ORIGINAL ARTICLE

Evaluating the Supplementary Effects of Vitamin C on Carbamazepine and Pentylenetetrazol-Induced Seizures and Preimplantation Loss in Pregnant Wistar Rats: Implications for Human Pregnancy

AKOREDE GANIU JIMOH[1,*], AMBALI SULEIMAN FOLORUNSHO[1], AGUNBIADE OLATUNBOSUN[1], OLATUNJI AISHAT OMOBOLANLE[1], AREMU ABDULFATAI[1], SHITTU MUFTAU[2], AFISU BASIRU[3], AMEEN SALIU AKANNI[4], SULEIMAN KOLAWOLE YUSUF[3], RAJI LUKMAN OLADIMEJI[5]

[1]Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Ilorin, Nigeria
[2]Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria
[3]Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Ilorin, Nigeria
[4]Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ilorin, Nigeria
[5]Department of Theriogenology and Production, Faculty of Veterinary Medicine, University of Ilorin, Nigeria

Abstract

Background: Epilepsy is a complex neurological disorder affecting millions worldwide and is the most common major neurological complication during pregnancy. Carbamazepine, a widely used antiepileptic drug, has been associated with oxidative damage. Vitamin C, a powerful antioxidant has shown potential in controlling seizures and improving fertility. This study aimed to evaluate the protective effects of vitamin C against carbamazepine and pentylenegtetrazole-induced seizures on pre-implantation loss in pregnant Wistar rats.

Methods: We randomly assigned Thirty pregnant rats into six groups of five animals each. Group received distilled water (2 ml/kg), Group 2 was administered pentylenetetrazol (PTZ) at a dose of 60 mg/kg, Group 3 received vitamin C (100mg/kg) along with PTZ (60 mg/kg), Group 4 was given carbamazepine (20 mg/kg), Group 5 received both carbamazepine (20 mg/kg) and PZT (60 mg/kg), and Group 6 was pretreated with vitamin C, carbamazepine and PZT at doses of 100 mg/kg, 20 mg/kg and 60mg/kg, respectively. Treatments were administered via gavage once daily for 11 consecutive days, while PTZ was administered intraperitoneally ones. Oxidative stress parameters were assessed in the pituitary, ovary and uterine tissues alongside hematological parameters and sex hormones concentrations. We also evaluated preimplantation loss.

Results: The results demonstrated that vitamin C effectively mitigated the adverse effects of oxidative stress, hormonal disruptions, preimplantation loss, and hematological changes induced by seizure and carbamazepine.

Conclusion: The study concludes that antioxidant properties of vitamin C at gestation contribute to its protective effects against seizures and carbamazepine-induced alterations in reproductive parameters. The findings may have implications for human exposure to antiepileptics during pregnancy.

Keywords: Seizure, oxidative stress, pentylenetetrazol, carbamazepine, preimplantation loss, vitamin C.

How to cite this article: Akorede GJ, Ambali SF, Agunbiade O, Olatunji A O, Aremu A, Shittu M, et al. Evaluating the Supplementary Effects of Vitamin C on Carbamazepine and Pentylenetetrazol-Induced Seizures and Preimplantation Loss in Pregnant Wistar Rats: Implications for Human Pregnancy. *Asia Pac J Med Toxicol* 2024; 13(2): 56-64.

INTRODUCTION

Epilepsy is a chronic neurological disorder characterized by recurrent unprovoked seizures [1] that affects individuals of all ages and both sexes and is prevalent worldwide [2]. The disorder has substantial detrimental effects on animal and human health, significantly impacting the quality of life and imposing a heavy burden on society [3]. The occurrence of epileptic seizures during pregnancy has been associated with complications such as increased incidence of stillbirths, malformations, spontaneous abortions, and neonatal deaths [4].

Pentylenetetrazol (PTZ) is a chemical compound used to induce seizures in animal models of epilepsy [5]. It acts by inhibiting the activity of gamma-aminobutyric acid (GABA), a key inhibitory neurotransmitter in the brain; PTZ causes hyperexcitability and seizures in the brain, making it a valuable tool for studying the mechanisms of epilepsy [6].

It is essential to treat epilepsy during pregnancy because seizures can lead to falls, injury, miscarriage and physical

*Correspondence to: Akorede Ganiu Jimoh, Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Ilorin, Nigeria.
Email: akorede.gj@unilorin.edu.ng, Tel: +2347033553270

stress that can endanger the health of the dam and fetus [7]. Treatment typically involves the use of anti-epileptic drugs (AEDs), alongside the management of underlying conditions that may contribute to the seizures [8].

Carbamazepine (CBZ) is an effective AED used for treating epilepsy and neuropathic pain; it is also employed as a second-line treatment for bipolar disorders and as an adjuvant treatment for schizophrenia [9]. CBZ exerts its effect via the reduction of sustained repetitive neuronal firing by blocking voltage-gated sodium channels and, in turn, inhibits repetitious brain neuronal activities [10]. CBZ and its active metabolite (10, 11 -epoxycarbamazepine) also evoke oxidative damage [11] by disrupting cellular antioxidant systems, thus causing cellular injury. Additionally, CBZ interacts with the reproductive system due to oxidative stress induction [12, 13, 14]. As a lipid-soluble compound, CBZ can cross the placenta barrier and other biological membranes, resulting in malformations such as craniofacial defects, heart defects, neural tube defects, growth retardation, developmental delays, and fetal death [15]. Studies have shown a higher incidence of spontaneous abortions in pregnant individuals undergoing CBZ therapy [16].

Vitamin C is an essential water-soluble antioxidant in the blood, lymph, cerebrospinal and other biological fluids that plays a critical role in intracellular lipid peroxidation, mitigating endothelial dysfunction, and replenishing vitamin E within lipoproteins and cell membranes. This vital nutrient protects cells and tissues from free radical production and lipid damage [17]. Vitamin C has been beneficial for infertile women with luteal phase defects and those experiencing recurrent abortions, as it plays a significant role in folliculogenesis, enhances fertility through pituitary hormonal secretions, and improves endometrial thickness and cervical receptivity [18].

This study aims to evaluate the protective effects of vitamin C on carbamazepine and pentylenegtetrazole-induced seizures on pre-implantation loss in pregnant Wistar rats.

METHODS

Animals acquisitions and preparations

We obtained fifty sexually mature male Wistar rats and fifty nulliparous female Wistar rats from the Department of Veterinary Pharmacology and Toxicology, at the University of Ilorin, Nigeria. The animals were kept housed separately in plastic cages and fed with commercial grower pellets (Chikun®, Nigeria). Water was available *ad libitum*.

