Metabolic depletion of synaptosomal enzymes linked with neurotoxicity and ovarian dysfunction by phenolic antioxidants of *Croton zambsicus* leaves in rats exposed to chronic mixture of anthropogenic toxicant

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ARTICLE INFO

ABSTRACT

A complex mixture of organic contaminants and metals is associated with neuron-fertility disorders and studies have demonstrated that phenolic antioxidants from herbal origin, possesses a strong protective potential. This study aimed to investigate the protection of phenolic croton zambesicus (C-ZAMB) leaves against neuro-ovarian damage in rats exposed to chronic mixture of anthropogenic toxicants (EOMABRSL). The animals were divided into five groups (n = 10): Group I was given 0.5 ml of distilled water only; Group II received 0.5 ml of EOMABRSL for 98 days; Group III received 0.5 ml of EOMABRSL for 70 days and withdrew for 28 days; Group IV received 0.5 ml of EOMABRSL for 70 days +400 mg/kg phenolic C-ZAMB for 28 days; Group V received 400 mg/kg C-ZAMB only for 28 days via oral route. Both non-withdrawal and withdrawal EOMABRSL-exposed animals exhibited neuro-ovarian impairment by up-regulating neuronal 5^1 eco-nucleotidase (5^1ENT), acetylcholinesterase (AChE), butrylcholinesterase (BuChE), synaptosomal monoamine oxidase-A (MAO-A) with altered cerebral antioxidants. Similarly, exposure to EOMABRSL for 98 and 70 days caused ovarian injury by amplifying the activity of 5^1ENT with corresponding decline of fertility index, lactate dehydrogenase (LDH) and Δ5 17β-hydroxyl steroid dehydrogenase (Δ517β-HSD). EOMABRSL intoxication also increased the neuro-ovarian MDA content with reduced numbers of neonates. Phenolic antioxidants from C-ZAMB leaves identified by High Pressure Liquid Chromatography (HPLC) ameliorated the chronic EOMABRSL intoxication. The treatment also prevented ovarian lesions by depleting MDA content and improved antioxidant status. Thus, confirming its neuro-ovarian protection.

1. Introduction

Universally, neuro-fertility challenges among women are progressively increasing in the biosphere which poses a serious threat to human healthiness [1]. It was empirically estimated that erratic inevitable springing of hysterical industrialization and work-related activities could expose the human population to mediators of numerous diseases including ovarian damage when triggered by neuronal dysfunction [2,3]. Fertility disorders associated with abnormal neuronal signaling molecules are speculated to be prevalent among womenfolk in industrialized countries due to consequential exposure to mixed-environmental chemicals. In the past, cancer of the testes, ovaries, prostate, varicoceles, brain, and CNS tumor were identified as the major challenge in human subjects exposed to mixed-metals and toxicants from drinks, foods, and workplaces [4–6]. Also, childlessness and sterility connected to ovarian dysfunction had been identified in females with a high risk of the psychiatric disorder [7]. The US National Institute of Mental Health (NIMH) appraised that some American female adults suffer from psychological ailments, with significant acquainted difficulty in child birth [7]. It was additionally stated that femininity is an essential variable in experimental and clinical neurological and psychiatric studies [7]. The report also suggested that Italian women

with menopause trigger metabolic changes in the brain which may promote Alzheimer's diseases [8]. This might be linked to unregulated exposure to chemical agents that have the potential to interact with the ovary and/or ovules as well as their receptors to initiate ovarian impairment [6,9].

Chronic neuro-ovarian disorder remains a major medical problem because currently available anti-infertility drugs in women have a limited range of activity and their severe adverse drug reactions tend to compromise patients' compliance [10]. Additionally, the prevalence of treatment-resistant types of female infertility and slow progressive action of the drug has also restricted the therapeutic efficacy of anti-infertility drugs [11] in women. Unfortunately, in vitro fertilization (IVF) is an expensive option for people struggling with infertility. It was estimated that 48% of IVF cycles resulted in pregnancy while 9% of IVF pregnancies ended in miscarriage among women of 43 years and older [12]. Also, most IVF clinics could not even provide treatment to women of 45 years or older. For these aforementioned reasons, research interest has migrated increasingly to natural products in pursuit of innocuous and effective new medications as a therapeutic substitute for chronic ovarian disorders.

Croton zambscus is a class in the family, Euphorbiaceae, members of which are known to possess potent anti-depressant and anticonvulsant activities [13]. In West African folk medicine, the root is appreciated for treating hypertension and urinary infections as well as several medicinal applications such as relieving malaria, diabetes and fundamentally speculated as epileptic seizures [13] Also, the aqueous leaf extract of *C. zambscus* promotes central nervous system (CNS) [14] and used as energizer of conditions associated with nervous weakness such as impotence, reduce libido and fatigue, which also characterize noticeable symptoms of neuro-ovarian damage [13]. It was also postulated that the leaves of *C. zambscus* might be useful for managing inflammation, cardiovascular conditions, arthritis, osteoporosis, hepatotoxicity, renal failure, infertility, retinopathy, and Alzheimer's disease. Furthermore, the leaves have been reported to contain various phytochemicals active compounds such as p-cymene including linalool and beta-caryophyllene, pinene, limonene linalool, menthol, carvone, thymol, alpha-humulene and ceisnerolidol [14]. Also, phenolic compounds are the most abundant naturally-occurring antioxidants because of their presence in most plant products. They have multifunctional antioxidant properties because of their ability to reduce the incidence of degenerative diseases such as ovarian cancer, Alzheimer's disease, barrenness as well as improving libido in females [15]. Pharmacologically, no detailed studies have been carried out to ascertain whether the leaf possesses inhibitory potential (in vivo) on biological markers linked to chronic ovarian toxicity when triggered by the neuronal disorder. Also, one potential environmental risk factor of ovarian damage that has not received serious attention is exposure to a mixture of toxic compounds. Hence, the present study was designed to investigate the metabolic inhibitory potential of phenolic leaf fraction of *Croton zambescus* using conventionally experimental models in female rats.

2. Materials and methods

Substrates AMP, acetylcholine iodide, butrylcholine iodide, benzylamine, Di-hydroxylepiandrosterone (DHEA), testosterone, nicotinamide adenosine dinucleotide (NAD+), NADPH, 5, 5-dithio-bis-2-nitrobenzoic acid (DTNB), GSH, hydrogen peroxide, trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were purchased from Sigma (St Louis, MO, USA). All other reagents were of analytical grade and were obtained from the British Drug Houses (Poole, Dorset, UK). Kit for lactate dehydrogenase activity was purchased from Random Laboratory Limited, United Kingdom.

2.1. Sample site, collection and preparation of leachate

Leachate collection and preparation was done following our previous

method [16]. After the preparation and selection, it was labelled as EOMABRSL and stored at 4 °C for further use. Briefly, 100 g of sample was dissolved in 100 ml distilled water producing 100% stock solution of leachate from Elewi Odo Municipal Battery Recycling site (EOMABRS). We then took 0.5 ml from the stock solution to give each animal due to the fact that the animals were exposed for a long time i.e. chronic exposure. This technique supports previous study [16].

2.2. Sample collection

The new *Croton zambsicus* (C-ZAMB) leaves were harvested from Ogundele Village farm at Kwara State, Ilorin, Nigeria. Leaves authentication was approved by the Department of Plant Biology, University of Ilorin, Nigeria with the voucher number of UIH001/1191. Thereafter, we quantified the phenolic compounds under gradient conditions using HPLC-DAD reverse-phase chromatography (Table 1 and Fig. 1) [17].

2.3. Preparation of phenolic antioxidant fraction

Precisely, 50 g of pulverized C-ZAMB leaves were macerated in 500 ml of methanol for 24 h in air tight clean flat-bottomed container at room temperature. It was repeatedly stirred on a shaker for a day following filtration by a Whatman filters paper through cotton plug. The phenolic fraction was fractionated by partitioning chromatography.

2.4. Animal management

Adult male Wistar rats (130–170 g) from the Central Animal House of the Kwara State University Malete, Nigeria were used in this experiment. The animals were maintained at a constant temperature (22 ± 2 °C) on a 12 h light/dark cycle with free access to food and water. Animal care and handling was performed according to the institutional guidelines.

2.5 Investigational protocol

The acclimatized rats for two weeks were arbitrarily divided into five groups of ten animals each (n = 10). Group 1 was the control and given distilled water only via oral route; Group 2 (NWD-EOMABRSL) served as neuro-ovarian damage group exposed to 0.5 ml of leachate from Elewi odo municipal auto-battery recycling site (EOMABRSL) for 98 days (chronic exposure); Group 3 (WD-EOMABRSL) served as neuro-ovarian damage group exposed to 0.5 ml of EOMABRSL for 70 days and was withdrawn for 28 days. Group 4 (EOMABRSL + C-ZAMB) served as neuro-ovarian damage group exposed to 0.5 ml of EOMABRSL for 70 days and post-treated with 400 mg/kg body weight of phenolic antioxidants from *Croton zambsicus* leaves fraction (C-ZAMB) for 28 days. Group 5 (C-ZAMB only) served as the treated group only given 400 mg/kg body weight of C-ZAMB for 28 days. Previous study had made use of

Table 1
The level of heavy metals in EOMABRSL in relation to WHO limit [2,3].

