2.5.3. Sperm morphology

For sperm morphology evaluation, smear of sperm cell suspension was prepared, dried and fixed (three volume of absolute methanol and one volume of glacial acetic acid), stained with haematoxylin for 15 min and then washed, follow by staining with 1 % eosin for 10 min, washed and left to dry at room temperature [26].

## 2.6. Relative pituitary and testicular gland weights

Pituitary gland and testes were carefully dissected out and immediately weighed using electronic weighing balance (Golden-Mettler, USA). The relative organ weight of each animal was then calculated as follows:

$$\text{Relative organ weight (\%)} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

## 2.7. Tissue preparations

The pituitary gland and testicular tissue were weighed, rinsed with cold saline to remove blood and each tissue was homogenized in a known volume (5 mg/ml) of ice-cold phosphate buffer to make 1:5 w/v. They were then centrifuged at 2000 g for 10 min and the supernatant were subsequently used for the analysis of levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).

## 2.8. Evaluation of oxidative stress parameters

### 2.8.1. Pituitary gland and testicular tissue lipoperoxidation

The pituitary gland and testicular malondialdehyde (MDA) concentrations were evaluated using the double heating method [21].

### 2.8.2. Pituitary and testicular superoxide dismutase activities

The superoxide dismutase activity was analysed using SOD Assay Kit-WST (SOD-19160 water-soluble tetrazolium salt, Sigma-Aldrich, USA) as previously described [22].

### 2.8.3. Pituitary and testicular catalase activities

The pituitary and testicular catalase activity was analysed using Catalase Activity Assay Kit (ab83464, UK) as previously described [23].

### 2.8.4. Pituitary and testicular glutathione peroxidase activities

The glutathione peroxidase activity was analyzed using the Fortress Diagnostic Glutathione Peroxidase Assay Kits Protocol BXC051A (Antrim, UK), as described by Paglia and Valentine [24].

## 2.9. Evaluation of sex hormone concentration

### 2.9.1. Serum follicle-stimulating hormone and luteinizing hormone

The serum FSH and LH concentrations were assayed using the Follicle Stimulating Hormone Enzyme Linked Immunosorbent Assay (ELISA) Kit (FS232 F, Calbiotech Inc. USA) and Luteinizing Hormone Elisa Kit (LH231 F, Calbiotech Inc. USA), respectively according to manufacturer's instruction.

### 2.9.2. Serum testosterone concentration

Sera samples collected were assayed for testosterone concentration using ELISA method based on competitive binding principle. The resulting colour was measured using a spectrophotometer (UV/VIS 752, Pec Medical, USA) at 450 nm.

## 2.10. Evaluation of gross and histopathological changes

The pituitary gland and testis of each rat were examined for any gross lesion. Tissue samples were then prepared using the modified method for histological examination [27].

## 2.11. Statistical analysis

The data obtained were expressed as mean  $\pm$  SEM and then subjected to one-way analysis of variance (ANOVA) followed by *Turkey's post-hoc* multiple comparison test. GraphPad prism version 5.03 for windows (GraphPad Software, San Diego, California, USA) was used to analyse all the data. Values of  $p < 0.05$  were considered significant.

**Table 1**

Effect of vitamin C on epididymal sperm cells count, motility and morphology of adult male Wistar rats exposed to chronic carbamazepine.

Parameters	DW	VC	CBZ	VC + CBZ
Sperm count ( $10^6$ /ml)	137.00 $\pm$ 2.33 <sup>b</sup>	120.00 $\pm$ 1.16	93.33 $\pm$ 3.71 <sup>a</sup>	108.00 $\pm$ 5.13
Motility (%)	93.33 $\pm$ 1.67 <sup>c</sup>	76.67 $\pm$ 3.33	66.67 $\pm$ 3.33 <sup>a</sup>	74.37 $\pm$ 3.33
Head Abnormality (%)	1.55 $\pm$ 0.18	2.04 $\pm$ 0.17	2.32 $\pm$ 0.07	2.16 $\pm$ 0.08
Mid-piece Abnormality (%)	4.07 $\pm$ 0.18	4.94 $\pm$ 0.23	6.02 $\pm$ 0.41 <sup>d</sup>	5.52 $\pm$ 0.25 <sup>e</sup>
Tail Abnormality (%)	4.52 $\pm$ 0.42 <sup>b,f</sup>	5.66 $\pm$ 0.26	6.68 $\pm$ 0.43	6.17 $\pm$ 0.04

Values are expressed as mean  $\pm$  SEM (standard error of mean).

