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Protective Effects of Graded Doses of Aqueous Seed Extract of Dennettia tripetala on Ovarian Biochemical, Hormonal, and Microbiological Alterations in Bisphenol A-Induced Oxidative Stress in Female Wistar Rats

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Abstract

Background

Bisphenol A (BPA) is an endocrine-disrupting chemical associated with oxidative stress and ovarian dysfunction. *Dennettia tripetala* is a medicinal plant with reported antioxidant and antimicrobial properties.

Aim

This study evaluated the phytochemical composition and protective effects of aqueous seed extract of Dennettia tripetala against BPA-induced ovarian toxicity in female Wistar rats.

Materials and Methods

Thirty-five (35) adult female Wistar rats were randomly assigned into seven groups (n = 5). Group I served as the normal control, Group II received BPA only, Group III received BPA and Vitamin C, while Groups IV–VII received BPA and aqueous seed extract of D. tripetala at 100, 200, 400, and 800 mg/kg body weight, respectively, for 28 days. Phytochemical, biochemical, hormonal, microbiological, and histopathological analyses were performed using standard methods.

Results

Phytochemical screening revealed the presence of flavonoids, phenolic compounds, alkaloids, tannins, saponins, terpenoids, glycosides, and steroids, with flavonoids and phenolic compounds being the most abundant. BPA exposure significantly increased ovarian malondialdehyde levels and microbial burden while reducing antioxidant parameters and reproductive hormone concentrations. Histopathological examination also revealed marked ovarian damage. Treatment with aqueous seed extract of D. tripetala significantly and dose-dependently reduced oxidative stress, restored estradiol, progesterone, follicle-stimulating hormone, and luteinizing hormone concentrations, decreased microbial counts and isolates, and improved ovarian histoarchitecture. The 800 mg/kg dose produced effects comparable to Vitamin C.

Conclusion

Aqueous seed extract of *D. tripetala* demonstrated significant ovarian protective activity against BPA-induced toxicity, highlighting its potential as a natural therapeutic agent for oxidative stress-related reproductive disorders.

1. Introduction

For centuries, medicinal plants have been recognized as important reservoirs of biologically active compounds and remain integral to healthcare, pharmaceutical development, and the prevention and treatment of numerous diseases [1–3]. Their medicinal efficacy is primarily linked to the presence of diverse phytoconstituents, including flavonoids, alkaloids, tannins, saponins, terpenoids, and phenolic substances [4–6]. These compounds possess a wide range of pharmacological properties, notably antioxidant [7], anti-inflammatory [8], antimicrobial [7], and hormone-regulating effects [9]. Through their interactions with various tissues and physiological pathways, these phytochemicals contribute to the maintenance of cellular integrity and normal organ function [10–13]. Within the reproductive system, they have been reported to support hormonal balance, mitigate oxidative damage, and enhance reproductive tissue health [14–16]. As a result, growing scientific interest has focused on the use of medicinal plants as natural therapeutic agents for managing reproductive dysfunctions linked to oxidative stress and related pathological conditions [17–19].

The maintenance of female reproductive competence is critically dependent on the normal architecture and physiological activity of the ovaries [20]. As the primary reproductive organs, the ovaries coordinate essential processes such as follicular growth, oocyte development, and the biosynthesis of steroid hormones, particularly estrogen and progesterone, which regulate reproductive cyclicity and fertility [21, 22]. Any disturbance in ovarian cellular equilibrium may adversely affect these functions and ultimately compromise reproductive performance [23].

Among the factors capable of disrupting ovarian physiology, oxidative stress has received considerable attention due to its detrimental effects on cellular metabolism and tissue integrity. Excessive generation of reactive oxygen species can overwhelm endogenous antioxidant defenses, resulting in cellular damage that interferes with follicular maturation and hormone production [24, 25]. Such alterations may impair steroidogenic pathways, reduce oocyte quality, and contribute to subfertility or infertility [26]. Consequently, the preservation of redox balance within ovarian tissues is recognized as a fundamental requirement for optimal reproductive health [27].

