

Carbamazepine evoked reproductive toxicity in male Wistar rats: protective properties of Moringa oleifera leaves methanolic extract

Ganiu Jimoh Akorede, Suleiman Folorunsho Ambali, Mikail Garba Hudu, Mohammed M. Suleiman, Kolawole Yusuf Suleiman, et al.

Comparative Clinical Pathology

ISSN 1618-5641

Comp Clin Pathol

DOI 10.1007/s00580-020-03169-x



Your article is protected by copyright and all rights are held exclusively by Springer-Verlag London Ltd., part of Springer Nature. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".



Carbamazepine evoked reproductive toxicity in male Wistar rats: protective properties of *Moringa oleifera* leaves methanolic extract

Ganiu Jimoh Akorede¹ · Suleiman Folorunsho Ambali¹ · Mikail Garba Hudu² · Mohammed M. Suleiman³ · Kolawole Yusuf Suleiman⁴ · Halimat Amin Abdulrahim⁵ · Lukman Oladimeji Raji⁶ · Isiaku AbdulMajeed⁷

Received: 31 August 2020 / Accepted: 21 September 2020
© Springer-Verlag London Ltd., part of Springer Nature 2020

Abstract

Carbamazepine is an effective chemotherapeutic agent used to manage individual with epilepsy and trigeminal neuralgia. Chronic use of carbamazepine has been incriminated to cause reproductive disorders. The present study investigates the protective property of *Moringa oleifera* leaves methanolic extract on chronic carbamazepine-evoked reproductive toxicity in male Wistar rats. Forty male Wistar rats (150–260 g) were randomly separated into four groups with 10 rats, given distilled water (2 ml/kg), *M. oleifera* (200 mg/kg), carbamazepine (20 mg/kg) and *M. oleifera* accompanied with carbamazepine, after 30 min. The treatments were administered once in a day for 15 weeks via gavage. The pituitary and testis were evaluated for parameters measuring oxidative challenge, sperm characteristics and histological changes. Sex hormone concentrations were also evaluated from sera samples. The result of the phytochemical analysis shows that *M. oleifera* leaves extract contain total phenolics (7.8%) and flavonoids (22.23%), which are considered higher in the plant. The results also revealed that exposure to *M. oleifera* leaves mitigates the disruption in oxidative stress parameters, sex hormone concentration, sperm characteristics and histological changes. This study revealed that chronic carbamazepine administration evokes oxidative stress, partly involved in the alterations of concentration of sex hormones, sperm characteristics and histoarchitecture of pituitary gland and testes. Treatment with leaf extract of *M. oleifera* protects against the adverse reproductive consequences of long-term exposure to CBZ, due to its antioxidant property.

Keywords Chronic exposure · Carbamazepine · Oxidative damage · Reproductive consequence · *Moringa oleifera* · Antioxidant

✉ Ganiu Jimoh Akorede
akorede.gj@unilorin.edu.ng

¹ Faculty of Veterinary Medicine, Department of Veterinary Pharmacology and Toxicology, University of Ilorin, Ilorin, Nigeria

² Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, University of Abuja, Abuja, Nigeria

³ Faculty of Veterinary Medicine, Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria, Nigeria

⁴ Faculty of Veterinary Medicine, Department of Veterinary Physiology and Biochemistry, University of Ilorin, Ilorin, Nigeria

⁵ Faculty of Basic Medical Sciences, Department of Medical Biochemistry, College of Health Sciences, University of Ilorin, Ilorin, Nigeria

⁶ Faculty of Veterinary Medicine, Department of Theriogenology and Production, University of Ilorin, Ilorin, Nigeria

⁷ Faculty of Veterinary Medicine, Department of Veterinary Medicine, University of Ilorin, Ilorin, Nigeria

Introduction

Carbamazepine is among the common and effective antiepileptics for seizure management, preferably as monotherapy due to high efficacy and minimal adverse effect (Al-Bishri et al. 2017). Carbamazepine (CBZ) is rapidly distributed throughout the body and binds between 75 and 78% to plasma proteins. CBZ decreases hyperexcitability of neurones and evokes its action via inhibition of neuronal sodium ion and other channels and transmitter system to a lesser degree. The inhibited sodium ion channel further minimizes monoamine and acetylcholine basal release (Zarghi et al. 2007). CBZ is converted to carbamazepine-10, 11 epoxides (active metabolite) by CYP₄₅₀ 3A4 in the liver that have both antiepileptic and toxic properties (Mittag et al. 2016). CBZ and its active metabolite evoke oxidative damage (Santos et al. 2008), via disruption of cellular antioxidant systems, thus causing cellular injury. CBZ and other antiepileptics have complex interactions with the reproductive system, due to oxidative stress

induction (Al-Bishri et al. 2017). The use of CBZ has been incriminated to cause reproductive disorders arising from tissue damage (Agarwal and Bedaiwy 2003; Yan et al. 2018; Akorede et al. 2020). Prolong exposure to CBZ has been reported to elevate sex hormone-binding globulin (SHBG) concentration in men (Isojarvi et al. 2005), causing reduced bioactivity of androgens and manifested as a lowered sexual function (Isojarvi et al. 1995).

Many plants have therapeutic activity on the presence of phytochemicals, including flavonoids and polyphenols, which have antioxidant properties (Hashem et al. 2019; Neamat-Allah et al. 2019a; Mahmoud et al. 2020). *Moringa oleifera* L is a good antioxidant's source (Asma et al. 2005), due to high plant contents of flavonoids and total phenolics (Iqbal and Bhanger 2006). Furthermore, *M. oleifera* has been shown to significantly increase testicular and epididymal weight gain (Priyadarshani et al. 2014) due to high content in vitamin B, potassium, calcium and protein, β -carotene and other secondary metabolites, antioxidant vitamins (A, C and E) and essential micronutrients (selenium/zinc) (Vongsak et al. 2013). Therefore, being a rich source of numerous antioxidants, the present study aimed at investigating the protective property of *M. oleifera* leaves on prolong CBZ-evoked reproductive toxicity in male Wistar rats.