<sup>a</sup> = (p < 0.05) Vs DW, VC and VC + CBZ groups.

<sup>b</sup> = (p < 0.05) Vs VC + CBZ group.

<sup>c</sup> = (p < 0.05) Vs VC and VC + CBZ groups.

<sup>d</sup> = (p < 0.05) Vs DW and VC groups.

<sup>e</sup> = (p < 0.05) Vs DW group.

<sup>f</sup> = (p < 0.05) Vs CBZ and VC + CBZ group.

### 3. Results

#### 3.1. Effect of treatment on epididymal sperm count

A significantly (p < 0.05) lower epididymal sperm count was recorded in CBZ group as compared to the DW, VC and VC + CBZ groups. Also a significant (p < 0.05) reduction in epididymal sperm count in VC + CBZ group was recorded when compared to the DW group (Table 1).

#### 3.2. Effect on sperm motility

The percentage sperm motility in CBZ group reduced significantly (p < 0.05) when compared to that of DW, VC and VC + CBZ groups. A significantly (p < 0.05) higher percentage sperm motility was observed in DW group when compared to VC and VC + CBZ groups (Table 1).

#### 3.3. Effect on percentage sperm head abnormality

Although, no significant (p > 0.05) change in percentage sperm head abnormality in all the groups, relative decreased values were recorded in DW (33 %) and VC (12 %) groups when compared to CBZ group (Table 1).

#### 3.4. Effect on percentage sperm mid-piece abnormality

Significantly (p < 0.05), the percentage sperm mid-piece abnormality noticed in CBZ group was higher than observed for DW and VC groups. Although no significant (p > 0.05) change in percentage mid-piece abnormality in CBZ group, a relative increase was recorded in the VC + CBZ (11 %) group. The percentage sperm mid-piece abnormality in VC + CBZ group was significantly (p < 0.05) higher as compared to the DW group (Table 1).

#### 3.5. Effect on percentage sperm tail abnormality

The percentage sperm tail abnormality was significantly (p < 0.05) lower in the DW group unlike those in the CBZ and VC + CBZ groups (Table 1).

#### 3.6. Effect on relative pituitary gland and testicular weights

The relative pituitary gland weight in CBZ group was significantly (p < 0.05) lower as compared to VC + CBZ group, while relative decreased values were recorded in the VC (10 %) and DW (23 %) groups. The relative testicular weight in CBZ group showed no significant (p > 0.05) change but relative decreased values were recorded as compared to DW (11 %) and VC (10 %) groups (Table 2).

#### 3.7. Effect on pituitary gland and testicular gland malondialdehyde concentration

The MDA concentrations in the pituitary gland in the CBZ group increased significantly (p < 0.05) unlike observed in DW group, but relative increased value was recorded as compared to VC (10 %) and VC + CBZ (17.7 %) groups. Also, a significantly (p < 0.05) lower MDA concentrations in the pituitary gland in the DW group was observed when compared to VC and VC + CBZ groups. A significant (p < 0.05) increase in testicular MDA concentrations in the CBZ group was recorded when compared to DW, VC and VC + CBZ groups (Table 3).

#### 3.8. Effect on pituitary and testicular gland superoxide dismutase activity

A significant (p < 0.05) reduction of SOD activity in the pituitary and testicular glands was observed in CBZ group as compared to VC + CBZ group, while other groups show insignificant (p > 0.05) change (Table 3).

#### 3.9. Effect on pituitary and testicular gland catalase activity

Although, not significant (p > 0.05), relative lower values were recorded in pituitary CAT activity in CBZ when compared to that of VC (12 %) and VC + CBZ (10 %) groups. The CAT activity in the testis of CBZ group reduced significantly (p > 0.05) as compared to DW, VC and VC + CBZ groups (Table 3).

#### 3.10. Effect on pituitary and testicular gland glutathione peroxidase activity

A relative reduction in the activity of GPx in the pituitary gland in CBZ group was observed when compared to DW (19.6 %), VC (32.3 %) and VC + CBZ (39 %) groups. However, a significant (p < 0.05) reduction in GPx activity of the testis was seen in CBZ group when compared to VC and VC + CBZ groups, while relative decreased value was recorded as compared to DW (11.3 %) group (Table 3).