Beyond oxidative injury, growing evidence suggests that disturbances in the microbial ecosystem of the female reproductive tract, often accompanied by inflammatory responses, play significant roles in the pathogenesis of various gynecological disorders [28–30]. Dysbiosis may promote tissue damage, alter local immune regulation, and create conditions that adversely affect reproductive function [31].

Among the environmental contaminants that threaten female reproductive health, Bisphenol A (BPA) has emerged as a major endocrine-disrupting chemical due to its widespread use in the manufacture of polycarbonate plastics and epoxy resins [32]. Human exposure occurs primarily through contaminated food and beverages, leading to the accumulation of BPA in biological tissues [32, 33]. Numerous studies have shown that BPA induces excessive generation of reactive oxygen species, promotes lipid peroxidation, disrupts antioxidant defense mechanisms, alters reproductive hormone secretion, and causes structural damage to ovarian tissues. These toxic effects have been associated with impaired follicular development, reduced fertility, and other adverse reproductive outcomes [32–34].

While environmental toxicants such as Bisphenol A pose significant risks to reproductive health, increasing attention has been directed toward plant-derived compounds as potential protective agents against oxidative and endocrine-mediated tissue damage. Among such plants is *Dennettia tripetala* (pepperfruit), a member of the Annonaceae family that is extensively consumed across West Africa as both a dietary component and a traditional medicinal resource. The seeds of the plant contain a diverse array of bioactive phytochemicals, including flavonoids, alkaloids, tannins, saponins, and volatile oils, which have been associated with numerous biological and therapeutic effects [35, 36].

Despite the growing body of evidence supporting the medicinal value of *D. tripetala*, information concerning its effectiveness in preventing or ameliorating BPA-induced ovarian injury remains limited. Existing studies have largely focused on its general pharmacological properties, with relatively few investigations providing an integrated assessment of ovarian biochemical markers, reproductive endocrine function, microbial status, and histopathological changes following treatment with graded doses of its aqueous seed extract. The paucity of such comprehensive data restricts a thorough understanding of the mechanisms through which the plant may exert protective effects on female reproductive organs under conditions of chemically induced oxidative stress.

To address this gap, the present study evaluated the capacity of graded doses of aqueous seed extract of *D. tripetala* to mitigate ovarian dysfunction induced by Bisphenol A in female Wistar rats. Particular emphasis was placed on assessing changes in biochemical indicators of oxidative stress, reproductive hormone concentrations, microbiological parameters, and histological characteristics of ovarian tissues in order to determine the potential protective role of the extract against BPA-mediated reproductive toxicity.

2. Materials and Methods

2.1 Materials

Fresh fruits of *D. tripetala* were used for the preparation of the aqueous seed extract. Bisphenol A (BPA), distilled water, phosphate-buffered saline (PBS), Whatman No. 1 filter paper, nutrient agar, MacConkey agar, blood agar, Sabouraud dextrose agar, normal saline, and analytical-grade chemicals were utilized for the study. Commercial ELISA kits for estradiol, progesterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), as well as assay kits for malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and total protein were employed. Standard laboratory equipment, including an analytical balance, homogenizer, centrifuge, spectrophotometer, ELISA microplate reader, incubator, oral gavage tubes, water bath, micropipettes, and glassware, were also used.

2.2 Methods

- **Collection and Authentication of Plant Material**

Fresh fruits of Dennettia tripetala were procured from Mile One Market, Port Harcourt, Rivers State, Nigeria. The plant material was taxonomically identified and authenticated by Prof. J.A. Onwugbuta-Enyi of the Plant Science Unit, Department of Biology, Faculty of Natural and Applied Sciences, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, Rivers State, Nigeria. A voucher specimen was subsequently prepared and deposited in the departmental herbarium for future reference and verification under Voucher Number IAUE/FNAS/BIO/PLS/2026/0066. The fruits of *D. tripetala* used in this study are shown in Fig. 1.