Drugs/chemicals acquisition and preparations

Pentylenetetrazol (PTZ) was purchased from Sigma-Aldrich (St. Louis, MO, USA), commercial grade carbamazepine (Tegretol®) and vitamin C were obtained from a reputable pharmaceutical store in Ilorin, Kwara State.

All other chemicals used, including phosphate buffer solution, ammonium sulphide, cold saline, thiobarbaturic acid, trichloroacetic acid and tris-buffered saline, were of analytical grade and obtained and sourced from a reputable chemical company.

Experimental design

Experimental protocol: Fifty sexually matured female nulliparous Wistar rats weighing between 150 and 180 grams were bred with 50 adult males in a 1:1 mating scheme overnight, following the on Organization for Economic Cooperation and Development (OECD) guidelines (1995) for reproductive toxicity studies. Evidence of mating was confirmed by the presence of spermatozoa deposit in the vagina or the presence of vaginal plug. Day 1 of gestation was defined as the day on which a copulation plug or spermatozoa were found in the vagina.

The thirty pregnant dams were randomly divided into six groups, with five animals in each group. The treatment regimens were as follows:

Group 1 received distilled water (2ml/kg); Group 2 was administered pentylenetetrazol (60mg/kg); Group 3 was exposed to vitamin C (100mg/kg) and pentylenetetrazol (60mg/kg) respectively; Group 4 was exposed to carbamazepine (20mg/kg) [19]; Group 5 was administered carbamazepine (20 mg/kg) and pentylenetetrazol (60mg/kg) respectively; while Group 6 was pretreated with vitamin C (100mg/kg) and carbamazepine (20mg/kg) and later exposed to pentylenetetrazol (60mg/kg) respectively.

The treatments were delivered via gavage once daily for 12 days consecutively, while pentylenetetrazol was administered intraperitoneally (i.p.), ones. The rats were then sacrificed via jugular venesection after being mildly anesthesized with ether. Blood samples were collected into sterile test tubes, containing anticoagulant, and stored in a refrigerator for subsequent hematological evaluations. Evaluation of treatment on serum hormonal concentration.

Blood samples were collected via the medial canthus of the eyes into plain bottles and centrifuged at 1000 × g for 10 minutes. Serum were decanted into clean sample bottles and used for the analysis of FSH, LH, progesterone and estradiol concentrations using standard ELISA kits as described by the manufacturers.

Evaluation of hematological parameters

For this purpose, blood parameters were analyzed, such as packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC) count, total differential leukocytes and platelets were evaluated. The analyses were performed on an auto-analyzer unit (Perlong HA6000 Auto Haematology Analyzer; China).

Evaluation of preimplantation loss

On day 12 day of pregnancy, the rats were anaesthetized with ether for examination. After anesthesia, the uterine horns were removed, and the number and distribution of implantation sites were recorded. The ovaries were also examined and the corpora lutea were counted. To confirm the embryonic implantation sites, the uterine horns were placed in Salewski reactive solution [20]. The percentage of preimplantation loss was calculated using the following formula:

$$\%\text{pre} - \text{implantation loss} = (\text{No. of corpura lutea} - \text{No. of implantation sites})/(\text{No. of corpura lutea}) \times 100$$

Tissue preparations

The pituitary gland, ovaries and uterus were weighed, rinsed with cold saline to remove blood, and homogenized in a known volume (5 mg/ml) of ice-cold phosphate buffer at a 1:5 w/v. The homogenates were then centrifuged at 2000 g

for 10 minutes, and the supernatants were used to analyze of the levels of malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx).

Pituitary, ovarian and uterine tissues lipoperoxidation

The Pituitary, ovarian and uterine tissues malondialdehyde (MDA) concentrations were evaluated using the double heating method [21].

Pituitary, ovarian and uterine tissues superoxide dismutase activities

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

Pituitary, ovarian and uterine tissues glutathione peroxidase activities

Glutathione peroxidase activity was assessed using the Fortress Diagnostic Glutathione Peroxidase Assay Kits Protocol (BXC0551A, Antrim, UK) [23].

Statistical analysis

The values are expressed as mean ± standard deviation (SD). Differences within the groups were analysed using *Tukey post-hoc* method following ANOVA, with statistical analyses performed using Graph-pad Prism version 8.03 (San Diego, California, USA).

RESULTS

Clinical signs

Clinical signs observed in Groups II, III, IV, V and VI, which were treated with pentylenetetrazol (PTZ) included, anxiety, immobilization, head nodding, myoclonus of facial, forelimb, or hind limb, continuous whole-body myoclonus, myoclonic jerks, a stiffly held tail, rearing, tonic seizure, falling on their sides, tonic-clonic seizures, falling on their backs, and wild rushing and jumping.

Effect of Treatment on Hormonal Concentration

Effect of treatment on follicle stimulating hormone concentration

Figure 1 shows no significant change in follicle-stimulating hormone (FSH) concentration among the groups ($p > 0.05$). However, the absolute mean FSH concentration in the PTZ group was relatively lower when compared to that of DW (40%), CBZ (67%), VC+PTZ (54%), CBZ+PTZ (56%) and VC+CBZ+PTZ (56%) groups. Also, reduction of FSH concentration when recorded in DW group when compared with VC+PTZ (22%), CBZ (19%), CBZ+PTZ (11%) and VC+CBZ+PTZ (11%) groups.

Effect of treatment on luteinizing hormone concentration

There was no significant ($p > 0.05$) change in the LH concentration in all the groups. However, the absolute mean LH concentration in DW group was relatively lower when compared to that of VC+PTZ (22%), CBZ (14%), CBZ+PTZ (14%) and VC+CBZ+PTZ (14%) groups (Figure 2).

Effect of treatment on progesterone concentration

There was significant ($p < 0.05$) reduction in progesterone level in the PTZ group compared to the DW and VC+CBZ+PTZ groups. Relative reductions were noted when comparing PTZ to the VC+PTZ (62%), CBZ (43%), CBZ+PTZ (32%) groups. Additionally, the CBZ group showed a significant reduction ($p < 0.05$) in progesterone compared to the VC+CBZ+PTZ group, with relative reductions noted compared to PTZ (43%) and VC+PTZ (55%) groups (Figure 3).

Effect of treatment on estradiol concentration

A significant ($p < 0.05$) decrease in estradiol concentrations was observed in the PTZ group compared to all other groups. A relative reduction ($p > 0.05$) in estradiol was observed in the CBZ group when compared to VC+PTZ (12%), CBZ+PTZ (21%) and VC+CBZ+PTZ (21%) groups (Figure 4).