Metal	Level	WHO Limit	% increase
Cu	0.341	2.00	–
Zn	0.010	3.00	–
Cd	0.006	0.003	100
Mn	7.842	0.40	1860
Co	0.049	0.05	–
Cr	0.068	0.05	36
Fe	2.667	0.30	789
Ni	0.051	0.02	150
Pb	0.015	0.01	50

All values are in milligrams per litre. Least observable effective concentration (LOEC) set by the World Health Organization [61]; **EOMABRSL**: Elewi Odo municipal battery recycling site leachate; **% increase**: Percent increase compared with the WHO permissible limits in drinking water.
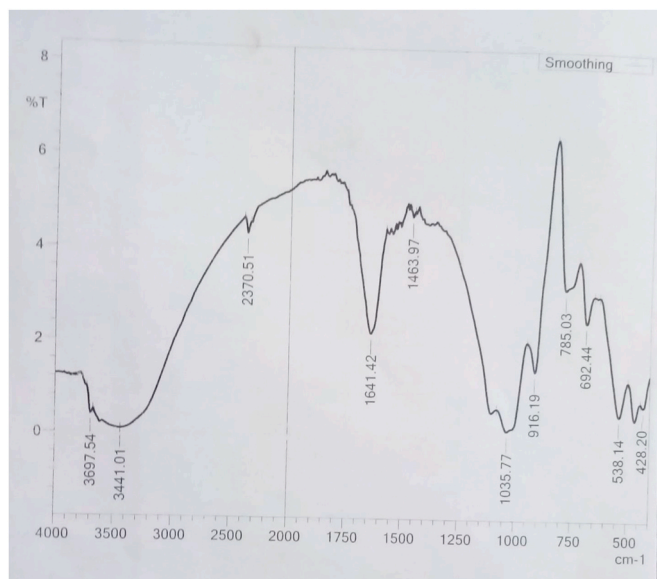
Fig. 1. The representative Fourier infra-red chromatography profile of solid sample from the EOMABRSL of Oyo State, Nigeria.

400 mg/kg body weight of *Croton zambsicus* leaves [14] and 0.5 ml of EOMABRSL was selected based on our preceding findings [2,3,16]. The rats were fed with the same standard food and had free access to drinking water throughout the entire experiment. The experiment lasted for 14 weeks (chronic exposure). These rats were euthanized 24 h after the last treatment session.

2.6. Mating of the female rats

Sequel to the exposure of female rats to EOMABRSL poisoning for 70 days, one male rat was randomly introduced into each cage containing 10 female rats, such that the male-female ratio in the cages was 1:10. After the introduction of males into the 5 mating cages, the mated females were followed up individually in the cages to ensure that unmated females were mated. The males were removed from the cages immediately pregnancy of the mated females occurred. Thereafter, fertility index and the numbers of neonates were calculated.

2.7. Preparation of post mitochondrial fraction (PMF) of ovary and cerebral cortex

The animals submitted to euthanasia being previously anesthetized with ethyl acetate and ovaries and brain were removed for ovarian and cerebral homogenate preparation, respectively. The ovaries were homogenized in 4 vol while cerebral in 10 vol of an ice cold medium, consisting of 1.15% potassium chloride and 50 mM Tris-HCl buffer with a pH 7.4 in a motor driven. Teflon-glass homogenizer. The supernatants were isolated at 4 °C and used for biochemistry assays.

2.8. Synaptosome preparation

After the treatment, the animals were submitted to euthanasia being previously anesthetized with ethyl acetate and synaptosome was extracted from the homogenate of the brain by differential centrifugation. Rats brain was homogenized in 10 ml Phosphate buffer, pH 7.4 (0.01 M) and centrifuged at 770 g for 5 min. The supernatant was centrifuged at 50, 000 g for 15 min. The supernatant was removed and the pellet re-suspended in 20 ml of Phosphate buffer, pH 7.4 (0.01 M). The pellet resulting from another centrifugation (50, 000 g, 15 min, 4 °C) was re-suspended in 1–2 ml of Phosphate buffer, pH 7.4 (0.01 M) and stored at 4 °C until use.

2.9. Lipid peroxidation assay

Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method described by Ohkawa et al. [18] and expressed as nmoles/mg protein.

2.10. Lactate dehydrogenase (LDH) assay

The homogenates were assayed for lactate dehydrogenase (LDH) activity using commercially available kit (Randox Laboratories UK). Assay was carried out according to the manufacturer's instructions [19].

2.11. Eco- 5^1-nucleotidase activity assay

The 5^1-nucleotidase activity (brain and ovary) was determined essentially by the method of Heymann et al. (1984) [20]. The released inorganic phosphate (Pi) was measured at 700 nm and enzyme activity was reported as nmol Pi released/min/mg of protein.

2.12. Biological antioxidant assays

Superoxide dismutase (SOD) activity was determined by measuring the inhibition of autooxidation of epinephrine pH 10.2, at 30 ± 1 °C according to Misra and Fridovich [21] (1989). The homogenate collected was used for the estimation of catalase (CAT) activity using hydrogen peroxide as the substrate according to the method of Clairborne [22] (1995). GST was assayed using 1-chloro-2, 4-dinitrobenzene as the substrate according to the method of Habig (1974) [23] and expressed as mole CDNB-GSH complex formed/min/mg protein. Reduced GSH was determined at 412 nm using the method described by Jollow et al. [24].

2.13. Assay for ovarian $\Delta^5 17\beta$-HSD activity

The ovarian $\Delta^{5-}17\beta$-HSD activity was measured by the method of Jarabak et al. [25]. One unit of enzyme activity is equivalent to a change in absorbance of 0.001/min at 340 nm.

2.14. Acetylcholinesterase activity assay

The acetylcholinesterase (AChE) an enzymatic assay was determined by the method of Ellman [26]. The enzyme activity was expressed in nmol AChE/min/mg of protein.

2.15. Butyrylcholinesterase activity assay

The butyrylcholinesterase (BuChE) an enzymatic assay was determined by the method of Ellman [26]. The enzyme activity was expressed in nmol BuChE/min/mg of protein.

2.16. Monoamine oxidase-A activity (MAO-A) assay

Monoamine oxidase-A (MAO-A) activity was estimated using benzylamine as the MAO substrate according to the method described by Kettler and colleagues [27]. Results were expressed in units per mg protein (Unit/mg protein).

2.17. Protein determination

Protein was measured by the Biuret method as described by Gornall et al. [28] using serum albumin as standard.

2.18. Pathological examination on the neuronal and ovarian cells

After the treatment, the cerebra and ovaries were separated and placed in 4% para-formaldehyde at 4 °C for 48 h. After dehydration,

transparency, paraffin immersion and paraffin embedding, the cerebrum was sliced along median antero-posterior axes at a thickness of 6 mm. The section was stained with hematoxylin and eosin for morphological observation and defining positions. Sections were read and images were captured using microscope.

2.19. Statistical analysis

All data were expressed as mean ± SD (standard deviation). The statistical analysis used was one-way ANOVA, followed by Duncan's multiple range tests at the significance level α = 0.05.

3. Results

3.1. Organic pollutants and mixed heavy metals levels in EOMABRSL

The Fourier infra-red chromatography showing mixed organic pollutants from EOMABRSL is presented Fig. 1. The EOMABRSL contains supplementary organic pollutants as reported by our previous finding [2,3]. They include acetonitrile, ethyl-4-chloro-2-cyanoacetoacetate, 3-methoxyphenylacetonitrile, 2, 4, 6-trihydroxyacetophenone, benzalacetone, phenylsulfonylacetonitrile, 3-methylacetophenone, ethyl acetohydroxamate, 1-acetonaphthone and P-tolyacetonitrile. Also, our previous finding had shown the levels of nine heavy metals (Cu, Zn, Cd, Mn, Cr, Co, Fe, Ni and Pb) in EOMABRSL (Table 1). These heavy metals were reported to be greater than permissible levels in drinking water [2, 3].

3.2. Phenolic antioxidants in C-ZAMB leaves

The HPLC profile of phenolic antioxidants from *Croton zambsicus* leaves is presented in Fig. 2 as observed from the previous finding (Table 2) [2,3].

3.3. Effect of phenolic C-ZAMB leaves on neuronal and ovarian MDA contents

Fig. 2 shows the effect of administration of phenolic C-ZAMB leaves (400 mg/kg body weight) and its administration in EOMABRSL induced neuro-ovarian damage of rat. Post hoc analysis of testing showed that non-withdrawal and withdrawal exposure of EOMABRSL markedly (P < 0.05) increased the MDA contents in both ovary and cerebral cortex of animals (Fig. 2A and B). However, post treatment and phenolic C-ZAMB leaves (400 mg/kg) significantly (p < 0.05) prevented these effects

Table 2
Composition of *Croton zambesicus* aqueous extracts, C-ZAMBAE.

Compounds	C-ZAMBAE (mg/g)	LOD (μg/mL)	LOQ (μg/mL)
Gallic acid	1.58 ± 0.01[a]	0.021	0.068
Caffeic acid	2.07 ± 0.02[b]	0.018	0.059
P-coumarin	ND	0.009	0.030
Rutin	ND	0.025	0.082
Quercetin	4.17 ± 0.04[c]	0.009	0.030
Luteolin	0.95 ± 0.01[d]	0.027	0.089
Apigenin	2.11 ± 0.01[b]	0.015	0.049

LOD = Limit of detection; LOQ = Limit of quantification. The results are expressed as mean ± SD of three determinations. Comparing various groups, different letters indicate statistically significant findings.
Adapted from Akintunde et al. [79]

(Fig. 2A and B). Thus, it was indicated that pharmacological treatment with phenolic C-ZAMB leaves may inhibit chronic cellular (cerebrum and ovary) membrane damage initiated by EOMABRSL intoxication (Fig. 2).