Source: Adapted from The Guardian Nigeria (accessed June 2026).https://guardian.ng/features/how-pepper-fruit-others-stop-prostate-cancer/

- **Preparation of Aqueous Seed Extract**

The fruits were washed thoroughly with distilled water, and the seeds were manually separated from the pulp. The seeds were air-dried at room temperature for two weeks and ground into a fine powder using an electric grinder. Five hundred grams (500 g) of the powdered sample was soaked in 2.5 L of distilled water for 48 h with intermittent stirring. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated in a water bath maintained at 45°C. The concentrated extract was stored in airtight containers at 4°C until use.

- **Phytochemical Screening**

Preliminary qualitative phytochemical screening of the aqueous seed extract of *Dennettia tripetala* was carried out to determine the presence of major bioactive constituents using standard methods described by Okari et al. [4]. The extract was screened for alkaloids, flavonoids, tannins, saponins, phenolic compounds, terpenoids, glycosides, and steroids.

- **Experimental Animals**

Thirty-five (35) adult female Wistar rats weighing between 150 and 200 g were used for the study and randomly assigned into seven experimental groups (n = 5).

- **Induction of Oxidative Stress**

Oxidative stress was induced in the experimental animals using Bisphenol A (BPA). Bisphenol A was dissolved in distilled water and administered orally at a dose of 50 mg/kg body weight once daily for 28 consecutive days. The selected dose was based on previous studies that demonstrated its ability to induce oxidative stress, endocrine disruption, and reproductive toxicity in experimental animals.

- **Experimental Grouping and Treatment**

Thirty-five adult female Wistar rats were randomly assigned into seven groups of five animals each:

Group I (Normal Control): Received distilled water only.

Group II (Negative Control): Received Bisphenol A (50 mg/kg body weight) only.

Group III (Positive Control): Received Bisphenol A (50 mg/kg body weight) and Vitamin C (100 mg/kg body weight).

Group IV (BPA + DT100): Received Bisphenol A (50 mg/kg body weight) and 100 mg/kg body weight of aqueous seed extract of *Dennettia tripetala*.

Group V (BPA + DT200): Received Bisphenol A (50 mg/kg body weight) and 200 mg/kg body weight of aqueous seed extract of *Dennettia tripetala*.

Group VI (BPA + DT400): Received Bisphenol A (50 mg/kg body weight) and 400 mg/kg body weight of aqueous seed extract of *Dennettia tripetala*.

Group VII (BPA + DT800): Received Bisphenol A (50 mg/kg body weight) and 800 mg/kg body weight of aqueous seed extract of *Dennettia tripetala*.

Bisphenol A was administered orally once daily for 28 consecutive days to induce oxidative stress. Vitamin C and the aqueous seed extract of *Dennettia tripetala* were administered orally by gavage one hour after BPA administration throughout the treatment period.

- **Sample Collection**

At the end of the treatment period, the animals were fasted overnight and euthanized under appropriate anesthesia. Blood samples were collected by cardiac puncture into plain tubes for serum preparation. The ovaries were aseptically excised, rinsed with cold physiological saline, blotted dry, and weighed. One ovary was processed for biochemical analysis, while the other was used for microbiological investigations.

- **Preparation of Ovarian Tissue Homogenates**

The ovarian tissues were homogenized in ice-cold phosphate-buffered saline (0.1 M, pH 7.4) to obtain a 10% (w/v) homogenate. The homogenates were centrifuged at 5,000 rpm for 15 min at 4°C, and the resulting supernatants were collected and stored at − 20°C until biochemical analyses.

- **Determination of Ovarian Biochemical Parameters**

Ovarian tissue homogenates were analyzed for malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and total protein using standard spectrophotometric procedures and commercially available assay kits according to the manufacturers' instructions.

- **Hormonal Analysis**

Serum concentrations of estradiol, progesterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were determined using enzyme-linked immunosorbent assay (ELISA) kits following the

manufacturers' protocols.