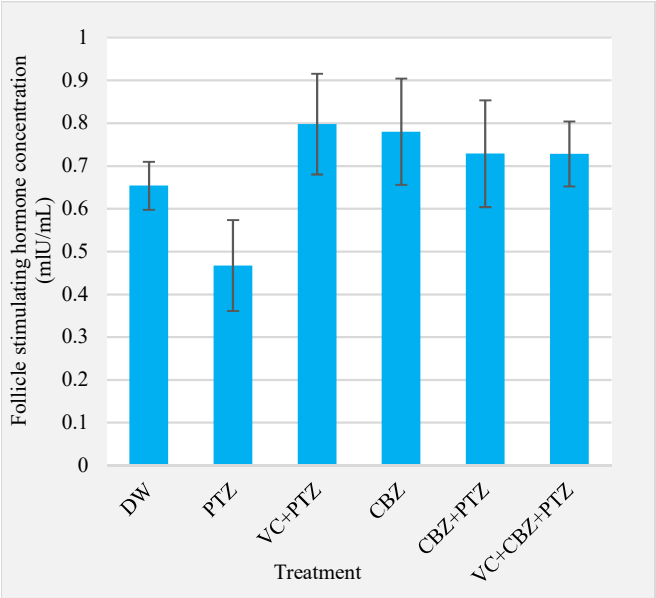

Figure 1. The effect of vitamin C on follicle-stimulating hormone (FSH) concentrations in pregnant Wistar rats exposed to carbamazepine and pentylenetetrazole

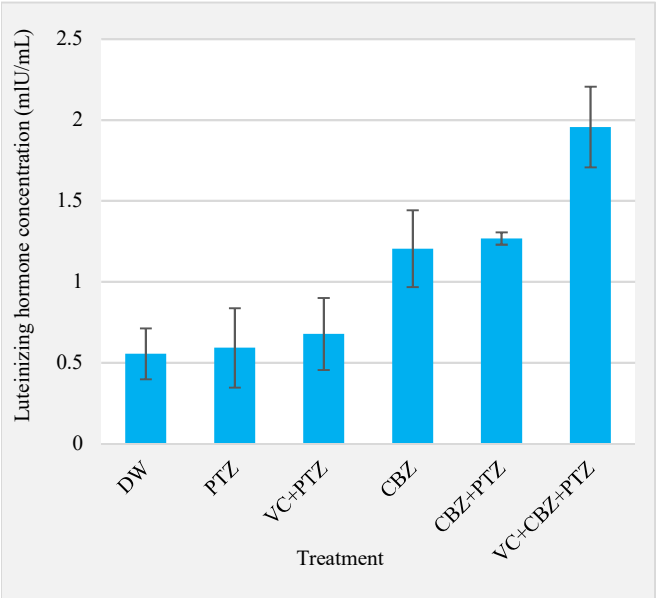

Figure 2. The ffect of vitamin C on luteinizing hormone (LH) concentrations in pregnant Wistar rats exposed to carbamazepine and pentylenetetrazole

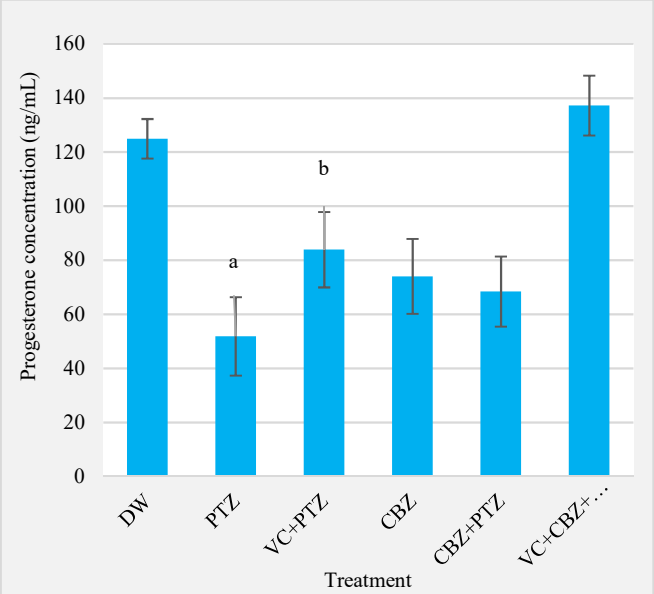

Figure 3. Th ffect of vitamin C on progesterone concentration in pregnant Wistar rats exposed to carbamazepine and pentylenetetrazole.

a = ($p < 0.05$) significantly lower compared to the DW and VC+CBZ+PTZ groups
b = ($p < 0.05$) significantly lower in the VC+CBZ+PTZ group.

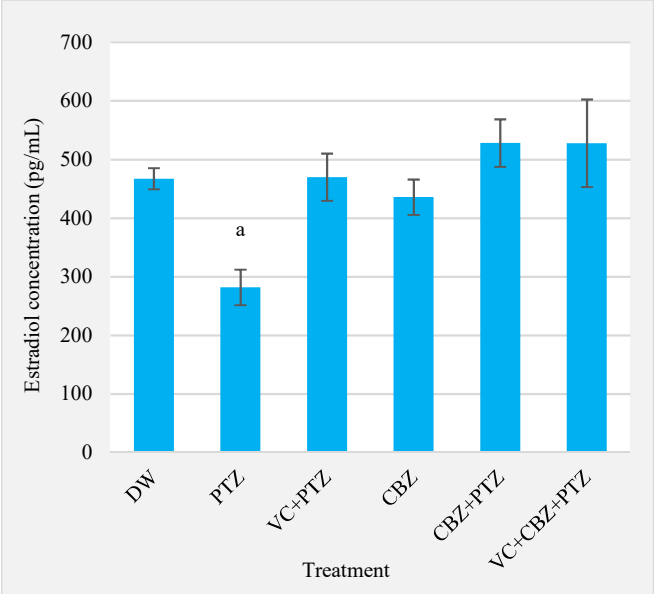

Figure 4. The effect of vitamin C on estradiol concentrations in pregnant Wistar rats exposed to carbamazepine and pentylenetetrazole.a = ($p < 0.05$) significantly lower compared to the DW, CBZ, VC+PTZ, CBZ+PTZ and VC+CBZ+PTZ groups

Effect of Treatment on Activity of Pituitary, Ovarian and Uterine Tissues Oxidative Stress Parameter

Effect of treatment on pituitary gland, ovarian and uterine tissues malondialdehyde concentrations

The MDA concentration in the pituitary gland showed no significant ($p > 0.05$) change within the groups. A relative increment was observed in pituitary MDA concentration in the PTZ group when compared to the DW (80%), VC+PTZ (61%), CBZ (36%), CBZ+PTZ (48%) and VC+CBZ+PTZ (58%) groups. Also, a relatively increase in pituitary MDA concentrations in the CBZ group was observed when compared to DW (68%), VC+PTZ (39%), CBZ+PTZ (19%) and VC+CBZ+PTZ (34%) groups (Table 1).