3.4. Antioxidant status in the brain and ovary

The antioxidant status in brain region and ovary post mitochondrial homogenate of the rats intoxicated with chronic EOMABRSL exposures (non-withdrawal and withdrawal) is presented in Figs. 3 and 4. Post hoc analysis showed that both neuronal and ovarian SOD activities were significantly (p < 0.05) elevated by chronic exposure to 0.5 ml EOMABRSL for 98 days (non-withdrawal) (Fig. 3A and C) when compared to their corresponding controls and other groups. A similar trend of neuronal and ovarian SOD activities (Fig. 3A and C) was observed in EOMABRSL exposure for 70 days (withdrawal for 28 days). In addition, brain CAT activity was significantly (p < 0.05) depleted by chronic exposure to 0.5 ml EOMABRSL for 98 days (non-withdrawal) as well as withdrawal for 28 days (Fig. 3B) when compared to the corresponding control and treated group. Conversely, chronic EOMABRSL exposure for 98 days (non-withdrawal) and 70 days (withdrawal for 28 days) noticeably (p < 0.05) increased the ovarian CAT activity when compared to the control and treatment groups. Generally, therapeutic treatment with phenolic antioxidants from C-ZAMB leaves (400 mg/kg) prohibited the alterations in both neuronal and ovarian (SOD and CAT activities) induced by mixture of organic pollutants and heavy metals (Fig. 3A–D).

Furthermore, neuronal GST activity was significantly (p < 0.05)
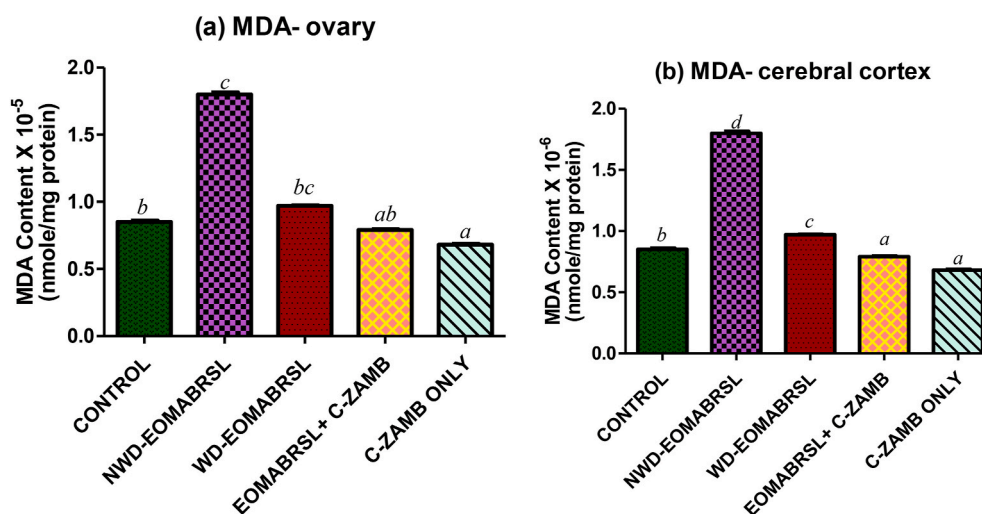
Fig. 2. (a and b): Effect of phenolic fraction from *Croton zambesicus* (C-ZAMB) on malonaldehyde (MDA) content in EOMABRSL induced neuro-ovarian damage of rat. Values represent mean ± SD, n = 10; Values with different superscript are significantly (P < 0.05) different.
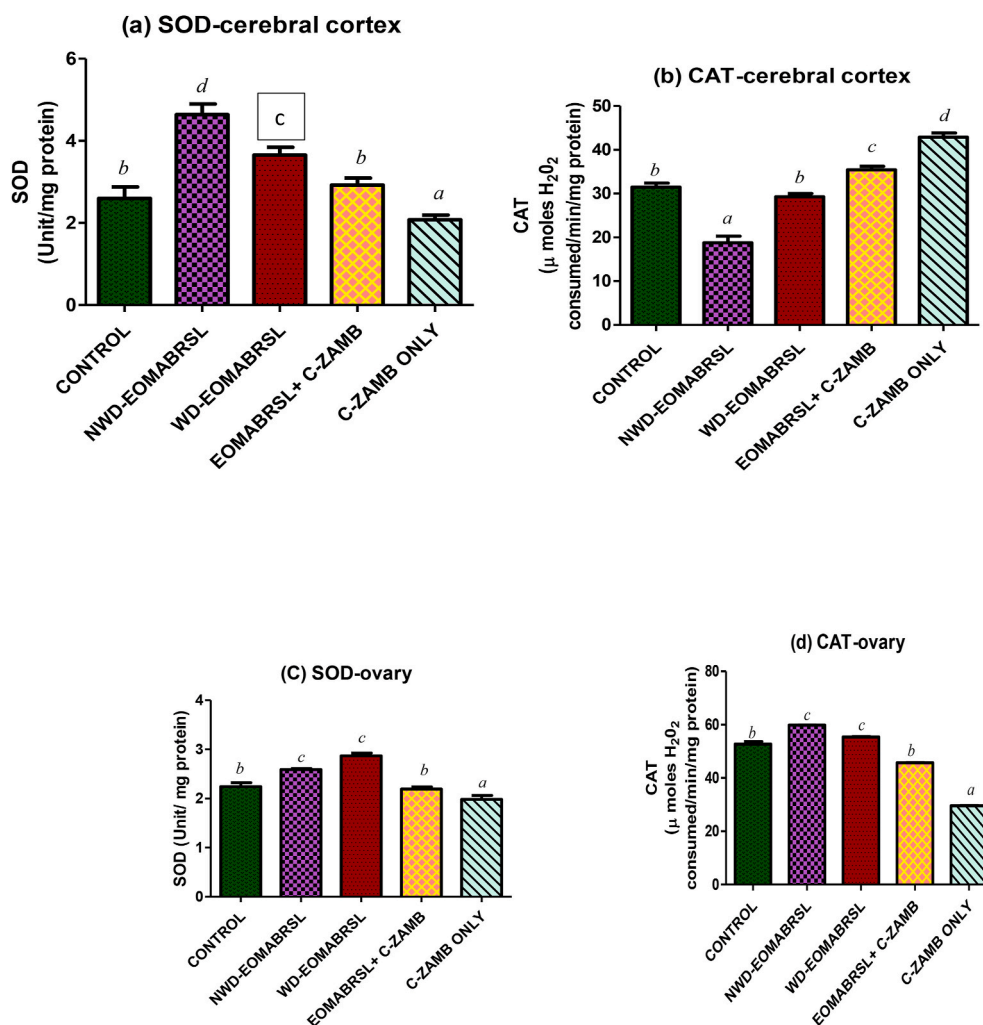
Fig. 3. (a, b, c and d): Effect of phenolic fraction from *Croton zambesicus* (C-ZAMB) on superoxide dismutase (SOD) and catalase (CAT) activities in EOMABRSL induced neuro-ovarian damage of rat. Values represent mean ± SD, n = 10; Values with different superscript are significantly (P < 0.05) different.

decreased by chronic exposure to 0.5 ml EOMABRSL for 98 days (non-withdrawal) as well as withdrawal for 28 days (Fig. 4A) when compared to the corresponding control and treated group. Inversely, chronic EOMABRSL exposure for both 98 days (non-withdrawal) and 70 days (withdrawal for 28 days) remarkably (p < 0.05) increased the ovarian GST activity when compared to the control and treatment groups (Fig. 4C). More so, post hoc analysis showed that both neuronal and ovarian GSH levels were significantly (p < 0.05) diminished by chronic exposure to 0.5 ml EOMABRSL for 98 days (non-withdrawal) (Fig. 4B and D) when compared to their corresponding controls and other groups. A similar trend in GSH levels (Fig. 4B and D) was observed in EOMABRSL exposure for 70 days (withdrawal for 28 days). Commonly, pharmacological treatment with phenolic antioxidants from C-ZAMB leaves (400 mg/kg) amended the variations in GST activities and GSH levels induced by chronic exposure to EOMABRSL (Fig. 4A–D).

3.5. Activity of LDH in the brain structures and ovary

Fig. 5 shows the effect of administration of phenolic C-ZAMB leaves (400 mg/kg body weight) on LDH activity in the brain structures and ovary. Post hoc analysis showed that LDH activity was considerably (p < 0.05) decreased by chronic exposure to 0.5 ml of EOMABRSL for 98 days (non-withdrawal) in the cerebral cortex and ovary (Fig. 5A and B) when compared to their corresponding control and other groups. Similarly, as observed from Fig. 5A and B, 0.5 ml EOMABRSL poisoning for

70 days (withdrawal for 28 days) significantly (p < 0.05) depleted the LDH activity in the cerebral cortex and ovary when compared to the control and treated groups. Conversely, post treatment (EOMABRSL + C-ZAMB) and phenolic C-ZAMB leaves (400 mg/kg) prevented this decrease in the cerebral cortex and ovary (Fig. 5A and B). Interestingly, the treatments with phenolic C-ZAMB leaves (400 mg/kg) were both effectual in brain structures (Fig. 5A) and the ovary (Fig. 5B).