Materials and methods

Plant collection, identification and preparation

Moringa oleifera leaves were harvested at the main campus of the University of Ilorin, Nigeria. The samples of the leaves were sent to the Department of Plant Biology (herbarium) for identification. Sample of the leaf was deposited at the herbarium for reference purpose, while voucher number UILH/001/1011 was given. The leaves were air-dried to constant weight at room temperature, pounded with ceramic mortar and pestle to powdered. The powdered samples were kept in a polythene bag until use.

Plant extraction

Two kilogrammes of the powdered *M. oleifera* leaf was extracted with methanol (10 l) using a cold extraction method in a glass funnel for 48 h, with periodic shaking, to enhance the extraction process. After that, the mixture was then filtered using Whatman No. 1 filter paper and the filtrate concentrated *in vacuo*, with the rotary evaporator coupled with thermocirculator. The residue was further air-dried to a constant weight and subsequently stored at 4 °C in a refrigerator. Four grammes of the extract was dissolved in 100 ml of distilled water to form a stock solution of 4% (w/v).

Phytochemical screening of *M. oleifera* leaf extract

The phytochemical analysis of *M. oleifera* leaf was evaluated as described by Trease and Evans (1978).

Quantitative analysis of methanol leaf extract of *M. oleifera* for flavonoids and total phenolics

The quantitative analysis of *M. oleifera* leaves total phenolics was performed using high-performance liquid chromatography method (Makkar et al. 1997), and aluminium chloride colourimetric assay method was used to measure total flavonoids (Zhishen et al. 1999).

Drug and preparation

Carbamazepine commercial grade (200 mg) marketed as Tegretol® (Novartis Farma, Italy) was acquired at a pharmaceutical store in Ilorin, Nigeria. Prior to usage, the drug was dissolved in a suitable amount of distilled water to form a solution.

Research animals

Forty male Wistar rats (150–250 g) were acquired at the Animal House of the Department of Veterinary Pharmacology and Toxicology, University of Ilorin, Nigeria. They were housed in plastic cages and fed grower's pelleted feed (Top Feed®, Nigeria), while water was given *ad libitum*. Before the commencement of the work, the rats were acclimatized for 14 days.

Treatment protocols

Animals were divided to random into 4 groups of ten rats each and were subjected to the following treatments: group I (DW) was given distilled water (2 ml/kg); group II (MO) was exposed to *M. oleifera* (200 mg/kg $\sim$ 1/25th of LD₅₀); group III (CBZ) was given 20 mg/kg of carbamazepine (Rajesh et al. 1991), while group IV (MO + CBZ) was exposed to *M. oleifera* (200 mg/kg) and then carbamazepine (20 mg/kg) after 30 min. The treatments were administered once a day for 15 weeks via gavage. The rats were weighed once weekly for dosage adjustment and on the last day of dosing period using a weighing scale (Sensor Disc Exclusive Tech, USA). Following light ether anaesthesia, the rats were sacrificed by jugular venesection, and blood was harvested into anticoagulant and plain container for serum. At the same time, pituitary and testicular tissues were harvested for analysis.

Preparation of pituitary and testicular tissue homogenate

Pituitary gland and testis were weighed, washed with cold saline to remove the blood and homogenized in 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 subsequently, the supernatants were used to evaluate the level of malondialdehyde, superoxide dismutase, catalase and glutathione peroxidase.

Evaluation of pituitary gland and testicular tissue lipoperoxidation

Lipoperoxidative changes in the testis and pituitary gland were assessed by measuring concentrations of malondialdehyde as described by Draper and Hardley (1978).

Evaluation of pituitary gland and testicular superoxide dismutase activities

The activity of superoxide dismutase in the pituitary and testicular tissue homogenates was evaluated with SOD Assay Kit-WST (SOD-19160, Sigma-Aldrich, USA) following the manufacturer's description.

Evaluation of pituitary and testicular catalase activities

Supernatant from the pituitary and testicular homogenates was evaluated for the activity of catalase using CAT kit (ab83464, UK) as described by the manufacturer.

Evaluation of pituitary and testicular glutathione peroxidase activities

Supernatant from the pituitary and testicular homogenates was evaluated for the activity of glutathione peroxidase using GPx kit (BXC0551A, Antrim, UK), adapted from the manufacturer's description.

Evaluation of serum sex hormone concentration

Follicle-stimulating hormone and luteinizing hormone

The concentrations of follicle-stimulating hormone and luteinizing hormone were determined using FSH Kit (FS232F, Calbiotech Inc., USA) and LH Kit (LH231F, Calbiotech Inc., USA) as instructed by the manufacturer.

Testosterone

The concentration of testosterone was measured in the sera samples with testosterone kit (TE187S, Calbiotech

Inc., USA), according to the instruction of the manufacturer.

Sperm characteristics evaluation

Sperm count

An epididymis was carefully removed from each rat, dried with blotting paper and instantly homogenized in 0.5% formal saline. One millilitre of the aliquot was diluted to 1:200 with the aids erythrocyte pipette. Counting was performed by using improved Neubauer counting chamber, while a light microscope at a magnification of $\times 40$ was used for cell counting (Verma and Chinoy 2002).

Sperm motility

The sperm motility was assessed using the standard method as described by Sonmez et al. (2005).

Sperm morphology

Sperm morphology was evaluated by preparing smear from sperm suspension, dried and fixed with fixative, stained with haematoxylin for 15 min and then washed, stained with 1% eosin for 10 min washed and allowed to dry at 25 °C (Wyrobek 1979).

Gross and histological changes evaluation

Each rat's pituitary and testis were evaluated for any macroscopic lesion. Tissue samples were prepared for histopathological examination using a modified standard method of Luna (1960).

Data analysis

Collected data were analysed as mean $\pm$ standard error of the mean (SEM) and subjected to one-way analysis of variance (ANOVA) then accompanied with Tukey's post hoc test. All data were analysed with GraphPad Prism version 5.03 (GraphPad Software, CA, USA). Value of $p < 0.05$ was assumed significant.