There was a significant ($p < 0.05$) increase in ovarian MDA concentration in the PTZ and CBZ groups when compared to the DW group. A substantial increment in ovarian MDA level was noticed in the PTZ group when compared to DW (67%), VC+PTZ (33%) and VC+CBZ+PTZ (28%) groups. A relatively increased ovarian MDA value was recorded in the CBZ group as compared to the VC+PTZ (29%) and VC+CBZ+PTZ (24%) groups (Table 1).

There was significant ($p < 0.05$) increase in uterine MDA concentration in the PTZ group when compared to DW and VC+PTZ groups, while relatively higher value was observed when compared with CBZ (15%), CBZ+PTZ (16%) and VC+CBZ+PTZ (38%) groups. The CBZ group showed an increase in the ovarian MDA concentration when compared with DW (47%), VC+PTZ (43%), VC+CBZ+PTZ (27%) groups (Table 1).

Effect of treatment on pituitary gland, ovarian and uterine tissues superoxide dismutase concentrations

As can be seen from Table 1, a significantly ($p < 0.05$) lower pituitary SOD concentration in the DW group when compared to PTZ, CBZ, CBZ+PTZ and VC+CBZ+PTZ groups.

There was no significant ($p > 0.05$) change in the ovarian SOD concentration in all the groups. A relative reduction was observed in the PTZ group compared to DW (15%) and VC+PTZ (23%) groups. A relatively decrease in ovarian SOD level was noted in the CBZ group when compared to DW (22%) and VC+PTZ (15%) groups (Table 1).

The uterine SOD concentration reduced significantly ($p < 0.05$) in PTZ group when compared with DW and VC+PTZ groups, while relative reduction was reported when compared with CBZ (45%), CBZ+PTZ (46%) and VC+CBZ+PTZ (52%) groups. There was a significantly ($p < 0.05$) lower uterine SOD level in the CBZ group when compared with DW group, but a relative reduction was noticed when compared with PTZ (45%), VC+PTZ (27%) and VC+CBZ+PTZ (12%) groups (Table 1).

Effect of treatment on pituitary gland, ovarian and uterine tissues glutathione peroxidase concentrations

There was a significant ($p < 0.05$) decrease in pituitary gland GPx concentration in the PTZ group when compared to VC+PTZ and VC+CBZ+PTZ groups, while relative reduction was recorded with DW (30%), CBZ (22%) and CBZ+PTZ (13%) groups. The pituitary GPx reduced ($p < 0.05$) significantly in the CBZ group when compared with the VC+CBZ+PTZ group, while relative decreased value was reported when compared with DW (10%), VC+PTZ (14%) and CBZ+PTZ (10%) groups (Table 1).

There was a significant ($p < 0.05$) decrease in ovarian GPx concentration in the PTZ group when compared to DW, VC+PTZ and VC+CBZ+PTZ groups, while a relative reduction was notice with CBZ (19%) and CBZ+PTZ (24%) groups. The ovarian GPx level reduced ($p < 0.05$) significantly in the CBZ group when compared to the VC+PTZ group, but relative lower value was recorded when

compared with the DW (19%) and VC+CBZ+PTZ (21%) groups.

There was a significant ($p < 0.05$) decrease in uterine GPx concentration in the PTZ group when compared to DW, VC+PTZ and VC+CBZ+PTZ groups, while a relative reduction was seen when compared to the CBZ (11%) and CBZ+PTZ (19%) groups. There was significant ($p < 0.05$) change in uterine GPx concentration in the CBZ group when compared with DW, CBZ+PTZ and VC+CBZ+PTZ groups (Table 1).

Effect of Treatment on Hematological Parameters

Effect on parked cell volume (PCV)

There was a no significant ($p > 0.05$) change in parked cell volume in all the groups. There was relative decrease in PCV level in the PTZ group when compared to DW (15%), VC+PTZ (33%), CBZ (55%) and CBZ+PTZ (16%). A relative reduction in PCV level was also observed in the CBZ group when compared to DW (55%), VC+PTZ (17%), CBZ+PTZ (55%) and VC+CBZ+PTZ (45%) (Table 2).

Effect red blood cell (RBC) count

Table 2 showed no significant ($p > 0.05$) change in red blood cell (RBC) count in all the groups. There was a relative decrease in RBC level in the PTZ group when compared to the VC+PTZ (35%), CBZ (68%) and CBZ+PTZ (46%). A relative reduction in RBC level was seen in the CBZ group when compared to DW (74%), VC+PTZ (68%) and CBZ+PTZ (15%).

Effect on hemoglobin concentration (Hb)

No significant ($p > 0.05$) change in hemoglobin concentration (Hg) was observed in any of the groups. There

Table 1. Effect of Vitamin C on MDA, SOD and GPx Activities in Pituitary Gland, Ovarian and Uterine Tissues nof Pregnant Wistar Rats Exposed to Carbamazepine and Pentylenetetrazole

Parameters	DW	PTZ	VC+PTZ	CBZ	CBZ+PTZ	VC+CBZ+PTZ
Pituitary MDA (µMol/mg protein	1.02±0.94	4.98±0.86	1.94±0.25	3.17±0.37	2.57±0.82	2.08±0.25
Ovarian MDA (µMol/mg protein)	1.01±0.92	3.09±0.51[b]	2.05±0.41	2.90±0.17[a]	2.79±0.21	2.21±0.64
Uterine MDA (µMol/mg protein)	1.55±0.15	3.44±0.81[c]	1.64±0.67	2.91±0.26	2.90±0.51	2.11±0.15
Pituitary SOD (U/L)	1.18±0.24[d]	0.84±0.03	0.91±0.09	0.84±0.03	0.85±0.01	0.88±0.03
Ovarian SOD (U/L)	0.65±0.08	0.74±0.36	0.91±0.08	0.79±0.07	0.81±0.05	0.81±0.07
Uterine SOD (U/L)	0.90±0.27	0.27±0.013[c]	0.67±0.03	0.49±0.09[a]	0.50±0.03	0.56±0.10
Pituitary GPx (U/L)	75.81±10.87	53.27±1.72[e]	79.34±0.56	68.08±18.05[f]	61.22±8.55	95.77±1.56
Ovarian GPx (U/L)	81.22±4.80	52.89±4.50[g]	87.26±9.61	65.69±5.37[h]	69.68±3.00	82.99±9.03
Uterine GPx (U/L)	80.50±2.10	56.27±5.16[g]	94.81±8.16	63.30±6.45[i]	69.86±2.15	83.13±2.82

Values are presented as mean ± standard deviation (SD)
a = ($p < 0.05$) significantly higher compared to the DW group.
b = ($p < 0.05$) significantly higher compared to the DW group.
c = ($p < 0.05$) significantly higher compared to the DW and VC+PTZ groups.
d = ($p < 0.05$) significantly higher compared to the PTZ, CBZ, CBZ+PTZ and VC+CBZ+PTZ groups.
e = ($p < 0.05$) significantly lower compared to the VC+PTZ and VC+CBZ+PTZ groups.
f = ($p < 0.05$) significantly lower compared to the VC+CBZ+PTZ group.
g = ($p < 0.05$) significantly lower compared to the DW, VC+PTZ and VC+CBZ+PTZ groups.
h = ($p < 0.05$) significantly lower compared to the VC+PTZ group.
i = ($p < 0.05$) significantly lower compared to the DW, CBZ+PTZ and VC+CBZ+PTZ groups.