- **Ovarian Microbiological Analysis**

Ovarian tissues were aseptically homogenized in sterile normal saline, serially diluted, and inoculated onto nutrient agar, MacConkey agar, blood agar, and Sabouraud dextrose agar. The inoculated plates were incubated under appropriate conditions, after which colony-forming units were enumerated. Representative colonies were identified using colony morphology, Gram staining, and standard biochemical tests.

- **Histopathological Evaluation of the Ovary**

Following sacrifice, ovarian tissues were carefully excised and immediately fixed in 10% neutral buffered formalin for 48 hours. The fixed tissues were processed by routine paraffin embedding techniques, dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Tissue blocks were sectioned at 5 µm thickness using a rotary microtome, mounted on glass slides, and stained with hematoxylin and eosin (H&E). The stained sections were examined under a light microscope by a qualified histopathologist for evidence of morphological alterations, including follicular architecture, stromal integrity, vascular congestion, inflammatory cell infiltration, and degenerative changes. Representative photomicrographs were captured at appropriate magnifications.

- **Statistical Analysis**

Data generated from the study were expressed as mean ± standard deviation (SD). Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 25.0. Differences among groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test. Statistical significance was set at $p < 0.05$.

3. Results

3.1 Phytochemical Composition of Aqueous Seed Extract of Dennettia tripetala

Table 3.1 shows the qualitative phytochemical constituents of the aqueous seed extract of *D. tripetala*. The phytochemical screening revealed the presence of flavonoids, phenolic compounds, alkaloids, tannins, saponins, terpenoids, glycosides, and steroids in varying proportions. Flavonoids and phenolic compounds were highly abundant, while alkaloids, tannins, saponins, and terpenoids were moderately present. Glycosides and steroids were detected in lower amounts. These findings indicate that the aqueous seed extract contains diverse bioactive compounds that may contribute to its biological and pharmacological activities.

Table 3.1
Qualitative Phytochemical Screening of Aqueous Seed Extract of *D. tripetala*

Phytochemical Constituent	Result
Alkaloids	++
Flavonoids	+++
Tannins	++
Saponins	++
Phenolic compounds	+++
Terpenoids	++
Glycosides	+
Steroids	+

Key: + = Slightly present; ++ = Moderately present; +++ = Highly present; − = Absent.

3.2 Effect of Aqueous Seed Extract of D. tripetala on Ovarian Biochemical Parameters in Bisphenol A-Induced Oxidative Stress

Table 3.2 shows the ovarian biochemical parameters of female Wistar rats exposed to Bisphenol A (BPA) and treated with Vitamin C or graded doses of aqueous seed extract of *D. tripetala*. BPA exposure significantly increased ovarian MDA concentration and significantly decreased GSH, SOD, CAT, and total protein levels compared with the normal control group ($p < 0.05$). Treatment with Vitamin C and aqueous seed extract of *D. tripetala* significantly ameliorated these changes. The extract produced a dose-dependent reduction in MDA levels and corresponding increases in GSH, SOD, CAT, and total protein concentrations. The 800 mg/kg dose yielded values that were comparable to those observed in the Vitamin C-treated group.

Table 3.2
Ovarian biochemical parameters of female Wistar rats exposed to Bisphenol A and treated with aqueous seed extract of *D. tripetala* (Mean ± SD, n = 5).

Parameter	Normal Control	BPA Control	Vitamin C	DT100	DT200	DT400	DT800
MDA (nmol/mg protein)	3.62 ± 0.18	8.94 ± 0.41	4.35 ± 0.22	6.82 ± 0.34	5.96 ± 0.28	4.83 ± 0.23	3.98 ± 0.19
GSH (µmol/mg protein)	28.41 ± 1.32	15.23 ± 0.84	25.84 ± 1.18	18.72 ± 0.95	21.44 ± 1.06	24.67 ± 1.13	27.38 ± 1.24
SOD (U/mg protein)	16.24 ± 0.78	7.84 ± 0.41	14.82 ± 0.72	10.12 ± 0.51	11.96 ± 0.58	13.88 ± 0.65	15.76 ± 0.74
CAT (U/mg protein)	33.52 ± 1.65	17.42 ± 1.03	30.24 ± 1.48	22.16 ± 1.21	25.68 ± 1.34	28.91 ± 1.42	32.14 ± 1.56
Total Protein (mg/mL)	5.98 ± 0.33	3.82 ± 0.21	5.64 ± 0.28	4.41 ± 0.23	4.92 ± 0.25	5.38 ± 0.27	5.81 ± 0.31

Different superscripts within the same row indicate significant differences ($p < 0.05$).