Results

Phytochemical screening of *M. oleifera* leaf extract

Phytochemical screening confirmed the availability of alkaloid, flavonoid, glycosides, phenols, steroids, tannin, lipids as

well as amino acids in *M. oleifera* leaf, while saponin was absent.

Flavonoid and phenolic contents of *M. oleifera* leaves

The quantitative analysis of the extract's total flavonoid showed that it contains 22.23% quercetin equivalent (QAE) of flavonoids. The results of UV-visible analysis of the extract also revealed that the extract contained phenols (7.8%), tannin (0.54%) and glycoside (0.004%).

Effect of treatment on the pituitary gland and testicular malondialdehyde concentration

The concentration of pituitary MDA in the CBZ group is substantially ($p < 0.05$) higher than in the DW and MO + CBZ groups, but a relative non-substantial ($p > 0.05$) increased value was revealed as compared with that of the MO (21%) group. Also, relative to DW and MO + CBZ groups, a substantially ($p < 0.05$) increased MDA concentration was observed in the pituitary of the MO group. The testicular MDA concentration in the CBZ group substantially ($p < 0.05$) increased relative to other groups, other than MO group which shows non-significant ($p > 0.05$) change (Table 1).

Effect on pituitary gland and testicular superoxide dismutase activity

A substantial ($p < 0.05$) reduction in pituitary SOD activity was seen in the CBZ group relative to that of the MO and

MO + CBZ groups. Compared with the MO + CBZ group, a substantial ($p < 0.05$) reduction in testicular SOD activity in the CBZ group was observed (Table 1).

Effect on the pituitary gland and testicular catalase activity

Although not significant ($p > 0.05$), a relative reduction in pituitary CAT activity in the CBZ group was observed when compared with MO (15%) and MO + CBZ (17%) groups. The testicular CAT activity reduced significantly ($p < 0.05$) in the CBZ group relative to the other groups. Besides, the testicular CAT activity in the MO group increased considerably ($p < 0.05$) relative to the DW and MO + CBZ groups (Table 1).

Effect on pituitary gland and testicular glutathione peroxidase activity

An insignificantly ($p > 0.05$) reduced GPx activity was observed in the CBZ group pituitary when compared with DW (20%), MO (27%) and MO+CBZ (35%) groups. A significantly ($p < 0.05$) lower testicular GPx activity was observed in CBZ group relative to MO group, but a relative non-significant ($p > 0.05$) decrease was observed when compared with DW (11.3%) and MO + CBZ (13%) groups. Also, substantial ($p < 0.05$) increase in testicular GPx activity was recorded in the MO group relative to DW and MO + CBZ groups (Table 1).

Table 1 Effect of *M. oleifera* leaves on pituitary gland and testicular malondialdehyde (MDA) concentrations, superoxide dismutase, catalase and glutathione peroxidase activities of adult male Wistar rats subjected to prolong carbamazepine administration

Parameters	DW	MO	CBZ	MO + CBZ
Pituitary MDA ($\mu\text{Mol/mg protein}$)	0.301 $\pm$ 0.11	0.460 $\pm$ 0.06 ^b	0.555 $\pm$ 0.12 ^a	0.279 $\pm$ 0.05
Testicular MDA ($\mu\text{Mol/mg protein}$)	0.528 $\pm$ 0.03	0.533 $\pm$ 0.02	0.700 $\pm$ 0.03 ^a	0.318 $\pm$ 0.02
Pituitary SOD (U/mg protein)	0.2324 $\pm$ 0.06	0.2337 $\pm$ 0.02	0.2321 $\pm$ 0.01 ^c	0.2370 $\pm$ 0.03
Testicular SOD (U/mg protein)	0.2313 $\pm$ 0.01	0.2315 $\pm$ 0.02	0.2308 $\pm$ 0.01 ^d	0.2323 $\pm$ 0.01
Pituitary CAT ($\mu\text{mg protein}$)	54.03 $\pm$ 1.59	65.03 $\pm$ 2.78	54.29 $\pm$ 2.30	63.72 $\pm$ 2.45
Testicular CAT ($\mu\text{mg protein}$)	52.91 $\pm$ 1.78	68.71 $\pm$ 2.05 ^b	38.48 $\pm$ 1.71 ^e	58.23 $\pm$ 2.79
Pituitary GPx (U/mg protein)	208.4 $\pm$ 6.75	221.5 $\pm$ 14.38	174.2 $\pm$ 16.69	234.3 $\pm$ 9.57
Testicular GPx (U/mg protein)	199.6 $\pm$ 8.62	259.4 $\pm$ 7.41 ^b	179.4 $\pm$ 10.81 ^f	202.3 $\pm$ 10.56

Values are presented as mean $\pm$ SEM

^a $p < 0.05$, higher vs MO + CBZ and DW groups

^b $p < 0.05$, higher vs MO + CBZ and DW groups

^c $p < 0.05$, lower vs MO + CBZ and MO groups

^d $p < 0.05$, lower vs MO + CBZ group

^e $p < 0.05$, lower vs MO + CBZ, MO and DW groups

^f $p < 0.05$, lower vs MO group

W distilled water, CBZ carbamazepine, MO *Moringa oleifera*

Effect on the concentration of follicle-stimulating hormone, luteinizing hormone and testosterone

A relative non-significant ($p > 0.05$) reduction in the concentration of FSH in the CBZ group was observed in the CBZ group as compared with MO (19%) and MO + CBZ (13%) groups. The mean concentration of LH in the CBZ population was however relatively low compared with other groups (DW, 13%; MO, 21%; and MO + CBZ, 32%). In the CBZ group, a relative non-substantial ($p > 0.05$) reduction in testosterone level was found compared with other groups (DW, 10%; MO, 19%; and MO + CBZ, 14%) (Table 2).

Effect on sperm count

The epididymal sperm count reduced ($p < 0.05$) significantly in the CBZ group relative to other groups. A substantially higher number of sperm count relative to MO and MO + CBZ groups was recorded in the DW group (Table 3).