3.6. Activity of eco-5^1-nucleotidase (E5^{1}NT) in the brain structures and ovary

Fig. 6 shows the effect of management of phenolic C-ZAMB leaves (400 mg/kg body weight) on eco-5^1-nucleotidase (E5^{1}NT) activity in the brain structures and egg encapsulated tissue (ovary). Post hoc analysis showed that E5^{1}NT activity was substantially (p < 0.05) amplified by chronic exposure to 0.5 ml EOMABRSL for 98 days (non-withdrawal) in the brain structures and ovary (Fig. 6A and B) when compared to their corresponding control and other groups. Alike, as experimented in Fig. 6A and B, 0.5 ml EOMABRSL exposure for 70 days (withdrawal for 28 days) profoundly (p < 0.05) inflamed the E5^{1}NT activity in the brain structures and ovary when compared to the control and cured groups. Contrariwise, post treatment (EOMABRSL + C-ZAMB) and phenolic C-ZAMB leaves (400 mg/kg) disallowed this hike in the cerebral cortex and ovary (Fig. 6A and B). However, when phenolic C-ZAMB leaves (400 mg/kg) was given to the EOMABRSL-exposed animals, it was
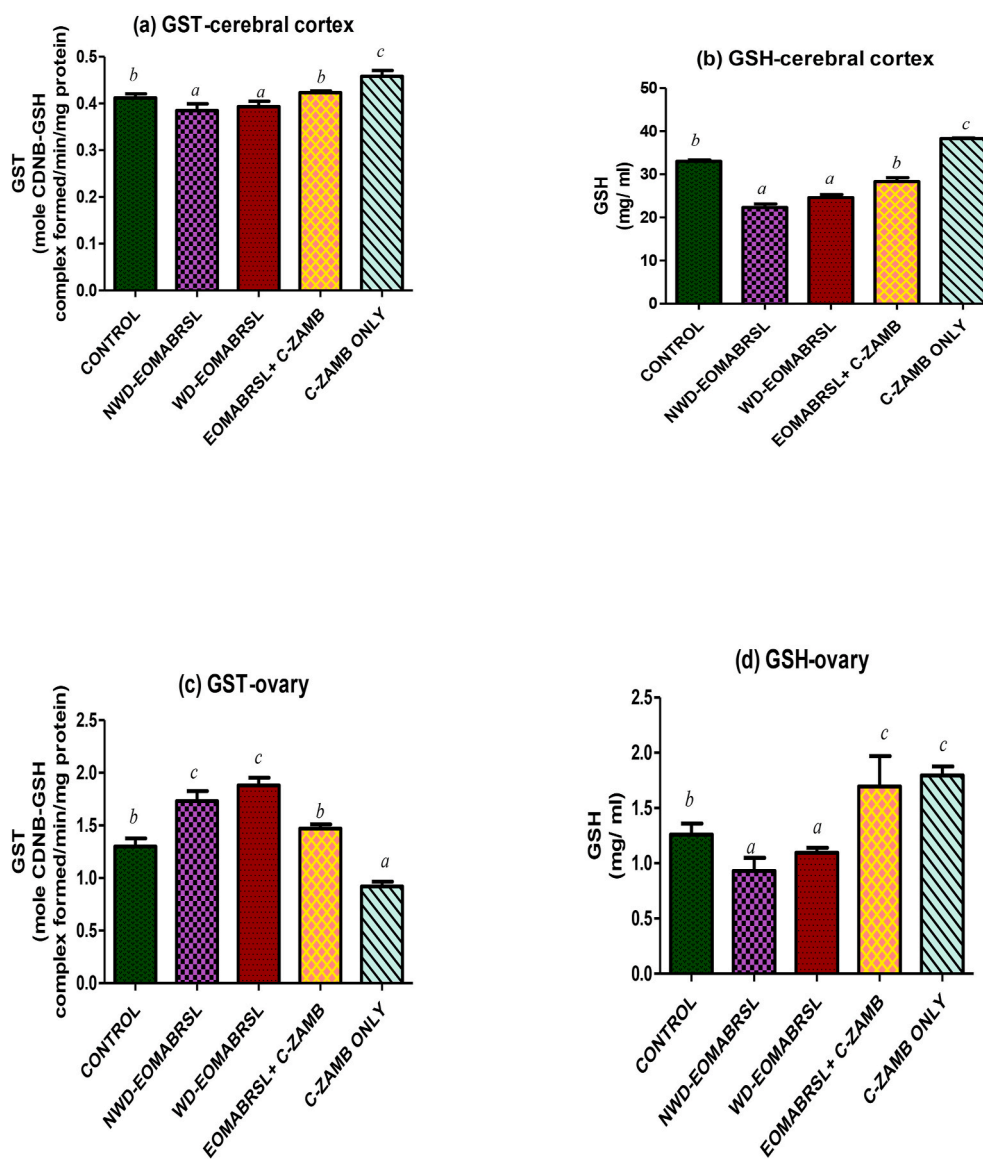
Fig. 4. (a, b, c and d): Effect of phenolic fraction from *Croton zambesicus* (C-ZAMB) on glutathione-S-transferase (GST) activity and reduced glutathione (GSH) content in EOMABRSL induced neuro-ovarian damage of rat. Values represent mean ± SD, n = 10; Values with different superscript are significantly (P < 0.05) different.

effective in subverting the increase in E5^{1}NT activity induced by mixture of organic pollutants and heavy metals (Fig. 6).

3.7. Effect of phenolic C-ZAMB leaves on biomarker linked to neuroendocrinology

Fig. 7 shows the effect of administration of phenolic C-ZAMB leaves (400 mg/kg body weight) and its administration in EOMABRSL exposed rats on withdrawal and non-withdrawal approaches. Post hoc analysis showed that non-withdrawal (98 days) and withdrawal (70 days) groups of animals were significantly (p < 0.05) depleted in activities of Δ^5-17β-HSD in relation to the control and treated group. Post-treatment with phenolic antioxidants from C-ZAMB leaves markedly (p < 0.05) prevented this outcome by up-turning the ovarian Δ^5-17β-HSD activity (Fig. 7). Statistical analysis showed significant increase between post-treatment (EOMABRSL + C-ZAMB) and C-ZAMB only; indicating its efficacy (Fig. 7).

3.8. Activities of AChE and BuChE in brain

Fig. 8 shows the effect of administration of phenolic C-ZAMB leaves (400 mg/kg body weight) on AChE and BuChE activities in the cerebral cortex. Post hoc examination showed that chronic 0.5 ml EOMABRSL intoxication for 90 days (non-withdrawal) and 70 days (withdrawal) significantly (p < 0.05) amplified neuronal AChE (Fig. 8A) and BuChE (Fig. 8B) activities in relation to control and treated groups. However, neuronal AChE (Fig. 8A) and BuChE (Figure B) activities were significantly (p < 0.05) decreased by phenolic antioxidants (400 mg/kg) from C-ZAMB leaves when compared to the intoxicated groups (Fig. 8A and B).

3.9. Effect of phenolic C-ZAMB leaves on mono amine oxidase-A (MAO-A) activity in post-mitochondrial fraction (PMF) and synaptosomes (SP) in EOMABRSL induced neuro-ovarian damage

Fig. 9 displays the effect of phenolic C-ZAMB leaves (400 mg/kg body weight) on monoamine oxidase-A (MAO-A) activity in the brain structure. Post hoc study showed that post mitochondrial fraction pmf-
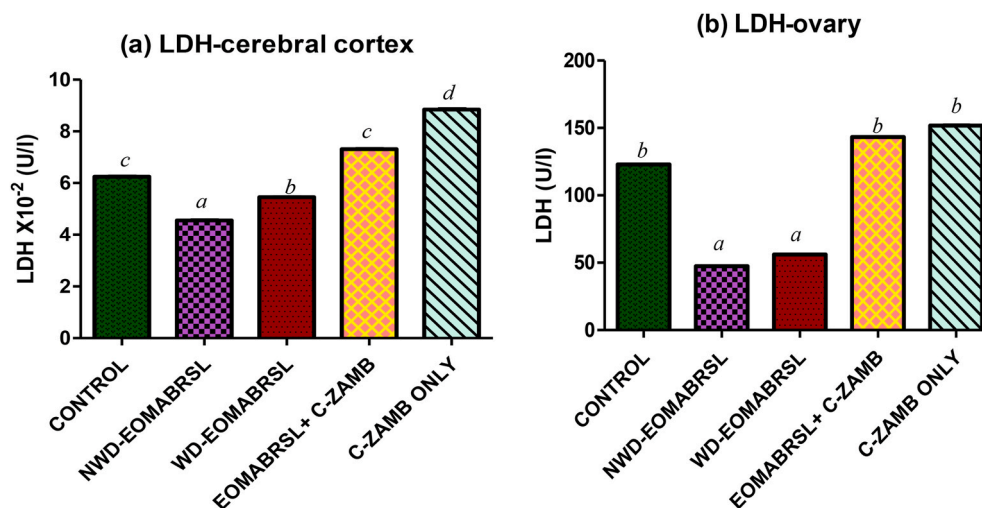
(a) LDH-cerebral cortex

(b) LDH-ovary

Fig. 5. (a and b): Effect of phenolic fraction from *Croton zambesicus* (C-ZAMB) on lactate dehydrogenase (LDH) activity in EOMABRSL induced neuro-ovarian damage of rat. Values represent mean $\pm$ SD, n = 10; Values with different superscript are significantly (P < 0.05) different.

(a) 5^1NT-cerebral cortex

(b) 5^1NT-ovary

Fig. 6. (a and b): Effect of phenolic fraction from *Croton zambesicus* (C-ZAMB) on 5^1 nucleotidase (5^1NT) activity in EOMABRSL induced neuro-ovarian damage of rat. Values represent mean $\pm$ SD, n = 10; Values with different superscript are significantly (P < 0.05) different.
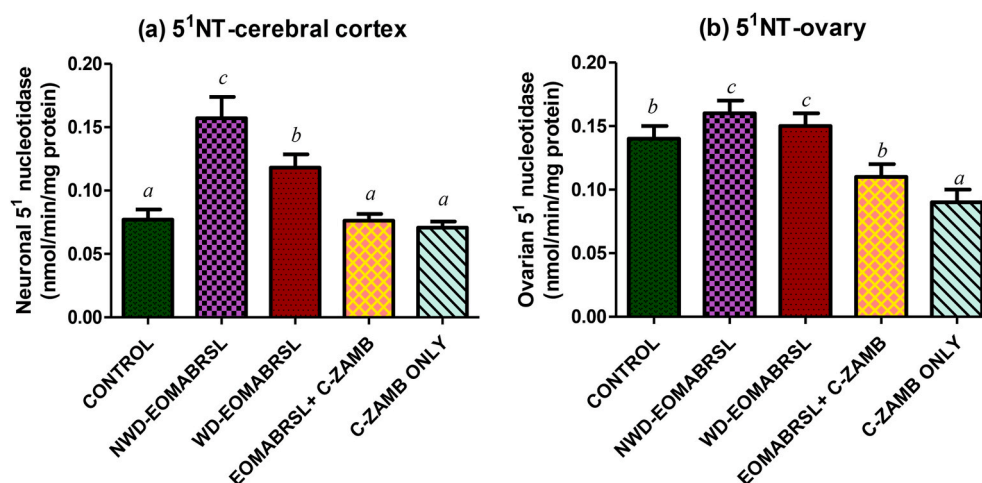
MAO-A activity was substantially (p < 0.05) elevated by chronic exposure to 0.5 ml EOMABRSL for 98 days (non-withdrawal) in the brain (Fig. 9A) when compared to the corresponding control and treated groups. In the same vein, as shown in Fig. 9A 0.5 ml EOMABRSL exposure for 70 days (withdrawal for 28 days) significantly (p < 0.05) exacerbated the pmf-MAO-A activity in the brain when compared to the control and preserved groups. Similarly, post hoc examination showed that chronic 0.5 ml EOMABRSL intoxication for 90 days (non-withdrawal) significantly (p < 0.05) increased snaptosomal sp-MAO-A activity in relation to control and treated groups (Fig. 9B). A similar trend was observed in sp-MAO-A activity in animals intoxicated with EOMABRSL for 70 days (withdrawal) (Fig. 9B). Fundamentally, post treatment (EOMABRSL + C-ZAMB) and phenolic C-ZAMB leaves (400 mg/kg) significantly (p < 0.05) depleted both pmf-MAO-A and sp-MAO-A activities in the whole brain (Fig. 9A and B).