Table 2. Effect of Vitamin C on the Hematological Parameters in Pregnant Wistar Rats Exposed to Carbamazepine and Pentylenetetrazole

Parameters	DW	PTZ	VC+PTZ	CBZ	CBZ+PTZ	VC+CBZ+PTZ
PCV (%)	38.67 ± 2.08	33.67 ± 2.08	25.33 ± 8.08	21.67 ±10.02	44.67 ±29.54	31.33 ± 4.16
RBC (mL/mm^3)	6.32 ± 0.18	6.11 ± 0.93	4.16 ±1.33	3.64 ± 1.35	4.20 ± 2.75	6.35 ± 1.58
Hb (g/dL)	11.82 ± 1.28	10.70 ± 1.01	6.42 ±1.53	7.47 ± 3.07	8.10 ± 5.10	8.70 ± 3.17
Total leukocytes (×10^9/L)	5.76 ± 0.48	7.77 ± 0.67	6.80 ± 2.31	6.96 ± 1.94	6.82 ± 1.60	5.93 ± 1.67
Neutrophils (×10^9/L)	30.33 ± 3.06	41.00 ± 1.00[a]	33.67 ± 5.51	39.33 ± 2.31[b]	39.00 ± 1.00	38.00 ± 2.00
Lymphocytes (×10^9/L)	58.33 ± 2.89	67.00 ± 2.65[c]	57.33 ± 1.16	63.67 ± 5.86	57.00 ± 3.61	56.33 ± 2.08
Monocytes (×10^9/L)	2.33 ± 1.53	2.00 ± 1.00	1.67 ± 1.16	2.00 ± 1.00	2.00 ± 1.00	1.33 ± 1.53
Platelets (×10^9/L)	198.0 ± 16.82	215.0 ±11.27	202.3 ± 3.22	337.3 ±37.29[d]	181.3 ±45.32	225.7 ± 7.02

Values are presented as mean ± standard deviation (SD). a = ($p < 0.05$) significantly higher compared to the DW group.
b = ($p < 0.05$) significantly higher compared to the DW group.
c = ($p < 0.05$) significantly higher compared to the VC+PTZ, CBZ+PTZ and VC+CBZ+PTZ groups.
d = ($p < 0.05$) significantly higher compared to the DW, PTZ, VC+PTZ and CBZ+PTZ groups.

was a relative decrease in Hb level in the PTZ group when compared to the DW (10%), VC+PTZ (40%), CBZ (30%), CBZ+PTZ (24%) and VC+CBZ+PTZ (17%) groups. A relatively reduced Hg level was recorded in the CBZ group compared to DW (37%) and VC+CBZ+PTZ (17%) groups (Table 2).

Effect on total leucocyte count

No significant ($p > 0.05$) change in total leucocyte count was observed in any of the groups. There was a relative increase in leucocyte count in the PTZ group compared to the DW (35%), VC+PTZ (13%), CBZ (10%) and VC+CBZ+PTZ (12%). A relative increment in leucocyte count was also recorded in the CBZ group compared to DW (21%) and VC+CBZ+PTZ (17%)(Table 2).

Effect on neutrophil count

A significantly ($p < 0.05$) higher neutrophil count was observed in the PTZ group when compared to the DW group, while a relative increment was reported when compared to VC+PTZ (18%) group. The neutrophil count in the CBZ group increased ($p < 0.05$) significantly when compared to DW group whereas, a relatively higher value was reported when compare to VC+PTZ group (14%) (Table 2).

Effect on lymphocyte count

A significantly ($p < 0.05$) higher lymphocyte count was observed in the PTZ group when compared to the VC+PTZ, CBZ+PTZ and VC+CBZ+PTZ groups. The lymphocyte counts in CBZ group showed a relative increased value when compared to the VC+PTZ (10%), CBZ+PTZ (10%) and VC+CBZ+PTZ (12%) groups (Table 2).

Effect on monocytes

There was no significant ($p > 0.05$) change in monocyte count in the all the groups (Table 2).

Effect on platelets count

Table 2 revealed a significantly ($p < 0.05$) higher platelets count in the CBZ group when compared to the DW, PTZ, VC+PTZ and CBZ+PTZ groups, while a relative increment to reported when compared with VC+CBZ+PTZ (49%) group. A relatively high platelets count was noticed in the PTZ group when compared to the CBZ+PTZ (19%) group (Table 2).

Effect of treatment on preimplantation loss

There was a significant ($p < 0.05$) increase in preimplantation loss in the PTZ group when compared to the DW and VC+CBZ+PTZ groups, while a relative increased value was recorded when compared with CBZ (12%), VC+PTZ (23%) and CBZ+PTZ (13%) groups. The CBZ group showed a significantly ($p < 0.05$) higher percentage preimplantation loss when compared to DW group, but a relatively increased value was noticed when compared with the VC+CBZ+PTZ group (Figure 5).

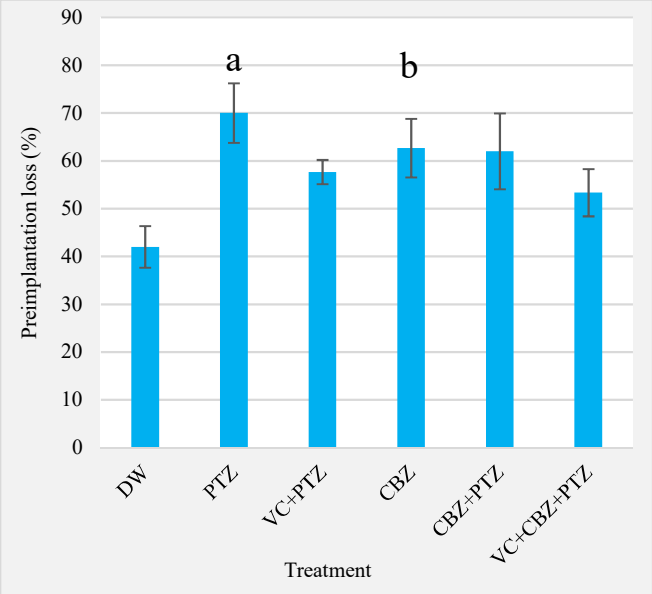

Figure 5. The effect of vitamin C on percentage preimplantation loss in Wistar rats exposed to carbamazepine and pentylenetetrazole

a= ($p < 0.05$) significantly lower compared to the DW and VC+CBZ+PTZ groups.
b= ($p < 0.05$) significantly higher compared to the DW group.