Effect on sperm motility

There was a considerable ($p < 0.05$) decrease in percentage sperm motility in the CBZ group relative to the DW group, but a relative non-significant ($p > 0.05$) decrease was observed compared with MO (16%) and MO + CBZ (18%) groups. The percentage of sperm motility in the DW group increased substantially ($p < 0.05$) to MO + CBZ and MO groups (Table 3).

Effect on sperm head abnormality

There was insignificant ($p > 0.05$) change in percentage abnormality of the sperm head in all the groups. The CBZ group, however, had the highest percentage of sperm head abnormalities (Table 3).

Effect on sperm mid-piece abnormality

The proportion of mid-piece sperm defect in the CBZ group showed a substantial increase ($p < 0.05$) relative to the DW group. Although not substantial ($p > 0.05$), a relatively higher value of percentage sperm mid-piece abnormality was

recorded in CBZ group compared with MO + CBZ (16%) and MO (18%) groups (Table 3).

Effect on sperm tail abnormality

A substantial ($p < 0.05$) increase in sperm tail percentage defect was showed in the CBZ group relative to the DW group. In contrast, relatively higher value (15%) was reported as compared with MO group. The percentage tail sperm defect in the DW group reduced substantially ($p < 0.05$) than in the MO + CBZ group (Table 3).

Effect of treatments on the pituitary gland and testicular histological changes

Figure 1 shows the effect of the treatments on the histology of the pituitary gland. The pituitary of the CBZ group demonstrated degenerative changes in the pituicyte, neutrophil infiltrations surrounding the pituicytes and cell projection loss. MO and MO + CBZ groups showed mild neutrophil infiltrations. There was no obvious histological abnormality in the group DW. Histological abnormality was observed in the testis of the CBZ group, which is characterized by degenerative changes in the seminiferous tubules as shown by spermatogenic arrest, inflamed (haemorrhage) and fibrosis of the Leydig cell. Other groups showed no apparent histological abnormality (Fig. 2).

Discussion

Phytochemical evaluation of *Moringa oleifera* (MO) leaf confirmed that alkaloids, glycosides, flavonoids, phenols, steroids, tannins, lipids and amino acids are present, while saponin is absent. This result corroborates the reports from earlier studies (Okorundu et al. 2013; Misra et al. 2014; Unuigbo et al. 2014). Total flavonoid content of MO leaf extract (22.23%) from this present study was considered very high and by far above 2.7%, 0.77% and 21.76% reported by Sreelatha and Padma (2009), Oluduro (2012) and Muhammad et al. (2017) respectively but lower than 29.5% (quercetin equivalent) reported by Moyo et al. (2013). The

Table 2 Effect of *M. oleifera* leaves on the concentrations of luteinizing hormone, follicle-stimulating hormone and testosterone of adult male Wistar rats subjected to prolonged carbamazepine administration

Parameters	DW	MO	CBZ	MO + CBZ
FSH (ng/mL)	0.835 ± 0.02	0.928 ± 0.05	0.781 ± 0.01	0.871 ± 0.04
LH (mIU/mL)	0.834 ± 0.04	0.941 ± 0.03	0.741 ± 0.01	0.979 ± 0.05
Testosterone (ng/mL)	2.387 ± 0.07	2.536 ± 0.16	2.138 ± 0.08	2.430 ± 0.13

Values are presented as mean ± SEM

W distilled water, CBZ carbamazepine, MO *Moringa oleifera*

Table 3 Effect of *M. oleifera* leaves on epididymal sperm cells count, motility and morphology of adult male Wistar rats subjected to prolonged carbamazepine administration

Parameters	DW	MO	CBZ	MO + CBZ
Sperm count ($10^6/\text{ml}$)	137.30 ± 2.33^b	108.00 ± 5.13	93.33 ± 3.71^a	106.10 ± 5.13
Motility (%)	93.33 ± 1.67^b	73.33 ± 3.33	63.33 ± 3.33^c	66.67 ± 3.33
Head abnormality (%)	1.55 ± 0.18	2.08 ± 0.09	2.32 ± 0.07	2.14 ± 0.30
Mid-piece abnormality (%)	4.07 ± 0.18	5.10 ± 0.11	6.20 ± 0.41^c	5.20 ± 0.25
Tail abnormality (%)	4.52 ± 0.42^d	5.68 ± 0.33	6.68 ± 0.43^c	6.11 ± 0.09

Values are presented as mean $\pm$ SEM

^a $p < 0.05$, lower vs MO + CBZ, MO and DW groups

^b $p < 0.05$, higher vs MO + CBZ and MO groups

^c $p < 0.05$, higher vs DW group

^d $p < 0.05$, lower vs MO + CBZ group

W distilled water, CBZ carbamazepine, MO *Moringa oleifera*

variation in the quantity of flavonoid content may be dependent on the soil, growing location, the season of collection, agricultural practices, processing techniques and analytical variability. Total phenolic content of 7.8% reported in this present study is considered very high and above that reported by Sreelatha and Padma (2009) (2.6%), Oluduro (2012) (0.29%) and El-Sohaimy et al. (2015) (4.83%), while the tannin content of the MO leaf extract of 0.54% from this present study is considered very low compared with 9.19% and 5.8% reported in previous studies (Nweze and Nwafor 2014; Unuigbo et al. 2014). The current study revealed increment of MDA levels of testis and pituitary gland in the CBZ group, in tandem with previous studies (Suleiman et al. 2015; Tarun et al. 2010). The MDA increment can be as a

result of the inability of the body innate antioxidant system to cope with increased activity of reactive oxygen species (ROS) provoked by chronic CBZ administration (Neamat-Allah et al. 2020). The significant reduction in MDA levels in the pituitary gland and testis of the MO-pretreated group is due to the antioxidant property of MO, which reduces free radical generation, thus preventing oxidative damage to both pituitary and testis. Flavonoids and other antioxidant compounds in MO have significant protective activity against oxidative damage induced by prolonged CBZ administration and played an essential role in reducing the MDA levels (Elham et al. 2017). The substantial reduction in the activity of SOD in pituitary and testicular tissues of CBZ group, as observed in this study, agreed with earlier works (Li et al.