3.10. Effect of phenolic antioxidants from C-ZAMB leaves on cerebral cortex and ovarian follicles in chronic EOMABRSL intoxication

Group of animals chronically intoxicated with 0.5 ml EOMABRSL for 98 days (non-withdrawal) showed congested cerebral blood vessels as discovered in Fig. 10. Also, exposed animals for 70 days (withdrawal)

depicted brain morphological alterations in relation to the control group (Fig. 10). Remarkably, experimental animals that were therapeutically dosed with phenolic C-ZAMB leaves (400 mg/kg) showed no visible lesions to the neuronal cells (NVL). Our observation further depicted multiple numbers of under-developed ovarian follicles following notable congestion of ovarian stroma blood vessels and large corpora tissue in animals intoxicated with 0.5 ml EOMABRSL for 98 days (non-withdrawal) as well as 70 days (withdrawal) (Fig. 11). The pragmatic disorders were prevented by administering 400 mg/kg phenolic C-ZAMB leaves (as shown in Fig. 11).

3.11. Effect of phenolic C-ZAMB leaves on female fertilizing potential and neonates in EOMABRSL induced neuro-ovarian toxicity

The result of fertility index and number of neonates is presented in Table 3. Chronic EOMABRSL intoxication for 98 days (non-withdrawal) declined the fertilizing index (FI) of the female rats in relation to the control and treated groups (EOMABRSL + C-ZAMB and C-ZAMB only) by 50%, 66.7% and 50%, respectively (Table 3). It was observed that EOMABRSL exposure for 70 days (withdrawal) showed no decrease in fertilizing index (Table 3). Additionally, the number of neonates was considerably few in animal chronically intoxicated with EOMABRSL for
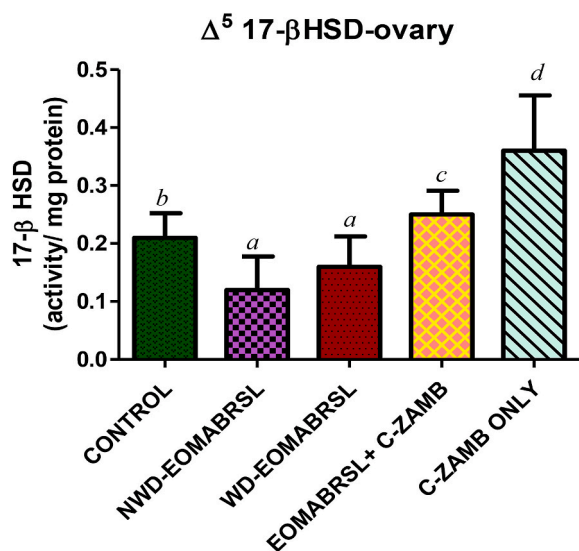
Δ^5 17-βHSD-ovary

Fig. 7. Effect of phenolic fraction from *Croton zambesicus* (C-ZAMB) on hydroxyl steroid dehydrogenase (17-βHSD) activity in EOMABRSL induced neuro-ovarian damage of rat. Values represent mean ± SD, n = 10; Values with different superscript are significantly (P < 0.05) different.

98 days (non-withdrawal) in comparison to the control and treated groups (Table 3). Similarly, animals exposed to EOMABRSL for 70 days showed the same trend regarding the number of neonates (Table 3). Proactively, both decreased fertility index and few neonates were noticeably prevented on post–administration with phenolic C-ZAMB leaves (Table 3).

4. Discussion

Exposure to a mixture of organic pollutants and mixed-metal poisoning was associated with neuronal ovariotoxicity, which could be a consequence of neurofertility disorders in females via the Central Nervous System (CNS) and ovarian organization. Fundamentally, shreds of evidence have demonstrated the connection of oxidative stress to the central nervous system i.e. pathology of neurological disorders [6] and the susceptibility of the somatic nervous system (cellular toxicity) to oxidative damage has been conventionally established [29,30]. Nonetheless, the mechanistic applications underlying the nervous system (neuronal toxicity) and cellular system (ovarian-toxicity) using the mammalian model during mixed exposure to environmental toxicants are largely unknown. However, examining the mediators of this cellular pathway may shed light on the causes of neurodegenerative disorders connected to ovarian damage as well as fertility imbalances among compromised women. In the present study, we investigated whether lipid peroxidation-mediated mixed toxicants could cause neuronal and ovarian pathology by (i) establishing the lipid peroxidation: oxidative damage marker both at the cerebral cortex and ovary, and (ii) examining whether the inhibitor of lipid peroxidation, phenolic C-ZAMB leaves, prevent the neuronal and ovarian MDA increase. In addition to checking the level of lipid peroxidation, we also measured the capacities of four antioxidant proteins, namely superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and reduced glutathione (GSH). This study revealed that oral exposure to 0.5 ml of EOMABRSL significantly increased both cerebral and ovarian MDA contents in female animals. The MDA increase occurred in both non-withdrawal (90 days exposure) and withdrawal (70 days exposure). This signifies a compromised or an impaired cellular membrane to the neurons and ovaries in the exposed animals. Furthermore, organic and/or inorganic toxicants could produce major metabolites that react with superoxide forming powerful free radicals. This mediates lipid peroxidation and protein and
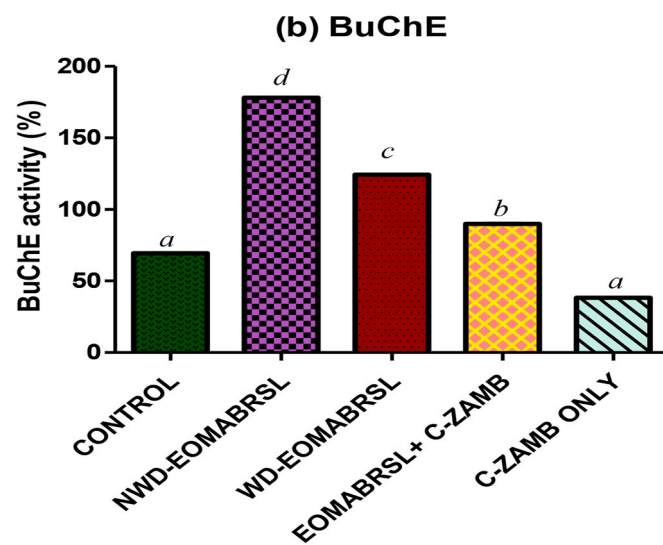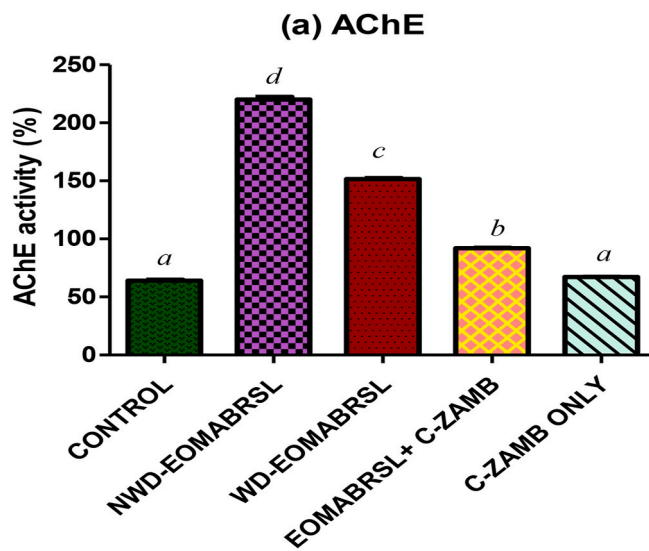
(a) AChE

(b) BuChE

Fig. 8. Effect of phenolic fraction from *Croton zambesicus* (C-ZAMB) on cholinesterase activities (a) AChE and (b) BuChE in EOMABRSL induced neuro-ovarian damage of rat. Values represent mean ± SD, n = 10; Values with different superscript are significantly (P < 0.05) different.