DISCUSSION

The convulsive behaviors observed in Group II (PTZ) rats indicate the occurrence of epileptic seizures, which is consistent with the results presented in a study by Feature [24], who classified PTZ-induced epileptic behaviors into freezing, myoclonic twitches, clonic seizures, and tonic-clonic seizures [25].

Malondialdehyde (MDA), a by-product of lipoperoxidation, is known to cause membrane damage by altering membrane fluidity and structural integrity and inactivating membrane-bound enzymes. The present study revealed significantly elevated concentrations of MDA in the pituitary, ovarian, and uterine tissues of the PTZ and CBZ groups, consistent with previous research [14, 26].

Our findings suggest that both seizures and carbamazepine administration contribute to oxidative stress, as evidenced by elevated MDA levels. The increase in MDA levels likely results from intensified oxidative challenges associated with reactive oxygen species (ROS) activity in animals exposed to PTZ and CBZ. Treatment with vitamin C has been shown to decrease MDA concentrations in the tissues above. This is likely due to its antioxidant properties, which reduce free radical generation and consequently mitigate oxidative damage [27].Moreover, the observed decrease in superoxide dismutase (SOD) activity in the pituitary, ovarian, and uterine tissues of the PTZ and CBZ exposed groups suggests that seizures and CBZ administration during pregnancy may contribute to reduced enzyme synthesis, increased metabolism activity, or enzyme inactivation due to elevated superoxide production. This supports previous findings. [28].

The magnified lipoperoxidation, indicated by the higher MDA concentrations in the PTZ and CBZ groups, have further diminished SOD activity, potentially due to the increased demand to counteract the oxidative damage resulting from PTZ and CBZ exposure, along with subsequent metabolic breakdown. SOD serves as the first line of defense against ROS catalytically converting superoxide radicals (O^{2-}) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) [29].

Notably, the elevated SOD activities in the pituitary, ovarian, and uterine tissues of vitamin C treated groups,

which donates electrons to prevent the oxidation of other compounds [30].

Our findings also highlight a substantial reduction in glutathione peroxidase (GPx) concentration in the pituitary, ovarian, and uterine tissues in the PTZ and CBZ groups, corroborating previous research [14,28,31].

It is likely that the depletion of GPx is a direct result of oxidative damage caused by CBZ and PTZ. As an essential antioxidant enzyme, Glutathione peroxidase plays a critical role in protecting the cytosol and plasma membrane from lipid peroxidation by catalyzing the reduction of hydrogen peroxide (H_2O_2) and organic hydroperoxides generated at the membrane [32].

The low GPx activity recorded in our study indicates an inadequate degradation of H_2O_2 and lipid peroxide, could form hydroxyl radicals (OH*) and lipid peroxyl radicals through interaction with transition metals [33]. We also showed vitamin C increased GPx activity, highlighting its protective antioxidant effect against cellular damage induced by PTZ and CBZ [14].

Blood profile provides important insights into the body's response to injury, lesion, deprivation and stress [34]. The observed reduction in packed cell volume (PCV) indicates that pregnant women with epilepsy undergoing CBZ therapy may be at an increased risk of anemia. This risk is likely due to the adverse effects of seizures and CBZ on the bone marrow, and could be linked to the decrease in tissue iron concentration caused by CBZ exposure, which impair hemoglobin (Hb) production and shorten the red blood cell (RBC) life cycle. The results align with earlier research [11]. Notably, the administration of vitamin C significantly improved the PCV levels of the animals supporting the beneficial effects of vitamin C on anemia [11,35]. Furthermore, this study provides experimental evidence that PTZ and CBZ exposure decreases red blood cell count and hemoglobin concentration. The observed decrease in hemoglobin may be attributed to reduced iron levels in the blood, stemming from seizures and carbamazepine, which can deplete iron stores. The reductions in RBC count may result from decreased production or pure red cell aplasia, potentially due to the swelling of RBCs and increasing oxygen demands in response to CBZ-induced hypoxia. Our current findings corroborate other reports [11, 36].

Treatment with vitamin C tends to enhance the oxygen-carrying capacity of RBCs reflected in increased Hb levels and RBC counts suggesting that vitamin C may enhance RBC survival by improving erythrocyte membrane stability and osmotic resistance [37].

White blood cells (WBCs) perform important roles in regulating the body's immune defense. Alterations in WBC counts may indicate a decline in nonspecific immunity [38]. The leukocytosis observed in the PTZ and CBZ treatment groups could be attributed to increased neutrophil release (neutrophilia), stimulating effects on the immune system, and lymphocytosis driven by lymphoid and myeloid tissues. Our data demonstrate that pretreatment with vitamin C mitigates PTZ- and CBZ-induced leukocytosis. The observed neutrophilia may partly result from the oxidative stress challenge that triggers the production of inflammatory cytokines, leading to cellular damages [39].

Additionally, the lymphocytosis seen in the CBZ group could be attributed to the formation of epoxides through the cytochrome P450 metabolism [40], which is covalently bind to macromolecules, acting as haptens that induce immunological responses and cause lymphocytosis. Our findings robustly indicate that vitamin C treatment effectively normalizes the differential WBC counts altered by CBZ and PTZ exposure, while enhancing leukocyte chemotaxis and phagocytosis, promoting efficient removal of damaged cells [36].

PTZ and CBZ groups exhibited thrombocytosis, reflecting elevated platelet count, consistent with previous reports [11]. Thrombocytosis may occur due to the induction of oxidative stress, which can lead to increased production of erythropoietin and contribute to iron-depleted anemia [41]. The observed thrombocytosis in our study may represent a reactive type induced by inflammatory responses with increased serum interleukin-6 levels. Importantly, pretreatment with vitamin C effectively normalized PTZ/CBZ-induced thrombocytosis, showcasing its protective properties on the hematopoietic system. Our results indicates that the PTZ and CBZ groups showed decreased concentrations of FSH and LH compared to the other groups. Research has demonstrated the adverse effects of antiepileptic drugs on the anterior pituitary, which secretes FSH and LH hormones crucial for oogenesis [14, 31]. The reduced FSH and LH levels in the PTZ and CBZ groups may be attributed to the detrimental impact of these substances on gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus, which is pivotal for gonadotropin release from the anterior pituitary [42]. The observed increases in LH and FSH concentrations in the vitamin C-treated groups clearly demonstrate the protective effects of vitamin C on gonadotropin levels.