Fig. 1 Histological changes in the pituitary gland of rats chronically exposed to distilled water (a), *M. oleifera* (b), carbamazepine (c) and *M. oleifera* + carbamazepine (d). Normal pituicyte (black arrow) with no noticeable histological changes, infiltrated neutrophils around the pituicytes (line arrow) and loss of cellular projections (triangle and down arrows) (H&E, $\times 400$)

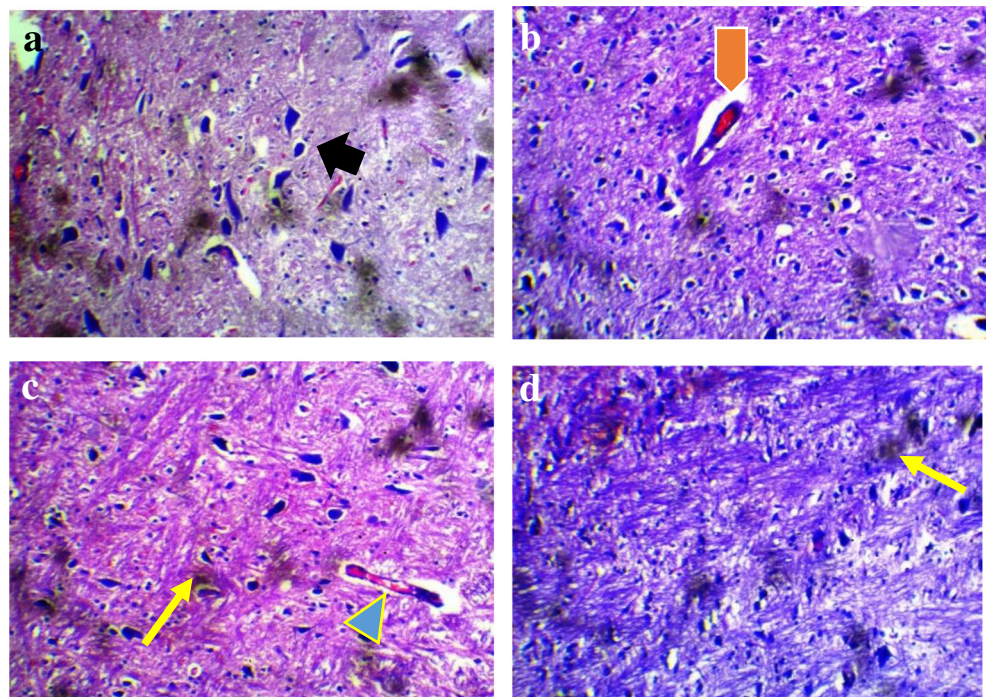
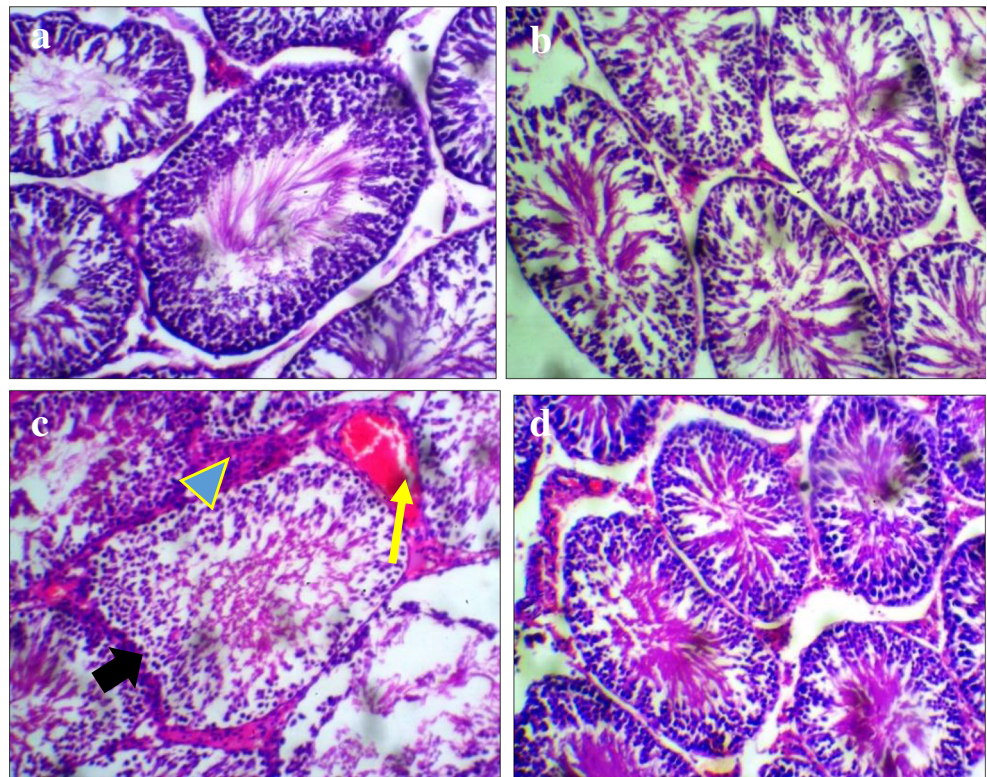


Fig. 2 Histological changes in the testis of rats chronically exposed to distilled water (a), *M. oleifera* (b), carbamazepine (c) and *M. oleifera* + carbamazepine (d). Seminiferous tubules undergo degenerative changes (block arrow) which indicate spermatogenic arrest (incomplete maturation of sperm cells), mild inflammation (haemorrhage) and fibrosis of the Leydig cell (triangle arrow) and haemorrhagic blood vessel (line arrow) (H&E, $\times 400$)