nucleic oxidation. Consequently, contributing to neuronal dysfunction and ovariotoxicity via poly ADP–ribose synthetase (PARS) activation, an enzyme that polymerizes ADP-ribose residues from NAD+, causing neuronal and ovarian ATP depletion, NADPH reduction, mitochondrial dysfunction, inflammation and finally, cell death [31–33]. The results also indicated that withdrawal from the chronic mixture of environmental toxicants after exposure may not be a perfect solution to the assaults. However, avoidance and/or prevention may be a healthier choice. This observation supports the other studies that have examined lipid membrane damage in the brain of toxicant-exposed rats [34]. They moreover reported that the body could not metabolize these anthropogenic toxicants particularly inorganic substances such as Pb, Hg, As, Ag, Ni, Fe, etc. However, the observed inhibition of MDA production by 400
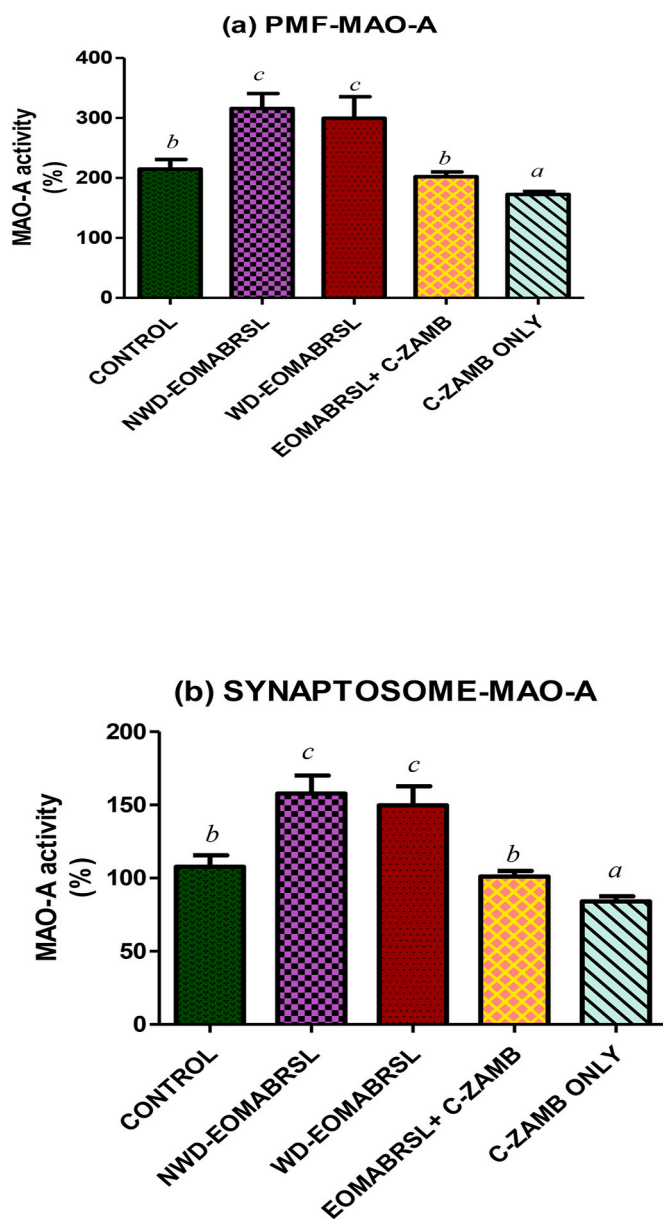
(a) PMF-MAO-A

(b) SYNAPTOSOME-MAO-A

Fig. 9. (a and b): Effect of phenolic fraction from *Croton zambesicus* (C-ZAMB) on mono amine oxidase-A (MAO-A) activities using brain (a) post-mitochondrial fraction (PMF) and (b) synaptosomes in EOMABRSL induced neuro-ovarian damage of rat. Values represent mean ± SD, n = 10; Values with different superscript are significantly (P < 0.05) different.

mg/kg phenolic C-ZAMB leaves in rat brain and ovary tissue homogenates during exposure to the complex mixture of pro-oxidant (EOMABRSL) gives credibility to the fact that the phenols from C-ZAMB leaves are strong antioxidant compounds (Table 3). As shown previously, inorganic metals particularly Fe^{2+} and Pb^{2+}; a constituent of EOMABRSL partake in Fenton's reaction leading to the generation of reactive oxygen species triggering damage to membrane lipids and eventually cell death. Elevated content of MDA in the brain and ovary had been linked to neuro-fertility impairment in women with elevated heavy metal levels localized in degenerate regions of Alzheimer's brain and dysfunctional ovary in both humans and animals [29,30].

More so, chronic exposure to toxicants have been reported to activate cellular expressing NMDA receptors and glutamate-type excitotoxicity [35,36], and oxidative stress results from overstimulation of NMDA receptors. Also, elevated levels of toxicants have been associated with several neuronal disorders linking female reproductive dysfunctions

[37,38]. In this study, we showed that SOD activity considerably increased in the cerebral cortex and ovarian homogenates of both NWD-EOMABRSL and WD-EOMABRSL. Following the exposure, CAT and GST activities were altered, while reduced GSH level was noticeably depleted in both NWD-EOMABRSL and WD-EOMABRSL. This result suggests that lipid peroxidation-mediated oxidative stress via chronic exposure to NWD-EOMABRSL and WD-EOMABRSL is one of the fundamental mediators of neuro-ovariotoxicity in female subjects. This observation supports studies that oxidative stress induced by the high concentration of ROS could initiate and propagates inflammation of the neurons and ovary as well as causing transcription and translational errors [39–41]. Also, a further study indicated that chronic cellular diseases have resulted from chronic exposure to pro-oxidants and oxidant-antioxidant imbalance [42]. Additionally, neurodegeneration due to oxidative stress could be implicated in the pathogenesis and progression of female fertility, with the diminution of cholinergic neurons in the brain [43]. Studies have reported that females with Alzheimer's disease were liable to severe oxidative stress [44], decreased cholinergic neurons [45] and depicted fertility disorders [46] with impaired ovariogenesis [41] followed by the reduced number of neonates [47]. Thus, augmentation of the body's antioxidant status through dietary means or ethno-pharmacotherapy could be a practical approach through which oxidative stress-induced neuroreproductive disorder is controlled. However, the inhibition of oxidative stress by 400 mg/kg phenolic C-ZAMB leaves in the rat brain and ovary homogenates are in line with former studies on herbal phytochemicals, which was alluded to their antioxidant properties [48].

Presently, a significant indicator to assess symptomatic pathology during the conversion of metabolic sugar into energy in mammalian cells is lactate dehydrogenase (LDH) [49]. Also, metabolic aberrations particularly aerobic glycolysis has been implicated in neuro-ovarian driven disorder [50]. Thus, we used chronic exposure to EOMABRSL (withdrawal and non-withdrawal) and phenolic C-ZAMB leaves via rat model to establish a biochemical link between neuronal LDH and ovarian LDH metabolism during neuro-reproductive process among females. Using the UV-spectrophotometry method, we found that brain and ovary lactate dehydrogenase activities were depleted in both chronic exposures to NWD-EOMABRSL and WD-EOMABRSL when compared to phenolic C-ZAMB leaves. The decrease in the LDH activities is indicative of low brain ATP levels, which may influence ovary dysfunctions [51], and that loss of LDH activity in the brain is linked to Alzheimer's disease [3]. Recent investigators that brain is a complex organ requiring high ATP for its functioning to regulate the entire body metabolism [52] supported this. Further studies have also suggested the performance of ovarian cells depends on variable levels of glycolysis or oxidative phosphorylation resulting in ATP generation within the human brain [53]. Interestingly, targeting brain versus ovary dysfunctions via oxidative phosphorylation is still current. Thus, to up-regulate the glycolytic capacity in this study, 400 mg/kg phenolic C-ZAMB leaves increased the LDH activity. This study, therefore, suggests that the active constituents including quercetin and luteolin may easily cross both brain-blood barrier (BBB) and ovarian blood-brain barrier (OBB) to elicit its pharmacology action. We can say that this study shows a direct correlation between neurons versus ovarian epithelial cells and lactate dehydrogenase activity. Recent work revealed that lactate dehydrogenase (LDH) may be a confirmatory prediction of brain tumor linking female reproductive dysfunctions [49]. Furthermore, previous studies have shown that abnormal LDH down-regulation is a common characteristic of brain tumors, which promotes an ovarian metabolic shift to ovarian carcinoma [52].