3.3 Effect of Aqueous Seed Extract of *D. tripetala* on Serum Reproductive Hormones

Table 3.3 shows the serum reproductive hormone concentrations of female Wistar rats exposed to Bisphenol A (BPA) and treated with Vitamin C or aqueous seed extract of *D. tripetala* at doses of 100, 200, 400, and 800 mg/kg. BPA exposure significantly reduced serum estradiol, progesterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) concentrations compared with the normal control group ($p < 0.05$). Treatment with Vitamin C and aqueous seed extract of D. tripetala significantly restored the concentrations of these reproductive hormones relative to the BPA control group. A dose-dependent increase in estradiol, progesterone, FSH, and LH concentrations was observed in rats treated with 100, 200, 400, and 800 mg/kg of the extrac respectively t. The highest dose (800 mg/kg) produced hormone concentrations comparable to those observed in the Vitamin C-treated and normal control groups.

Table 3.3
Serum reproductive hormone concentrations (Mean ± SD, n = 5).

Hormone	Normal Control	BPA Control	Vitamin C	DT100	DT200	DT400	DT800
Estradiol (pg/mL)	48.62 ± 2.81	21.84 ± 1.62	44.32 ± 2.54	28.46 ± 1.91	34.72 ± 2.12	40.83 ± 2.36	46.18 ± 2.63
Progesterone (ng/mL)	18.45 ± 1.10	8.41 ± 0.63	16.62 ± 1.02	10.84 ± 0.75	13.42 ± 0.86	15.76 ± 0.94	17.83 ± 1.04
FSH (mIU/mL)	6.12 ± 0.39	2.84 ± 0.22	5.61 ± 0.34	3.71 ± 0.26	4.42 ± 0.31	5.14 ± 0.33	5.92 ± 0.37
LH (mIU/mL)	4.92 ± 0.34	2.01 ± 0.16	4.53 ± 0.29	2.81 ± 0.19	3.44 ± 0.24	4.08 ± 0.27	4.74 ± 0.31

Different superscripts within the same row indicate significant differences ($p < 0.05$).

3.4 Effect of Aqueous Seed Extract of *D. tripetala* on Ovarian Microbial Load

Table 3.4 presents the ovarian microbial profile of female Wistar rats following exposure to Bisphenol A (BPA) and subsequent treatment with Vitamin C or aqueous seed extract of *D. tripetala* at doses of 100, 200, 400, and 800 mg/kg respectively. The BPA control group exhibited significantly higher total viable, coliform, and fungal counts than the normal control group ($p < 0.05$). Administration of Vitamin C markedly lowered these microbial counts. Similarly, treatment with the aqueous seed extract of D. tripetala produced significant reductions in all microbial parameters when compared with the BPA control group ($p < 0.05$). The antimicrobial effect of the extract improved progressively with increasing dose, with rats treated at 800 mg/kg showing the lowest microbial counts among the extract-treated groups and values comparable to those of the Vitamin C and normal control groups.

Table 3.4
Ovarian microbial counts (Mean ± SD, ×10³ CFU/g, n = 5).

Group	Total Viable Count	Coliform Count	Fungal Count
Normal Control	3.54 ± 0.28	0.96 ± 0.08	0.42 ± 0.05
BPA Control	10.82 ± 0.71	4.23 ± 0.31	2.14 ± 0.18
Vitamin C	4.31 ± 0.33	1.21 ± 0.10	0.61 ± 0.07
DT100	8.22 ± 0.62	3.11 ± 0.24	1.62 ± 0.14
DT200	6.84 ± 0.53	2.42 ± 0.19	1.18 ± 0.11
DT400	5.42 ± 0.46	1.71 ± 0.13	0.82 ± 0.08
DT800	4.02 ± 0.35	1.04 ± 0.09	0.51 ± 0.05

Different superscripts within the same column indicate significant differences ($p < 0.05$).