#### 3.11. Effect of treatments on follicle stimulating hormone, luteinizing hormone and testosterone concentrations

An insignificant (p > 0.05) change in the FSH concentration was recorded in all the groups. A relative reduction in LH concentration in CBZ group was observed when compared to DW (12.5 %), VC (13.2 %) and VC + CBZ (17 %) groups. There was a significant (p < 0.05) reduction in testosterone concentration in the CBZ group when compared to VC + CBZ group, while a relatively reduced value was recorded as compared to DW (11.6 %) and VC (11.1 %) groups (Table 4).

**Table 2**

Effect of vitamin C on relative pituitary and testicular organ weights of adult male Wistar rats exposed to chronic carbamazepine.

Parameter	DW	VC	CBZ	VC + CBZ
Pituitary (g)	0.080 ± 0.006	0.093 ± 0.007	0.103 ± 0.01 <sup>a</sup>	0.152 ± 0.011
Testis (g)	0.923 ± 0.032	0.937 ± 0.041	1.037 ± 0.029	1.050 ± 0.093

Values are expressed as mean ± SEM (standard error of mean).

<sup>a</sup> = (p < 0.05) Vs VC + CBZ group.

### 3.12. Effect on pituitary gland and testicular histological changes

Fig. 1 shows the effect on pituitary gland histology. The pituitary gland in CBZ group showed degenerative changes, characterized by neutrophil infiltrations surrounding the pituicytes and loss of cellular projections. Mild neutrophil infiltration was recorded in VC + CBZ group. No apparent histological abnormality was recorded in the DW and VC groups.

The testis of the CBZ group showed histological abnormality, characterized by degenerative changes as indicated by the spermatogenic arrest in the seminiferous tubules and presence of haemorrhage of the Leydig cell. Mild spermatogenic degeneration was recorded in VC group. No apparent abnormality was seen in VC + CBZ and DW groups (Fig. 2).

## 4. Discussion

The present study showed higher concentrations of MDA in the pituitary gland and testicular tissue of CBZ group, this conformed with previous findings [28,29]. MDA, is a by-product of lipoperoxidation, causing membrane damage, alters membrane fluidity and structural integrity, and inactivate membrane-bound enzymes [30,31]. The higher concentration of MDA might be as a result of increased oxidative challenge provoked by reactive oxygen species (ROS) activity in CBZ exposed animals. Pre-treatment with vitamin C shown decrease MDA concentrations in pituitary gland and testis apparently due to its antioxidant effect [32]. The free radical scavenging property of vitamin C is via abstract of hydrogen from ascorbate, to become monodehydroascorbate, which later attract electron and form dehydroascorbate [33]. The ROS is reduced to water, the oxidized ascorbate is relatively stable, unreactive and cause no damage [34].

The reduction in the pituitary and testicular SOD activity in CBZ group indicates that chronic administration of CBZ can induced reduction in the synthesis of the enzyme, increased its metabolism or inactivation simply due to increased superoxide production. The persistently higher lipoperoxidation as demonstrated by high level of MDA concentration in the CBZ group, may have caused low SOD activity due

**Table 3**

Effect of vitamin C on pituitary and testicular malondialdehyde (MDA) concentrations, superoxide dismutase, catalase and glutathione peroxidase activities of adult male Wistar rats exposed to chronic carbamazepine.

Parameters	DW	VC	CBZ	VC + CBZ
Pituitary MDA (µMol/mg protein)	0.301 ± 0.11 <sup>b</sup>	0.498 ± 0.06	0.555 ± 0.12 <sup>a</sup>	0.454 ± 0.03
Testicular MDA (µMol/mg protein)	0.528 ± 0.03	0.482 ± 0.02	0.700 ± 0.03 <sup>c</sup>	0.441 ± 0.02
Pituitary SOD (U/L)	0.232 ± 0.06	0.234 ± 0.02	0.232 ± 0.01 <sup>d</sup>	0.237 ± 0.04
Testicular SOD (U/L)	0.2313 ± 0.01	0.2314 ± 0.02	0.2308 ± 0.01 <sup>d</sup>	0.2318 ± 0.01
Pituitary CAT (µ/mg protein)	56.03 ± 1.59	60.26 ± 1.01	54.29 ± 2.30	59.77 ± 2.16
Testicular CAT (µ/mg protein)	52.91 ± 1.78	61.01 ± 0.75	38.48 ± 1.71 <sup>c</sup>	53.74 ± 2.39
Pituitary GPx (U/L)	208.4 ± 6.75	230.5 ± 21.28	174.2 ± 16.69	242.3 ± 11.12
Testicular GPx (U/L)	199.6 ± 8.62	236.9 ± 9.93	179.4 ± 10.81 <sup>e</sup>	228.6 ± 8.82