2010; Suleiman et al. 2015). The considerable improvement in the level of SOD in MO-pretreated group showed that MO has an antioxidant effect in preventing CBZ-induced oxidative challenge. This is apparently due to the phenolic and flavonoid contents in MO, known to be a viable free radical scavenger. The lower activities of CAT in pituitary gland and testis of CBZ group revealed in this investigation can be ascribed with the recurrent lipoperoxidation evoked by prolonged exposure to the drug and reduction in the activity of SOD as reported earlier that reduces the conversion rate of O_2^- to H_2O_2 the substrate for CAT. This finding corroborates with an earlier report (Elham et al. 2017). The apparent restoration of pituitary and testicular CAT activities observed in MO-pretreated group demonstrated the antioxidant activity of MO in boosting endogenous antioxidant reserve (Abdallah et al. 2019) that nullify or ameliorate the removal of ROS caused by chronic exposure to CBZ. The current research showed decreased activities of testicular and pituitary GPx of CBZ group; this agreed with earlier findings (Li et al. 2010). GPx might be used up in response to CBZ-provoked oxidative injury (Neamat-Allah et al. 2019b). However, pretreatment with MO was able to protect both the testicular and the pituitary gland GPx activities, further reinforcing the antioxidant effect of the plant (Abdallah et al. 2020). The current research exhibited decreased concentrations of LH and FSH in CBZ group relative to other groups; this can be as a result of CBZ inhibitory effect on the GnRH synthesis and secretions from the hypothalamus that aid

anterior pituitary to release LH and FSH. Previous studies have shown that antiepileptic agents cause a reduction in LH and FSH secretions, which play vital roles in spermatogenesis (Vijay et al. 2009; Li et al. 2010). Improvement in the concentration of LH and FSH seen in MO + CBZ group shows noticeable restoration in the level of gonadotropin by MO. The reduced concentration of testosterone in CBZ-treated group recorded in the present study corroborates the report of earlier researchers (Suleiman et al. 2015; Osuntokun et al. 2017). This reduction can be attributed to the inhibitory action of CBZ on the secretion of pituitary gonadotropins. The reduced level of testosterone can also be caused by the antiepileptic drug's ability to increase the activity of the aromatase enzyme in the liver, which transforms testosterone to estradiol, the hormone that further decreases the level of testosterone (Vijay et al. 2009). Oxidative damage to the testes caused by CBZ administration, as demonstrated by the impaired antioxidant defence system and elevated lipoperoxidation, produced an undesirable state for Leydig cells to produce testosterone. Changes in histoarchitecture of Leydig cells in the CBZ group intensified undesirable condition in testosterone biosynthesis. Hence, the reduced concentration of testosterone in the CBZ group can be as a result of damage to both pituitary gland and testis. The increase in the concentration of testosterone observed in group MO + CBZ indicates the protecting role of MO on pituitary and testicular damage caused by long-term CBZ administration. The significant decrease in

sperm count of the CBZ group is in accordance with the result from other studies (Asadi-Pooya et al. 2015; Suleiman et al. 2015; Osuntokun et al. 2017). The lower sperm count in the CBZ group could be caused by oxidative injury to pituitary gland and testis, as shown by increased lipoperoxidation, decreased antioxidant status and alteration of cytoarchitecture observed in the present study. Therefore, the impaired production of LH, FSH and testosterone and testicular damage evoked by CBZ have contributed immensely to the reduced epididymal sperm count. The substantial improvement in epididymal sperm count in the MO-pretreated group has shown that MO mitigated the CBZ-induced low sperm count. This indicates that MO effectively overturns the sperm count decline evoked by CBZ, likely by boosting the level of antioxidants. Reduction of sperm motility percentage in the CBZ group observed in this study agrees with previous findings (Suleiman et al. 2015; Osuntokun et al. 2017). The increment in sperm motility percentage of MO-pretreated group showed that MO mitigates CBZ-induced low sperm motility. Prolonged exposure to CBZ in the present study has been shown to induce sperm abnormality. Osuntokun et al. (2017) recorded adverse effects of CBZ and gabapentin (GBP) on sperm morphology. MO extract was able to mitigate altered sperm morphology evoked by prolonged CBZ administration apparently as a result of its antioxidant property, which creates a conducive atmosphere for complete spermatogenesis.

Conclusion

This study showed that chronic exposure to carbamazepine causes adverse reproductive outcomes because of oxidative injury indicated by hormonal, biochemical and histological parameters. Availability of flavonoids, phenolics and other antioxidant compounds of *M. oleifera* makes it useful in mitigating the harmful reproductive outcome in animals or individuals under prolonged CBZ therapy. It is recommended that parameters measuring oxidative stress status should be constantly monitored in individuals or animals that are on prolonged CBZ use. Intervention with plants rich in nutrients and antioxidants such as *M. oleifera* is advocated.

Acknowledgements The authors appreciated colleagues from the University of Ilorin, Department of Veterinary Pharmacology and Toxicology, for their assistance. We sincerely appreciate the support of Mr. Denis O. of Veterinary Pharmacology and Toxicology Department, Ahmadu Bello University, Zaria.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This research was performed as specified by the ethical review committee, Ahmadu Bello University (ABUCAUC/2016/040) and conformed to the National Research Council's Guide to Laboratory Animal Care and Use (2011).