Pregnancy loss occurs in approximately 15-25% of pregnancies [43]. Low levels of estradiol and progesterone indicate poor pregnancy outcome. Progesterone and estrogen are essential for normal pregnancy; thus, low levels, may compromise pregnancy viability. Estrogen stimulates endometrial hyperplasia and myometrium thickness, enhancing blood supply and uterine contractibility [44, 45]. Elevated estradiol levels during early pregnancy correlate with the dominant follicle's quality and the corpus luteum optimal function, both crucial for maintaining a healthy pregnancy [46].

Conversely, progesterone provides endocrine support, promotes a calming effect, and reduces uterine contraction intensity and frequency, extending the endometrial secretion period and coordination [47]. The marked decrease in estradiol and progesterone levels in the PTZ and CBZ groups are directly linked to the reproductive impairments caused by oxidative stress by these substances, supporting earlier studies [43]. The observed rise in progesterone and estradiol levels in the vitamin C treated groups demonstrates the protective effect of antioxidant vitamin C on sex steroid hormone concentrations, enhancing endometrial thickness during the luteal phase and priming the uterus for implantation. Furthermore, vitamin C boosts progesterone

secretion from corpus luteum tissues [18]. The substantial rise in preimplantation loss percentages in rats exposed to PTZ and CBZ provides compelling confirmation of our earlier findings. The increased oxidative stress, changes in blood composition, and reduced sex hormone levels in the PTZ and CBZ groups may have adversely impacted the preimplantation stage, resulting in higher preimplantation loss rates. Consequently, this may influence overall pregnancy rates. Conversely, the vitamin C treated group exhibited lower percentages of preimplantation loss, underscoring the protective and reproductive enhancement properties of vitamin C as an antioxidant.

CONCLUSION

In conclusion, the findings of this study indicate that seizures and the use of antiepileptic drugs, particularly carbamazepine, may increase the risk of pregnancy loss in individuals with epilepsy. This risk appears to be linked to oxidative damage observed in pituitary, ovarian, and uterine tissues.

Oxidative stress significantly impacts the levels of female sex hormones, hematological parameters, and the rate of preimplantation loss. Notably, Vitamin C has demonstrated protective effects against the alterations in sex hormone concentrations, oxidative stress markers, and hematological indices, as well as preimplantation loss induced by carbamazepine and seizures in Wistar rats. The incidence of seizures and the use of antiepileptic drugs during pregnancy, as well as the risk of overdose, represent important public health issues. These conditions are often associated with severe side effects, including hemorrhage, abortion, and stillbirth. The results of this study elucidate the biological pathways associated with these challenges [48,49]. Additionally, the protective effects of vitamin C identified in our research could offer valuable insights for developing strategies to mitigate the adverse effects of antiepileptic drugs in pregnant women exposed to

Funding and Support: The authors acknowledged the financial support provided by the University of Ilorin.

Conflict of interest: The authors declare no conflicts of interest related to this study.

REFERENCES

1. Lu H, Chang W, Huang ACW, Shyu BC. Detection of pentylenetetrazol-induced seizure activity in the 19-21 Hz beta range using a magnetic coil induction method. Front Biosci (Landmark Ed). 2022;27(3):1-12.
2. Beghi E. The epidemiology of epilepsy. Neuroepidemiology. 2020;54(2):185-91.
3. Farghaly WMA, Hamdy NE, Tarek AR, Eman MM, Nabil AM, Ghaydaa AS, et al. Epidemiology of uncontrolled epilepsy in the Al-Kharga District, New Valley, Egypt. Seizure. 2013;22(9):611-6.
4. Chen Y, Chiou H, Lin H, Lin H. Effect of seizures during gestation on pregnancy outcomes in women with epilepsy. Arch Neurol. 2009;66(8):979-84.
5. Wang Y, Wei P, Yan F, Luo Y, Zhao G. Animal models of epilepsy: a phenotype-oriented review. Aging Dis. 2022;13(1):215-31.
6. Shimada T, Yamagata K. Pentylenetetrazole-induced kindling mouse model. J Vis Exp. 2018;(136):1-10.
7. Szabo CA. Patient page. Risk of fetal death and malformation related to seizure medications. Neurology. 2006;67:6-7.
8. Bhatti SFM, De-Risio L, Muñana K, Penderis J, Stein VM, Tipold A. International veterinary epilepsy task force consensus proposal: medical treatment of canine epilepsy in Europe. BMC Vet Res. 2015;11(1):176.
9. Maan JS, Duong TH, Saadabadi A. Carbamazepine. StatPearls. 2023 [cited 2023 Nov 25]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482455/
10. Mittag N, Meister S, Berg AM, Walther UI. A case report of a carbamazepine overdose with focus on pharmacokinetic aspects. Pharmacopsychiatry. 2016;49(2):76-8.
11. Akorede GJ, Ambali SF, Olatunji AO, Aremu A, Ahmed AO, Basiru A, et al. Vitamin C protects against blood and thyroid toxicities induced by the chronic use of carbamazepine in rats. Iran J Toxicol. 2023;17(2):147-56.
12. Al-Bishri WM, Abdel-Reheim ES, Zaki AR. Purslane protects against the reproductive toxicity of carbamazepine treatment in pilocarpine-induced epilepsy model. Asian Pac J Trop Biomed. 2017;7(4):339-46.
13. Yan S, Wang M, Zha L, Li W, Luo Q, Sun J, et al. Environmentally relevant concentrations of carbamazepine caused endocrine-disrupting effects on non-target organisms, Chinese rare minnows (Gobiocypris rarus). Environ Sci Technol. 2018;52(2):886-94.
14. Akorede GJ, Ambali SF, Hudu MG, Olatunji AO, Shittu M, Aremu A, et al. Protective effect of vitamin C on chronic carbamazepine-induced reproductive toxicity in male Wistar rats. Toxicol Rep. 2020;7:269-76.
15. Artama M, Auvinen A, Raudaskoski T, Isojärvi I, Isojärvi J. Antiepileptic drug use of women with epilepsy and congenital malformations in offspring. Neurology. 2005;64(11):1874-8.
16. Bech BH, Kjaersgaard MI, Pedersen HS, Howards PP, Sørensen MJ, Olsen J, et al. Use of antiepileptic drugs during pregnancy and risk of spontaneous abortion and stillbirth: population-based cohort study. BMJ. 2014;349:5159.
17. Ibitoroko GM, Adebayo AO, Confidence W. Effect of vitamins C and E on hematological parameters in Albino rats treated with gasoline. Glob Vet. 2011;7(4):347-52.
18. Wasan GA. Vitamin C supplementation in relation to subfertility and ovarian function. Karbala J Med. 2014;7(1):1-8.
19. Rajesh KR, Surenda R, Thangam J. Effect of valproic acid and carbamazepine on learning and memory in rats. Indian J Pharmacol. 1991;23(3):185-9.
20. Damasceno DC, Volpato GT, Sartori TCF, Rodrigues PF, Perin EA, Calderon IMP, et al. Effects of Annona squamosa extract on early pregnancy in rats. Phytomedicine. 2002;9(7):667-72.
21. Draper HH, Hardley M. Malondialdehyde deformation as an index of lipid peroxidation. Methods Enzymol. 1990;186:421-31.
22. Martin JP, Dailey M, Sugarman E. Negative and positive assays of superoxide dismutase based on haematoxylin autooxidation. Arch Biochem Biophys. 1987;225:329-36.
23. Paglia DE, Valentine WN. Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967;70:158-69.
24. Features D. Pentylenetetrazol models of chemically-induced acute seizures. Funct MRI Epilepsy. 2006;14:127-52.
25. Pylvänen V, Pakarinen A, Knip M, Isojärvi JI, Lönnqvist J. Carbamazepine affects weight but not leptin secretion in epileptic patients. Pharmacol Toxicol. 2002;90(2):71-4.
26. Sener U, Uygur R, Aktas C, Uygur E, Erbaş O. Oxidative stress in carbamazepine use: a meta-analysis. Seizure. 2003;12(7):497-502.