One of the imperative indices with a fundamental role in ATP stability and its hydrolysis is the eco-51 nucleotidase enzyme [54]. Studies have proven that some compounds present in diets and polluted water, such as organic pollutants and heavy metals, are associated with heightened E51NT activity and ATP diminution [51,54]. Therefore, we hypothesized that the up-regulation of this enzyme could be one of the
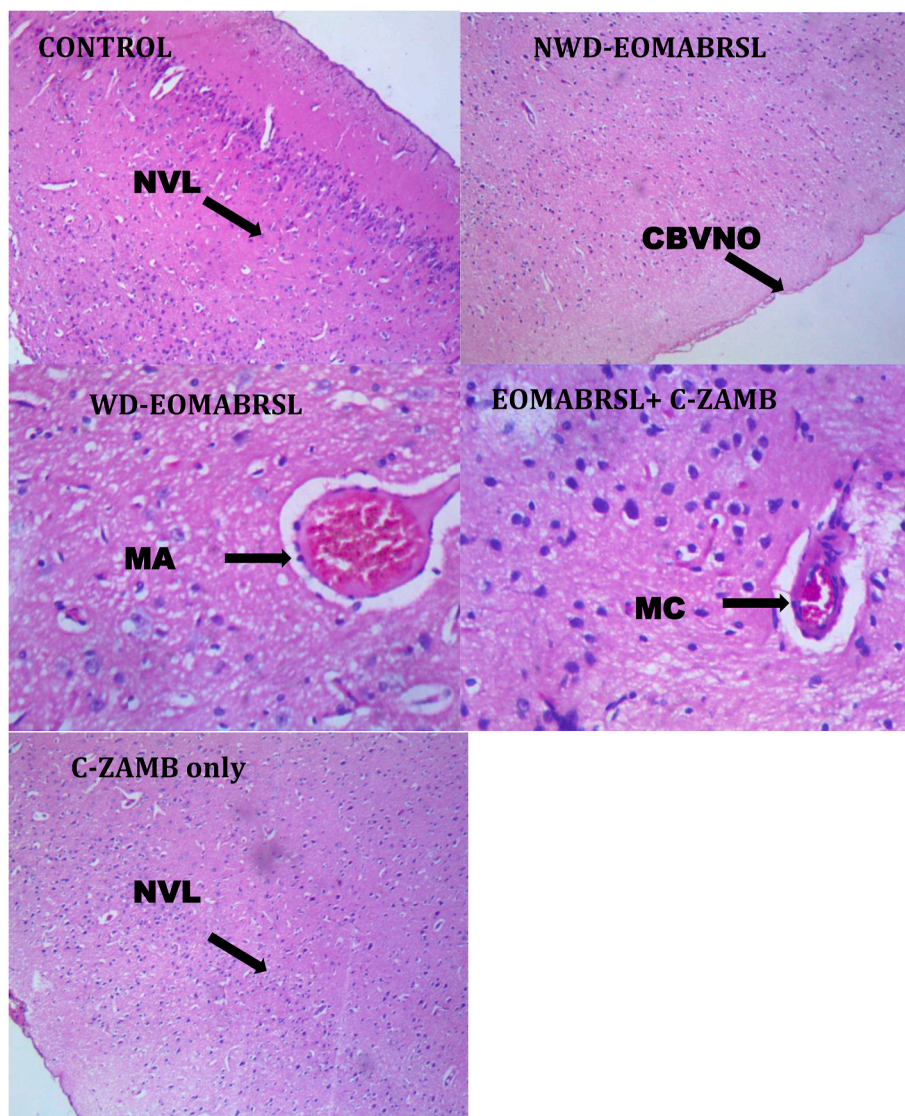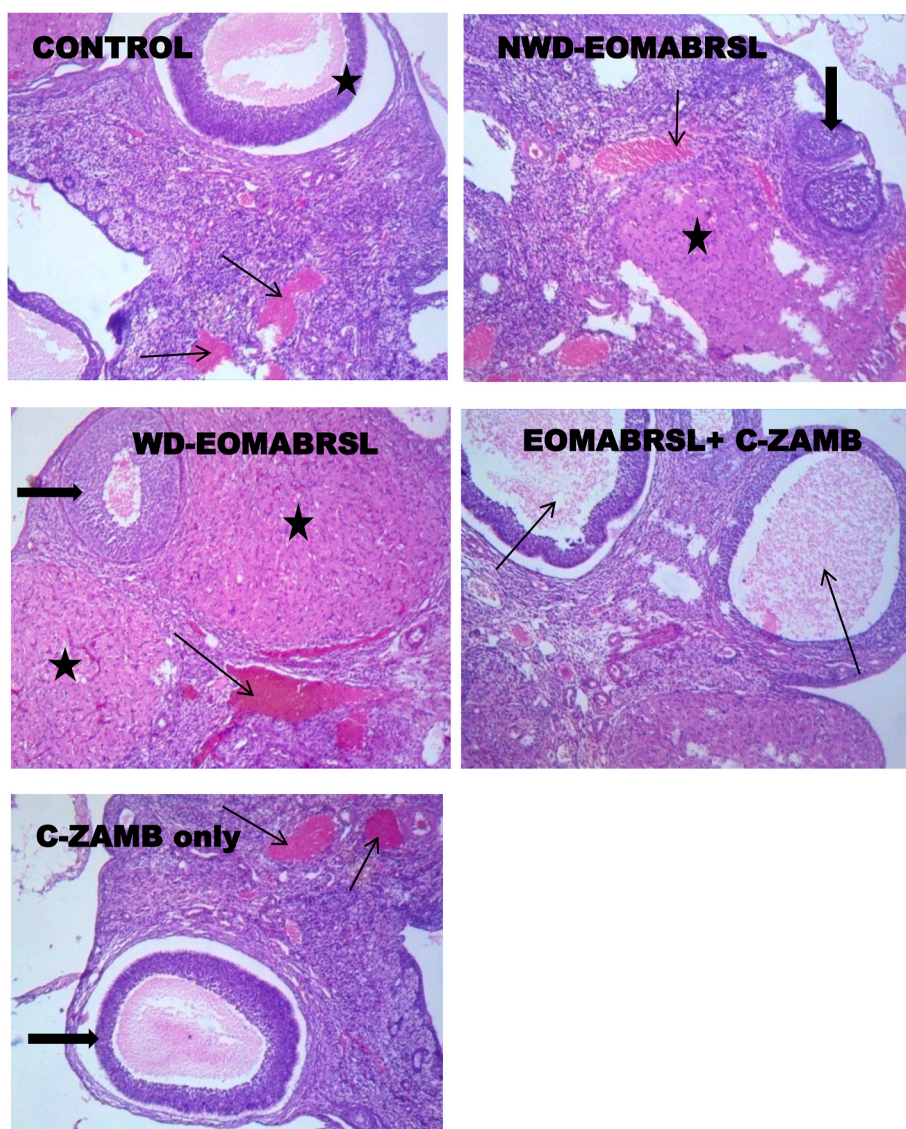
Fig. 10. Cerebral cortex histopathology changes on rat treated with C-ZAMB in EOMABRSL induced neuro-ovarian damage (original magnification ×400). **Control**: no visible lesions to the cerebrum or neurons (NVL). **NWD-EOMABRSL**: The cerebral blood vessels were noticeably overcrowded (CBVNO) **WD-EOMABRSL**: depicted morphological alterations (MA) when compared with the control. **EOMABRSL + C-ZAMB**: This group also showed morphological changes (MC) in relation to the control. **C-ZAMB only**: No visible lesions to the neuronal cells (NVL).

pathological bases for cerebro-ovarian toxicity when exposed to mixtures of toxicants. The metabolic inhibitory test had been adopted to evaluate neuro-reproductive wellness in rats and mice [55,56]. In our study, we found a significant increase in the activity of both neuronal and ovarian E51NT following chronic exposure to NWD-EOMABRSL and WD-EOMABRSL, suggesting fast hydrolysis of ATP and neuro-ovarian dysfunction in these animals. These results are in agreement with other studies that have examined the levels of ATP in toxicant-exposed rats [57]. Also, further studies had observed that low neuronal levels of ATP in females could trigger dementia, induced abortion and generally promoted sub-fertility problems [37]. However, when mix-toxicants exposed rats were orally treated with phenolic C-ZAMB leaf fraction (400 mg/kg body weight) for 28 days, the high activity of E51NT was depleted similar to that of rats from the control and C-ZAMB groups. These outcomes indicate that treatment with phenolic C-ZAMB leaf fraction was able to avert low levels of ATP in both the brain and ovary as well as sub-fertility challenges instigated by EOMABRSL exposure. Our results, therefore, demonstrated that 400 mg/kg of C-ZAMB have the potential to inhibit the activity of E51NT (a marker of ATP hydrolysis) initiated by dissimilar agents, such diabetic induced agents [58], acetonitrile, AlCl3 associated with D-galactose and anti-psychotic drugs [51,54]. It is imperative to mention that super addition or synergistic interaction of gallic acid, caffeic acid, quercetin, luteolin, and apigenin

could be responsible for the metabolic inhibition of eco-5^1-nucleotidase activity.

The regulatory key marker linked with oogenesis via progesterone production in females is ovarian Δ5-17β-HSD enzyme [59]. To confirm this possibility, we assessed the activity of Δ5-17β-HSD to ascertain whether cerebral dysfunction caused by EOMABRSL has an impact on progesterone performance. Our results demonstrated that both withdrawal and non-withdrawal exposure to EOMABRSL significantly inhibited the activity of ovarian Δ5-17β-HSD in relation to control, EOMABRSL-exposed group treated with C-ZAMB and C-ZAMB treated only. Thus, the oogenesis and progesterone might have been reduced, suggesting female infertility and reduced number of neonates. These data established the likelihood that neuronal disorder may contribute to the alteration of ovarian Δ5-17β-HSD enzyme in EOMABRSL-exposed rats, consequently acting as an interrupter of oogenesis and progesterone level. Several studies corroborated our data, reporting that chronic exposure to anthropogenic toxicants possesses Δ5-17β-HSD inhibitory activity [60] in females, which leads to infertile ovum causing delay or no offspring [61]. However, the observed increase in the enzyme activities when the phenolic C-ZAMB leaf fractions were introduced could be as a result of the antagonistic or competitive effect of gallic acid, caffeic acid, quercetin, luteolin, and apigenin. The existence of quinic acid moiety on the phenolic acid structure could have promoted their

Fig. 11. Ovarian histopathology changes on rat treated with C-ZAMB in EOMABRSL induced neuro-ovarian damage (original magnification ×400). **Control:** showed adequate numbers of developing ovarian follicles (star) with few congestion of stromal blood vessels (thin arrows). **NWD-EOMABRSL:** depicted multiple numbers of under-developed ovarian follicles (thick arrow) with remarkable congestion of stroma blood vessels (thin arrow) and large corpora tissue (star). **WD-EOMABRSL:** There are few under-developing ovarian follicles (thick arrow) with foci of haemorrhages in the ovarian stroma (thin arrows) and large corpora tissue (star). **EOMABRSL + C-ZAMB:** This showed numerous ovarian follicles (thin arrows) **C-ZAMB only:** There were mature and developing ovarian follicles (thick arrow) with little congestion of stromal blood vessels (thin arrows).

Table 3

Fertilizing index (pregnant rats) and numbers of neonates in control and EOMABRSL exposed rats.