References

- Abdallah AAM, Nasr El-Deen NM, Neamat-Allah ANF, Abd El-Aziz HI (2019) Evaluation of the hematoprotective and hepato-renal protective effects of *Thymus vulgaris* aqueous extract on thermally oxidized oil-induced hematotoxicity and hepato-renal toxicity. *Comp Clin Pathol* 29:451–461. <https://doi.org/10.1007/s00580-019-03078-8>
- Abdallah AAM, Nasr El-Deen NAM, Abd El-Aziz HI, Neamat-Allah ANF (2020) Effect of the aqueous root extract of *Curcuma longa* L. (turmeric) against thermally oxidized oil-induced haematological, biochemical and histopathological alterations. *Comp Clin Pathol* 29: 837–845. <https://doi.org/10.1007/s00580-020-03108-w>
- Agarwal RA, Bedaiwy MA (2003) Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertil Steril* 79:829–843. [https://doi.org/10.1016/s0015-0282\(02\)04948-8](https://doi.org/10.1016/s0015-0282(02)04948-8)
- Akorede GJ, Ambali SF, Hudu MG, Olatunji AO, Shittu M, Aremu A, Afisu B, Biobaku KT, Ahmed AO, Ameen SA (2020) Protective effect of vitamin C on chronic carbamazepine-induced reproductive toxicity in male Wistar rats. *Toxicol Rep* 7:269–276. <https://doi.org/10.1016/j.toxrep.2020.01.017>
- Al-Bishri WM, Abdel-Reheim ES, Zaki AR (2017) Purslane protects against the reproductive toxicity of carbamazepine treatment in pilocarpine-induced epilepsy model. *Asian Pac J Trop Biomed* 7(4):339–346. <https://doi.org/10.1016/j.apjtb.2017.01.003>
- Asadi-Pooya A, Farazdaghi M, Ashjzadeh N (2015) Effects of carbamazepine on semen parameters in men with newly diagnosed epilepsy. *Iran J Neurol* 14(3):168–170
- Asma S, Farooq A, Maleeha M, Ammara F (2005) Antioxidant activity of different solvent extracts of *Moringa oleifera* leaves under accelerated storage of sunflower oil. *Asian J Plant Sci* 4(6):630–635. <https://doi.org/10.3923/ajps.2005.630.635>
- Draper HH, Hardley M (1978) Malondialdehyde deformation as an index of lipid peroxidation. *Methods Enzymol* 186:421–431. [https://doi.org/10.1016/0076-6879\(90\)86135-i](https://doi.org/10.1016/0076-6879(90)86135-i)
- Elham MYE, Zahran HA, Aboelfetoh MA (2017) A comparative study of the effects of three *Moringa* species on obesity-induced oxidative stress state in liver tissue. *Int J Pharm Biol Sci* 8(2):572–584. <https://doi.org/10.22376/ijpbs.2017.8.2.b572-584>
- El-Sohaimy SA, Hamad GM, Mohamed SE, Mohamed HA, Al-Hindi RR (2015) Biochemical and functional properties of *Moringa oleifera* leaves and their potential as a functional food. *Glob Adv Res J Agric Sci* 4(4):188–199
- Hashem MA, Neamat-Allah ANF, Hammza HEE, Abou-Elnaga HM (2019) Impact of dietary supplementation with *Echinacea purpurea* on growth performance, immunological, biochemical, and pathological finding in broiler chickens infected by pathogenic *E. coli*. *Trop Anim Health Prod* 52:1599–1607. <https://doi.org/10.1007/s11250-019-02162-z>
- Iqbal S, Bhanger MI (2006) Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. *J Food Compos Anal* 19:544–551. <https://doi.org/10.1016/j.jfca.2005.05.001>
- Isojarvi JI, Repo M, Pakarinen AJ, Luuarmen O, Myllyla VV (1995) Carbamazepine, phenytoin, sex hormones and sexual function in men with epilepsy. *Epilepsia* 36:366–370
- Isojarvi JI, Tauboll E, Herzong AG (2005) Effect of antiepileptic drugs on reproductive endocrine function in individuals with epilepsy. *CNS*