Values are expressed as mean ± SEM (standard error of mean).

<sup>a</sup> = (p < 0.05) Vs DW group.<sup>b</sup> = (p < 0.05) Vs VC and VC + CBZ groups.<sup>c</sup> = (p < 0.05) Vs DW, VC and VC + CBZ groups.<sup>d</sup> = (p < 0.05) Vs VC + CBZ group.<sup>e</sup> = (p < 0.05) Vs VC and VC + CBZ groups.**Table 4**

Effect of vitamin C on follicle stimulating hormone, luteinizing hormone and testosterone concentrations of adult male Wistar rats exposed to chronic carbamazepine.

Parameters	DW	VC	CBZ	VC + CBZ
FSH (ng/mL)	0.835 ± 0.02	0.851 ± 0.03	0.781 ± 0.01	0.853 ± 0.03
LH (mIU/mL)	0.834 ± 0.04	0.839 ± 0.02	0.741 ± 0.01	0.866 ± 0.08
Testosterone (ng/mL)	2.387 ± 0.07	2.375 ± 0.09	2.138 ± 0.08 <sup>a</sup>	2.730 ± 0.02

Values are expressed as mean ± SEM (standard error of mean).

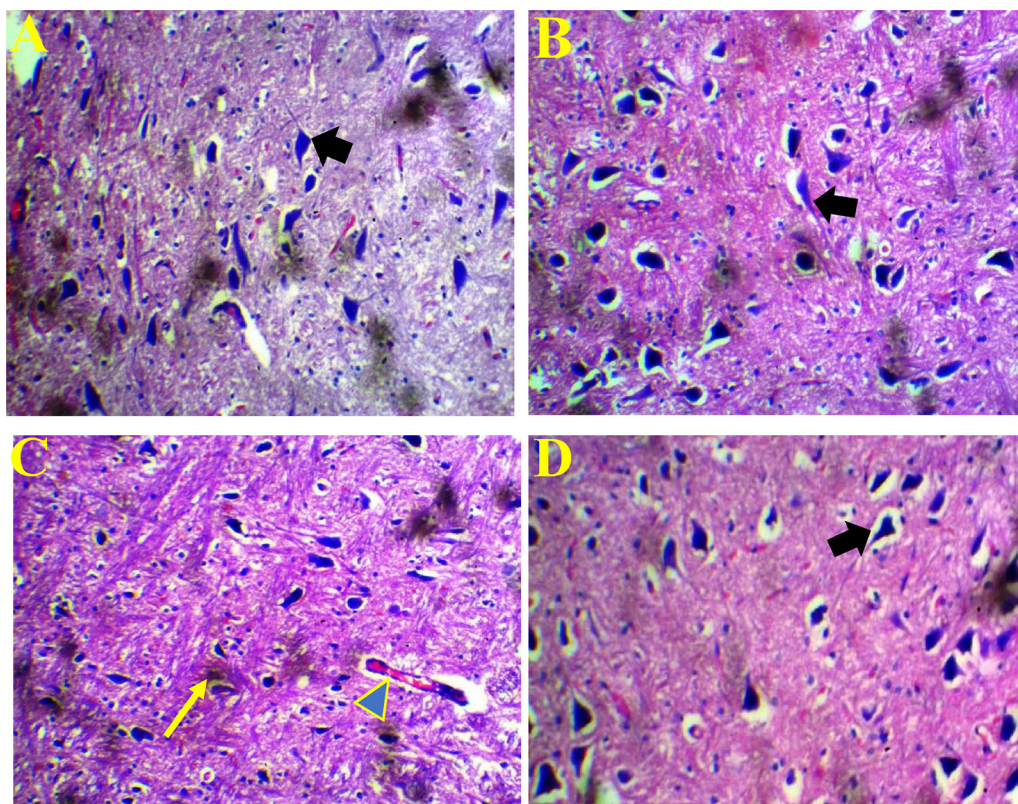
<sup>a</sup> = (p < 0.05) Vs VC + CBZ group.