Parameter	Control	NWD-EOMABRSL	WD-EOMABRSL	EOMABRSL + C-ZAMB	C-ZAMB only
Number of Animals in Each group	10	10	10	10	10
Mated Animal (n)	10	10	10	10	10
Fertility Index (%)	20	10	30	30	20
Neonates (n)	12	5	10	17	17

$$\text{Fertility index (\%)} = \frac{\text{Number of pregnant animals}}{\text{Number of animals that copulated}} \times 100$$

interactions at the active site of Δ5-17β-HSD, provoking substantial elevation of the enzyme activity. This may further clarify the high enzyme up-regulatory outcome when only phenolic C-ZAMB leaf fraction was included. Accordingly, our previous investigation reported that

functional oil that contains these phenolic acids could prevent neuronal disorder in the rat model, which eventually influence reproductive wellness [51]. Also, it has been validated that ovarian Δ5-17β-HSD activity is a committed step during the production of neuroendocrine hormones particularly progesterone [62]. However, it is unnecessary in this study to measure the levels of LH, FSH, and progesterone because of the increase in the activity of ovarian Δ5-17β-HSD by pharmacological treatment with phenolic C-ZAMB leaves suggested elevated levels of progesterone, LH and FSH for the physiological female reproductive system [63,64].

The significance of the signaling molecules for the cholinergic system in both brain and ovary performance cannot be overemphasized. The alteration of AChE and BuChE activities including acetylcholine neurotransmitter levels had been neurochemically connected with female reproductive deficits [65]. In this study, we discovered increased AChE and BuChE activities in both the ovary and cerebrum of female rats following sub-chronic exposure to 0.5 ml of EOMABRSL (withdrawal and non-withdrawal). The increase in AChE and BuChE activities additionally revealed that female rats that were chronically exposed to environmental toxicants depict symptomatic signs of Alzheimer's disease and followed by complicated ovarian dysfunctions. Further reports suggested that high activities of signaling enzymes particularly neurotransmitter enzymes were indicated among infertile women [66].

Furthermore, the use of AChE and BChE inhibitors to retard the hydrolysis of acetylcholine in mammal was predicted as a pharmacologic method in managing infertility linked-neurodegeneration in females. Also, studies have advocated that the combined inhibition of AChE and BuChE was preferred to the selective inhibition of AChE in treating AD [67] and reproductive abnormalities [68]. Our present finding demonstrates that phenolic C-ZAMB leaf fraction was effective in inhibiting the augmentation of AChE and BuChE activities induced by EOMABRSL in the ovary and the cerebra examined. Hence, it is noteworthy to say that phenolic C-ZAMB leaf fraction produced an amelioration of the EOMABRSL-induced cerebro-ovarian imbalance.

Furthermore, the use of MAO-A inhibitors and their underlying mechanism of action in the management of diseases associated with ovarian dysfunctions and dementia have been conventionally established [69]. Uncontrolled MAO activity, essentially MAO-A, contributes to neurodegeneration causing dementia progression in both males and females [70]. Additionally, excessive MAO-A activity has been linked to increased generation of free radicals in the brain and consequently ovarian damage [71]. Therefore, inhibiting MAO-A activity via the aminergic metabolic system in the present study is a therapeutic target in the management of abnormalities among female and male individuals. Hence, either pmf MAO-A activity in the cerebral structure was significantly increased following sub-chronic exposure to EOMABRSL during withdraw or non-withdrawal at the dose of 0.5 ml. Validating these findings, EOMABRSL showed a similar effect on brain spMAO-A activity in vivo. We speculate in this study that chronic exposure to 0.5 ml of EOMABRSL may cause brain excitotoxicity, which might explain its damage to the ovary functions. This may be because neurotransmitters such as gamma amino butyric (GABA), serotonin, noradrenalin, and dopamine produced in the neurons at the synaptic gap which is responsible for relaying signals had been deactivated by the mixture of toxicants (EOMABRSL). It had been reported that metal mixture particularly divalent heavy metals (M2+) have the permeability potential across the brain-blood barrier to initiating neuronal damage and glial cell necrosis [3]. This pattern of MAO-A activity was found previously by other research groups [69,70]. This further suggests that the superadditive effect of organic pollutants following EOMABRSL exposure triggers the increase in the number of receptors on the surface of neurotransmitter cells, making the cells hypersensitive to its hormone or other agents. Also, studies have shown that high activities of MAO-A were implicated in abnormal oogenesis and depression in mice and rats [72,73] when repeatedly intoxicated with nicotine, agricultural chemicals, and insecticides. Interestingly, phenolic treatment from C-ZAMB leaf fraction significantly depleted both pmf-MAO-A and sp-MAO-A activities in the whole brain. This suggests that excitatory neurotransmitters regulating ovarian functions, oogenesis as well as the entire female reproductive system have been invigorated by 400 mg/kg phenolic C-ZAMB leaf fraction. Prior study reported that croton zambsicus leaf extract possessed anticonvulsant and other CNS depressant activities which were linked to its interactions between serotonergic and GABAeric transmissions [13,14]. Although, this study may merit further investigation on both brain and ovarian culture cells to ascertain these results, nevertheless, we can still juxtapose that assessing pmf or sp MAO-A activity will extrapolate data for female reproductive system particularly ovarian functions.

Histologically, animals that were chronically exposed to 0.5 ml of EOMABRSL for 98 days (non-withdrawal) and 70 days (withdrawal) showed congested cerebral blood vessels, abnormal development of ovarian follicles with notable congested ovarian stroma blood vessels and large corpora tissue. This suggested that the Purkinje, ganglia cells and blood-brain barrier (BBB), as well as ovarian follicles, had been compromised. This indicates that the dysfunction of cerebral blood vessels could initiate the anomalous growth of ovarian follicles in the rat. Also, these findings suggest that chronic exposure to EOMABRSL may induce long-term dopaminergic neuronal damage in the substantia nigra facilitated by the activation of inflammatory responses in the nigrostriatal system [74]. The observed cerebral cortex and ovaries of the exposed animal additionally suggested demyelination of the CNS, mutilation of brain nerve cells and obstruction of ovarian functions [75]. This is because the pituitary gland functions as the principal regulatory organ for all other peripheral tissues [76]. However, the psychopharmacological treatment of phenolic C-ZAMB leaf fraction showed a similar protective effect to that found in its essential oil [77]. Recent studies revealed that essential oil from C-ZAMB contains p-cymene, linalool, and beta-caryophyllene which could prevent electroshock induced convulsions and potentiate marked sedative effects at the CNS [13,14].

The birth index reflects the relationship between the fertility index and the total numbers of neonates born alive. Sub-chronic exposure of EOMABRSL (withdrawal and non-withdrawal) to female rats declined their fertility potential and the number of neonates. This may be because the mixture toxicants interfere directly with the ova or probably prevented the fertilization of the ovum. Thus, a decrease of the fertilizing index in the group of mated females treated with EOMABRSL can be attributed to ovarian and neuronal dysfunctions which resulted in multiple deaths of neonates. Also, other perceived factors that may alter the neuro-reproductive process in females include disruption of maternal neurons and hormonal response which may hamper the viability of embryos and fetuses; thereby impeding the development and maintenance of pregnancy. Recent studies had shown that the decreased pituitary response (hypogonadism) to gonadotropin (GnRH) in patients with hypothalamic disorders was linked to the chronically understimulation of pituitary gland [78]. This study, therefore, suggested that the phenolic C-ZAMB leaf fraction potentiated the GnRH in the hypothalamus of the brain, resulting in normal regulation of the ovogenesis by androgen receptors to cause high fertilizing index and multiple neonates.

Ultimately, this investigation reveals that there is no major difference in the consequence of neuro-ovarian toxicity among the animals exposed to 98 days (chronic exposure) and 70 days (withdrawal for 28 days). This validates that ingestion with mixture of metals from anthropogenic activities may bioaccumulate and bio-magnify [79] especially in the brain and ovary tissues to elicit their etiology. However, it is interesting to ascertain here that metals and organic toxicant exposure for short (70 days) and long (90 days) provoked similar neuro-ovarian toxicity in male rats.

5 Conclusion

The finding showed that chronic (non-withdrawal) and sub-chronic (withdrawal) EOMABRSL-exposed animals exhibited damage to neuro-ovarian follicles and significant increases in cellular biochemical parameters related to neurological disorders, including 5^1 eco-nucleotidase (5^1ENT), acetylcholinesterase (AChE), butrylcholinesterase (BuChE), synaptosomal monoamine oxidase-A (MAO-A), and cerebral antioxidants. They also had elevated levels of markers associated to ovarian injury, such as 5^1 eco-nucleotidase (5^1ENT) and decreased biomarkers of fertility index, lactate dehydrogenase (LDH) and $\Delta 5$ 17β-hydroxyl steroid dehydrogenase ($\Delta 5 17\beta$-HSD) activities. Furthermore, EOMABRSL intoxication increased the neuronal and ovarian lipid peroxidation and altered antioxidant proteins with reduced numbers of neonates. Phenolic antioxidants (quercetin, apigenin, caffeic acid and gallic acid) from C-ZAMB leaves ameliorated the chronic EOMABRSL intoxication. The treatment also amended cerebral blood vessels and ovarian follicles, improved endogenous antioxidant status, and reduced lipid peroxidation. Fundamentally, they exerted synergistic/additive neuroprotective and ovarioprotective effects. Finally, the present study demonstrates that phenolic C-ZAMB was effective in preventing chronic EOMABRSL-induced neuro-ovariotoxicity in rats. Nevertheless, further studies particularly molecular mechanistic applications regarding each phenolic antioxidant from C-ZAMB leaves are desirable to juxtapose the efficacy of this phenolic medication.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

CRediT authorship contribution statement

J.K. Akintunde: Conceptualization, Methodology, Supervision, Investigation, Software, Writing – review & editing. **L.B. Ibrahim:** Formal analysis. **O.D. Omotosho:** Formal analysis. **A.A. Boligon:** Formal analysis.

Declaration of competing interest

Akintunde J.K declares that he has no conflict of interest. Ibrahim L.B declares that she has no conflict of interest. Omotosho O.D declares that he has no conflict of interest. Boligon A.A declares that she has no conflict of interest.

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