- Drugs 19:207–223. <https://doi.org/10.2165/00023210-200519030-00003>
- Li ZH, Li P, Randak T (2010) Effect of human pharmaceutical carbamazepine on antioxidant responses in the brain of a model teleost *in vitro*: an efficient approach to biomonitoring. *J Appl Toxicol* 30:644–648. <https://doi.org/10.1002/jat.1534>
- Luna GH (1960) Manual of histologic staining method of armed forces institute of pathology, 35th edn. McGraw-Hill Book Company, New York, p 258
- Mahmoud EA, El-Sayed BM, Mahsoub YH, El-Murrc AI, Neamat-Allah ANF (2020) Effect of *Chlorella Vulgaris* enriched diet on growth performance, hemato-immunological responses, antioxidant and transcriptomics profile disorders caused by deltamethrin toxicity in Nile tilapia (*Oreochromis niloticus*). *Fish Shellfish Immunol* 102: 422–429. <https://doi.org/10.1016/j.fsi.2020.04.061>
- Makkar HPS, Baker K, Abel H, Pawelzik E (1997) Nutrient contents, rumen protein degradability and anti-nutritional factors in some colour-and white-flowering cultivars of *Vicia faba* beans. *J Sci Food Agric* 75:511–520. [https://doi.org/10.1002/\(SICI\)1097-0010\(199712\)75:4<511::AID-JSFA907>3.0.CO;2-M](https://doi.org/10.1002/(SICI)1097-0010(199712)75:4<511::AID-JSFA907>3.0.CO;2-M)
- Misra A, Srivastava S, Srivastava M (2014) Evaluation of the anti-diarrheal potential of *Moringa oleifera* (Lam) leaves. *J Pharmacogn Phytochem* 2(5):43–46
- Mittag N, Meister S, Berg AM, Walther UI (2016) A case report of a carbamazepine overdose with focus on pharmacokinetic aspects. *Pharmacopsychiatry* 49:76–78. <https://doi.org/10.1055/s-0035-1569268>
- Moyo B, Masika PJ, Hugo A, Muchenje V (2013) Nutritional characterization of *Moringa* (*Moringa oleifera* Lam.) leaves. *Afr J Biotechnol* 10(60):12925–12933. <https://doi.org/10.5897/AJB10.1599>
- Muhammad M, Rahmatullah Q, Muhammad A, Muhammad SA, Masroor I (2017) Preliminary phytochemical screening, antifungal and cytotoxic activities of leaves extract of *Moringa oleifera* Lam. from salt range, Pakistan. *Pak J Bot* 49(1):353–359
- Neamat-Allah ANF, El-Murr AI, Abd El-Hakim Y (2019a) Dietary supplementation with low molecular weight sodium alginate improves growth, haematology, immune reactions and resistance against *Aeromonas hydrophila* in *Clarias gariepinus*. *Aquac Res* 1:1–10. <https://doi.org/10.1111/are.14031>
- Neamat-Allah ANF, Mahmoud EA, Abd El-Hakim Y (2019b) Efficacy of dietary Nano-selenium on growth, immune response, antioxidant, transcriptomic profile and resistance of Nile tilapia, *Oreochromis niloticus* against *Streptococcus iniae* infection. *Fish Shellfish Immunol* 94:280–287. <https://doi.org/10.1016/j.fsi.2019.09.019>
- Neamat-Allah ANF, Abd El-Hakim Y, Mahmoud EA (2020) Alleviating effects of β -glucan in *Oreochromis niloticus* on growth performance, immune reactions, antioxidant, transcriptomics disorders and resistance to *Aeromonas sobria* caused by atrazine. *Aquac Res* 1:1–12. <https://doi.org/10.1111/are.14529>
- Nweze NO, Nwafor FI (2014) Phytochemical, proximate and mineral composition of leaf extracts of *Moringa oleifera* Lam. from Nsukka, South-Eastern Nigeria. *J Pharm Biol Sci* 9(1):99–103
- Okorundu SI, Akujobi CO, Okorundu JN, Anyado-Nwadike SO (2013) Antimicrobial activity of the leaf extracts of *Moringa oleifera* and *Jatropha curcas* on pathogenic bacteria. *Int J Biol Chem Sci* 7(1): 195–202. <https://doi.org/10.4314/IJBCS.V7I1.16>
- Oluduro AO (2012) Evaluation of antimicrobial properties and nutritional potentials of *Moringa oleifera* Lam. leaf in South-Western Nigeria. *Malay J Microbiol* 8(2):59–67. <https://doi.org/10.21161/mjm.02912>
- Osuntokun OS, Olayiwola G, Oladele A, Ola I, Ayoka AO (2017) Chronic administration of gabapentin and a gabapentin-carbamazepine combination reversibly suppress testicular function in male Wistar rats (*Rattus norvegicus*). *Pathophysiology* 24:63–69. <https://doi.org/10.1016/j.pathophys.2017.01.004>
- Priyadarshani N, Varma MC, Bhagapur TM (2014) Effect of *Moringa oleifera* leaf powder on sperm count, histology of testis and epididymis of hyperglycaemic mice *Mus musculus*. *Am Int J Res Formal Appl Nat Sci* 7(1):7–13
- Rajesh KR, Surenda R, Thangam J (1991) Effect of valproic acid and carbamazepine on learning and memory in rats. *Indian J Pharm* 23(30):185–188
- Santos NA, Medina WS, Martins NM, Rodrigues MA, Curtis C, Santos AC (2008) Involvement of oxidative stress in the hepatotoxicity induced by aromatic antiepileptic drugs. *Toxicol in Vitro* 22(8): 1820–1824. <https://doi.org/10.1016/j.tiv.2008.08.004>
- Sonmez M, Turk G, Yuce A (2005) The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats. *Therio* 6:2063–2072. <https://doi.org/10.1016/j.theriogenology.2004.10.003>
- Sreelatha S, Padma PR (2009) Antioxidant activity and total phenolic content of *Moringa oleifera* leave in two stages of maturity. *Plant Foods Hum Nutr* 64(4):303–311. <https://doi.org/10.1007/s11130-009-0141-0>
- Suleiman I, Kawu MU, Tanko Y, Shittu M (2015) Effect of co-administration of aqueous extract of *Hibiscus sabdariffa* Linn (Malvaceae) calyx and vitamin E on carbamazepine-induced testicular changes in adult Wistar rats. *Int J Novel Res Life Sci* 2(1):22–33
- Tarun A, Ashish K, Krishna KS, Pramod KM, Basu DB, Gobind RG, Amit KS (2010) Effect of carbamazepine and lamotrigine on cognitive function and oxidative stress in the brain during chemical epileptogenesis in rats. *B Clin Pharmacol Toxicol* 106(5):372–377. <https://doi.org/10.1111/j.1742-7843.2009.00499.x>
- Trease GE, Evans WC (1978) Pharmacology, 11th edn. Bailliere Tindall Ltd, London, pp 60–75
- Unuigbo CA, Henry AO, Emmanuel EO, Dominic AO (2014) Phytochemical and antioxidant evaluation of *Moringa oleifera* (Moringaceae) leaf and seed. *J Pharm Biores* 11(2):51–57. <https://doi.org/10.4314/jpb.v11i2.4>
- Verma RJ, Chinoy NJ (2002) Evaluation of sperm characteristics. *Asian J Androl* 4(1):77–78
- Vijay P, Yeshwanth R, Bairy KL (2009) Effect of phenytoin sodium on the biochemical parameters of reproductive function in male albino Wistar rats. *J Physiol Biomed Sci* 3:14–18. <https://doi.org/10.9781/470>
- Vongsak B, Sithisarn P, Gritsanapan W (2013) Simultaneous HPLC quantitative analysis of active compounds in leaves of *Moringa oleifera* Lam. *J Chromatogr Sci* 4:5–11. <https://doi.org/10.1093/chromsci/bmt093>
- Wyrobek AJ (1979) Changes in mammalian sperm morphology after X-ray and chemical exposure. *Genetics* 59:105–119
- Yan S, Wang M, Zha L, Li W, Luo Q, Sun J, Wang Z (2018) Environmentally relevant concentrations of carbamazepine caused endocrine-disrupting effects on non-target organisms, Chinese rare minnows (*Gobiocypris rarus*). *Environ Sci Technol* 52(2):886–894. <https://doi.org/10.1021/acs.est.7b06476>
- Zarghi A, Praveen RP, Knaus EE (2007) Synthesis and biological evaluation of methanesulfonamide analogues of rofecoxib: replacement of methanesulfonyl by methanesulfonamido decreases cyclooxygenase-2 selectivity. *Bioorg Med Chem* 15:1056–1061. <https://doi.org/10.1016/j.bmc.2006.10.023>
- Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effect on superoxide radicals. *Food Chem* 64:555–559. [https://doi.org/10.1016/S0308-8146\(98\)00102-2](https://doi.org/10.1016/S0308-8146(98)00102-2)