to its increased usage to combat CBZ-evoked oxidative injury and then its subsequent metabolic degradation. SOD is the first line of defence that combat the deleterious effect of ROS [35]. The intracellular SOD functions by catalytically convert  $O_2^{\cdot-}$  to oxygen ( $O_2$ ) and hydrogen peroxide ( $H_2O_2$ ) [36]. The higher pituitary and testicular SOD activities in VC + CBZ was related to the antioxidant effect of vitamin C. Vitamin C (tends to donate its electrons, thereby preventing other compounds from being oxidized [37].

The lowered CAT activity in the pituitary and testicular tissues as seen in CBZ group may be partly due to the persistent long-term lipoperoxidative change induced by chronic administration of CBZ, as the rate of synthesis was not commensurate with the rate of utilization due to high amounts of free radicals in the biosystem. The apparent restoration in CAT activities observed in the VC + CBZ group is a demonstration of the antioxidant activity of vitamin C [38], which nullifies or mitigates the pro-oxidant effect of CBZ and accelerate its removal.

Our current data which showed decrease in pituitary and testicular GPx activity in CBZ group was in consistent with the report of Li et al. [39]. The GPx may have been depleted due to its response to oxidative injury induced by CBZ. The GPx is an antioxidant enzyme that protects the cytosol and plasma membrane from lipid peroxidation by catalyzing both the reduction of  $H_2O_2$  and organic hydroperoxides produced at the membrane level of water or corresponding alcohols [40]. The low GPx activity in the CBZ group indicates that  $H_2O_2$  and lipid peroxide are not