27. Kadar-Ali SH, Raja WA. The effect of antioxidants in acute amitriptyline poisoning. Toxicol Rep. 2019;6:380-8.
28. Nasr NA, Abd El Aal MF, El-Sokkary GH. Carbamazepine-induced oxidative stress in epileptic children: the role of SOD1 and GPx1 gene polymorphisms. Eur J Paediatr Neurol. 2013;17(6):594-600.
29. Fridorich I. Superoxide radicals and superoxide dismutases. Annu Rev Biochem. 1992;64:97-112.
30. Aprioku JS. Pharmacology of free radicals and the impact of reactive oxygen species on the testis. J Reprod Infertil. 2013;14(4):158-72.
31. Li ZH, Li P, Randak T. Effect of a human pharmaceutical carbamazepine on antioxidant responses in brain of a model teleost in vitro: an efficient approach to biomonitoring. J Appl Toxicol. 2010;30:644-8.
32. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: a review. Eur J Med Chem. 2015;97:55-74.
33. Tahbet F, Touyz RM. Reactive oxygen species, oxidative stress and vascular biology in hypertension. Compr Hypertens. 2007;3:337-47.
34. Afshar M, Moallem MSA, Mohammadpour AH, Shiravi A, Jalalian SM, Golalipour MJ. Teratogenic effects of carbamazepine on embryonic eye development in pregnant mice. Cutan Ocul Toxicol. 2010;29(1):10-5.
35. Akunne TC, Okafor SN, Igweze Z, Njoku CN, Ojapinwa OO. Toxicological profile of carbamazepine and levetiracetam on some biochemical and hematological parameters in rats. Pharma Biosci J. 2017;5(4):30-7.
36. Aliyu H, Ayo JO, Ambali SF, Zezi AU. Effects of administration of carbamazepine and/or phenytoin on serum biochemical parameters in Wistar rats. Afr J Pharm Pharmacol. 2013;7(23):1585-91.
37. Gowthami V, Sudha P, Suresh T, Mounika P, Dhivya K. Prospective study on the effect of monotherapy antiepileptics on haematological parameters and various biochemical parameters in epilepsy patients. J Chem Pharm Res. 2017;9(5):277-81.
38. Ambili TR, Saravanan M, Ramesh M, Abhijith DB, Poopal RK. Toxicological effects of the antibiotic oxytetracycline to an Indian major carp Cirrhinus mrigala under tropical conditions. Chemosphere. 2013;91(2):206-16.
39. Rivera-Rodriguez KM, Rodríguez-Rivera AV, Roman-Julia R, Morales-Borges R. Effectiveness of vitamin C in the treatment of anemia in patients with chronic diseases: A case study. Res Rev Pharm Pharm Sci. 2016;5(4):31-5.
40. Kavitha C, Malarvizhi A, Senthil-Kumaran S, Ramesh M. Toxicological effects of arsenate exposure on hematological, biochemical, and liver transaminases activity in an Indian major carp, Catla catla. Food Chem Toxicol. 2010;48(10):2848-54.
41. Çağ I, Altun Y, Altunışık E. Do antiepileptic drugs have any effect on neutrophil/lymphocyte and platelet/lymphocyte ratio? Med Sci Discov. 2019;6(4):76-81.
42. Osuntokun OS, Olayiwola G, Oladele A, Ola I, Ayoka AO. Chronic administration of gabapentin and a gabapentin-carbamazepine combination reversibly suppresses testicular function in male Wistar rats (Rattus norvegicus). Pathophysiology. 2017;24:63-9.
43. Deng W, Sun R, Du J, Wu X, Ma L, Wang M, Lv Q. Prediction of miscarriage in first trimester by serum estradiol, progesterone, and β-human chorionic gonadotropin within 9 weeks of gestation. BMC Pregnancy Childbirth. 2022;22(112):23-32.
44. Verma P, Verma R, Nair RR, Budhwar S, Khanna A, Agrawal NR, Sinha R, Birendra R, Rajender S, Singh K. Altered crosstalk of estradiol and progesterone with myeloid-derived suppressor cells and Th1/Th2 cytokines in early miscarriage is associated with early breakdown of maternal-fetal tolerance. Am J Reprod Immunol. 2019;81(2)
45. Zhang W, Tian Y, Xie D, Miao Y, Liu J, Wang X. The impact of peak estradiol during controlled ovarian stimulation on the cumulative live birth rate of IVF/ICSI in non-PCOS patients. J Assist Reprod Genet. 2019;36(11):2333-44.
46. Gao Q, Wang M, Rong T. Serum estradiol level in pregnant women of four to eight weeks and its relationship with threatened abortion. J Shandong Univ. 2008;46(9):884-6.
47. Di-Renzo GC, Giardina I, Clerici G, Brillo E, Gerli S. Progesterone in normal and pathological pregnancy. Horm Mol Biol Clin Investig. 2016;27(1):35-48.
48. Sane N, Goudarzi F. Seizure in patients with antiepileptic drug overdose: study on patients admitted to Shoushtari Hospital in Shiraz. Asia Pac J Med Toxicol. 2013;2:101-4.
49. Ajam M, Reyhani T, Roshanravan V, Zare Z. Increased miscarriage rate in female farmers working in saffron fields: a possible effect of saffron toxicity. Asia Pac J Med Toxicol. 2014;3:73-5.