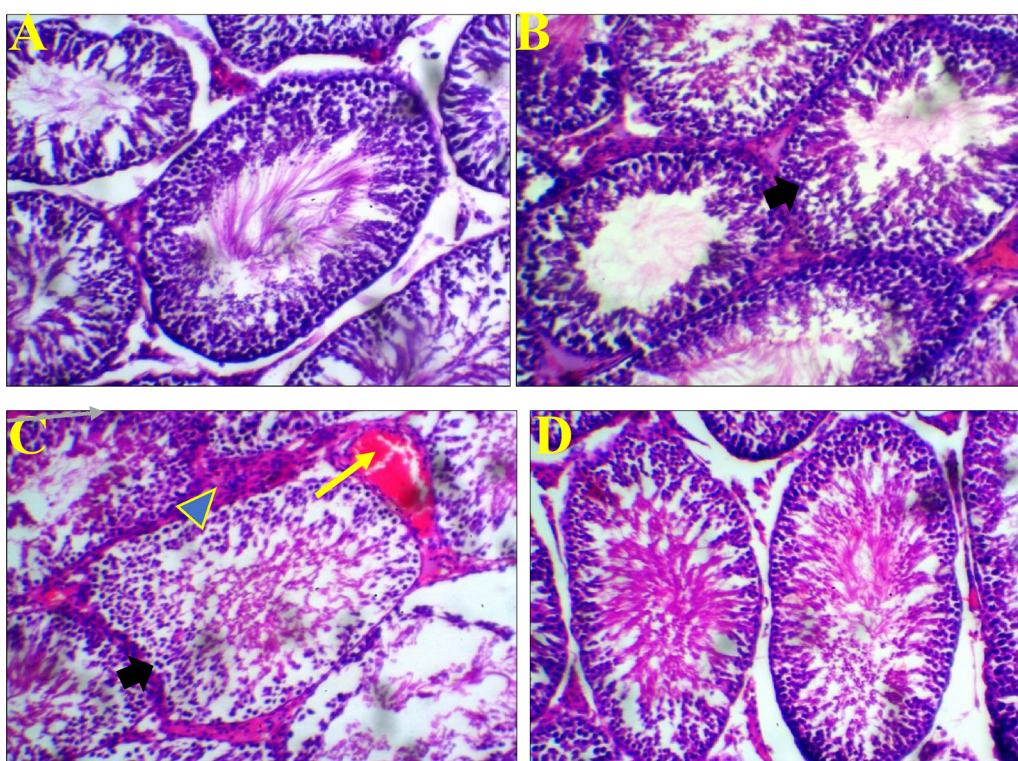




**Fig. 1.** Histological changes in the pituitary gland of rats chronically exposed to Distilled water (A), Vit-C (B), CBZ (C) and Vit-C + CBZ (D) H & E x 400. A: Normal pituicyte (block arrow). B: Normal pituicyte (block arrow) with no apparent histopathological lesions. C: Neutrophil (line arrow) infiltrations surrounding the pituicytes (line arrow), as well as loss of cellular projections lesion (triangle arrow). D: Normal pituicyte (block arrow) with no apparent histopathological lesions.

degraded and may be converted to OH<sup>•</sup> radicals and lipid peroxyl radicals respectively, by transition metals [41]. The result of our study showing increased activity of GPx following pre-treatment with vitamin C is a clear demonstration of the antioxidant effect of the vitamin. This effect protects against cellular damage and terminates harmful effects induced by CBZ [42].

In the current study, there was reduction in FSH and LH concentrations in the CBZ group when compared to other groups. Studies have shown that antiepileptic drugs have adverse effect on anterior pituitary which secretes FSH and LH responsible for spermatogenesis [39]. The lower serum FSH and LH concentrations in the CBZ group may be linked to the adverse effect of CBZ on gonadotropic releasing



**Fig. 2.** Histological changes in the testis of rats chronically exposed to Distilled water (A), Vit-C (B), CBZ (C) and Vit-C + CBZ (D). H & E x 400. A: No apparently histological lesions. B: Mild spermatogenic degeneration. C: Seminiferous tubules undergo degenerative changes (block arrow), indications of spermatogenic arrest (incomplete maturation of sperm cells), with mild haemorrhage of the Leydig cell (triangle arrow), as well as presence of fibrotic and haemorrhagic cells (line arrow). D: No apparently histological lesions.



hormone (GnRH) secretion from the hypothalamus, which aid in the release of gonadotropins (FSH and LH) from the anterior pituitary [43]. Loss of cellular projection of pituicytes, as well as neutrophil infiltrations of the pituicytes suggestive of on-going inflammatory process in the pituitary glands of the CBZ group, may have affected the secretion and release of the gonadotropins (LH and FSH). The lesions in the pituitary gland may be partly due to CBZ-provoked oxidative injury. The relative increased in LH and FSH concentrations recorded in VC + CBZ group indicates apparent protection of the gonadotropin level by vitamin C. The development of accessory reproductive organs and spermatogenesis are androgen dependent. Reduced biosynthesis of androgen will enhance reduction in the number of mature Leydig cells and their functional status [43]. FSH regulates the growth of seminiferous tubules and supports the function of Sertoli cells, which in play an important role in spermatogenesis [44] and sperm maturation [45]. Reduction in LH or FSH secretion can result in hypogonadism. This condition is typically seen in males as decrease number of sperm production [45]. LH enhances testosterone secretion in Leydig cells, which in turn act on both Sertoli and peritubular cells of the seminiferous tubules and indirectly stimulates spermatogenesis [44,46].

Our current study showed a lower level of serum testosterone in the CBZ group. This result corroborates the findings from earlier studies [29,43]. The lowered serum testosterone concentration in the CBZ group may be due to adverse effect of CBZ on the gonadotropin (FSH and LH) secretion. The lower testosterone concentration produced may also be partly due to the potentials of antiepileptics to enhance the activity of aromatase enzyme, which converts testosterone to estradiol, a hormone known to further reduce testosterone concentration [47]. Furthermore, CBZ-induced oxidative injury to the testes, as shown by the altered antioxidant status and increased lipoperoxidation may have created unfavorable condition for testosterone production by the Leydig cells. In fact, histological changes in the Leydig cells in the CBZ group further reinforced the unfavorable environment for testosterone production. Therefore, the lower level of serum testosterone in the CBZ group may be due to oxidative injury to the pituitary glands and testes. The significant increase in concentration of serum testosterone seen in the group pretreated with vitamin C, suggests its protective effect on the pituitary and testicular tissues. This further substantiates histoprotective function of vitamin C as revealed by an apparently normal pituitary gland and testicular histoarchitecture in VC + CBZ group. This implies that gonadotropins (LH and FSH) which are produced optimally are able to stimulate apparently normal Leydig cells in the testes to produce testosterone [48,49].

The present study has however shown that pretreatment with vitamin C protected from CBZ-induced low sperm count. This may be due to antioxidant activity of vitamin C apparently acting to reduce free radical load in the testis, thereby improving gonadotropin and testosterone production, and tissue injury resulting in provision of conducive environment for spermatogenesis.

Sperm motility is a useful measure to predict the fertilizing capability of spermatocyte. Fertilizing ability of spermatozoa would be seriously affected by negative impact on their motility [50]. The present study showed lower percentage sperm motility in the CBZ group as compared to other groups. This result agrees with the reports other researchers [29,43,51]. Maturation as well as development of progressive motility and fertilizing capacity of spermatozoa depend on the present of testosterone and epididymal proteins [52]. Therefore, the lower concentration of serum testosterone in CBZ group could be responsible for the reduced progressive motility. In addition, CBZ-evoked oxidative injury to the spermatozoa could be as a result of excessive proteins in the epididymis and testis, secondary to androgen deprivation, causing high production of sperm abnormality [53]. This may have contributed to the reduced sperm motility in the CBZ group since high ROS production have been correlated with reduced motility parameters, which have negative effects on fertility [54–56]. CBZ causes changes in the quality of semen and low sperm motility and high

frequency of sperm cells abnormality [57]. Vitamin C administration has been shown to mitigated CBZ-induced low sperm motility in the present study. Vitamin C acts efficiently as a reducing agent and an antioxidant at physiological pH, thus destroying ROS effect in the body [58]. It has beneficial effect on sperm motility from this study. It has been reported vitamin C enhances sperm motility in goat [59] and ram [60]. It also protects membranes against oxidative stress [61] and has been reported to increase sperm motility and improve semen quality and fertility in animals [62].

Chronic CBZ administration in the present study has adverse consequence on sperm morphology. Osuntokun et al. [43] reported that CBZ and gabapentin (GBP) caused adverse effect on sperm morphology. The low level testicular antioxidant enzymes reported earlier in this study, in CBZ group might be due to elevated ROS that may damage the sperm membrane resulting in altered morphology. The relative decreased in sperm abnormality reported in the VC + CBZ group showed the protective nature of vitamin C on CBZ-evoked sperm abnormality.

The current study showed reduction in relative pituitary gland and testicular weight in CBZ group. Osuntokun et al. [43] reported a decrease in the relative brain weight in CBZ-treated rats when compared to that of GBP and CBZ-GBP combination. The reduced changes in relative or absolute organ weight after exposure to an agent, is indicative of the toxic effect of such agent [63]. The lowered relative pituitary and testicular gland weight observed in rats exposed to CBZ could be caused by lipoperoxidation thereby depleting the body mass or impaired cellular metabolism from oxidative injury. VC + CBZ group showed a progressive rise in relative organ weight compared to those exposed to CBZ only, indicating the function of oxidative stress in the mechanism of CBZ-evoked alteration in organ weight. Our study has shown that vitamin C protects the pituitary and testicular weight against adverse changes provoked by chronic CBZ administration.

## 5. Conclusions

In conclusion, the present study demonstrated that long-term CBZ administration induces adverse reproductive outcome partly due to oxidative damage as revealed by biochemical, hormonal and histological parameters. Therefore, vitamin C may be useful in protecting against the adverse reproductive consequence in individuals or animals on long-term CBZ therapy due to its antioxidant and histoprotective properties. It is recommended that markers of oxidative stress should be constantly evaluated in individuals or animals that are on long-term CBZ therapy, and concomitant administration of vitamin C is advocated.

## Conflict of interest

All the authors declare no conflicting interest.

## Author statement

The author wish to state that this manuscript 'Protective Effect of Vitamin C on Chronic Carbamazepine-induced Reproductive Toxicity in Male Wistar Rats' is an original research article aimed at protecting individual on long-term Carbamazepine therapy to be taking vitamin C due to its antioxidant property.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.toxrep.2020.01.017>.

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