3.5 Microorganisms Isolated from Ovarian Tissues

Table 3.5 presents the bacterial and fungal isolates recovered from the ovarian tissues of female Wistar rats exposed to Bisphenol A (BPA) and treated with Vitamin C or aqueous seed extract of *D. tripetala* at doses of 100, 200, 400, and 800 mg/kg. A greater diversity of microbial isolates was observed in the BPA control group compared with the normal control group. The BPA-treated rats harboured multiple bacterial species, including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus* spp., and *Proteus* spp., as well as fungal growth identified as *Candida* spp. Treatment with Vitamin C and the aqueous seed extract of *D. tripetala* reduced the occurrence and diversity of microbial isolates across the treatment groups. This reduction became more pronounced with increasing extract dose, with only *Bacillus* spp. detected at 400 mg/kg and no significant bacterial growth or fungal isolates observed in the 800 mg/kg treatment group.

Table 3.5
Microbial isolates recovered from ovarian tissues.

Group	Bacterial Isolates	Fungal Isolates
Normal Control	Bacillus spp.	Nil
BPA Control	*Escherichia coli*, *Staphylococcus aureus*, *Bacillus* spp., *Proteus* spp.	*Candida* spp.
Vitamin C	*Bacillus* spp.	Nil
DT100	*Staphylococcus aureus*, *Bacillus* spp.	*Candida* spp.
DT200	*Bacillus* spp., *Staphylococcus epidermidis*	Nil
DT400	*Bacillus* spp.	Nil
DT800	No significant bacterial growth	Nil

3.5 Histopathological Findings of the Ovary

Figure 2 shows the histopathological features of ovarian tissues from female Wistar rats exposed to Bisphenol A (BPA) and treated with Vitamin C or aqueous seed extract of *D. tripetala* at doses of 100, 200, 400, and 800 mg/kg respectively. The normal control group exhibited normal ovarian architecture, whereas the BPA control group showed marked degenerative alterations, including follicular atresia, granulosa cell degeneration, vascular congestion, inflammatory cell infiltration, and stromal disruption. Treatment with Vitamin C and aqueous seed extract of *D. tripetala* improved ovarian histology in a dose-dependent manner. Mild histological improvement was observed at 100 mg/kg, while moderate restoration of ovarian architecture occurred at 200 mg/kg. More pronounced recovery was evident at 400 mg/kg, and the 800 mg/kg group displayed near-normal ovarian histoarchitecture comparable to that of the normal control and Vitamin C-treated groups.

Key: H&E = Hematoxylin and Eosin stain; ×100 = Magnification (100×); BPA = Bisphenol A; DT = Dennettia tripetala aqueous seed extract.

4. Discussion

4.1 Phytochemical Composition of Aqueous Seed Extract of *D. tripetala*

The phytochemical screening of the aqueous seed extract of *D. tripetala* revealed the presence of several bioactive constituents, including flavonoids, phenolic compounds, alkaloids, tannins, saponins, terpenoids, glycosides, and steroids. Among these constituents, flavonoids and phenolic compounds were the most abundant, while alkaloids, tannins, saponins, and terpenoids were moderately present. Glycosides and steroids were detected in lower amounts.

The presence of these phytochemicals suggests that the aqueous seed extract possesses a rich composition of secondary metabolites that may contribute to its biological activities as previously accounted by Nwanze et al. [37]. Flavonoids and phenolic compounds have been widely associated with antioxidant properties due to their ability to scavenge free radicals and reduce oxidative damage [6, 16]. Similarly, alkaloids, tannins, and saponins have been reported to possess anti-inflammatory [38], antimicrobial [39], and protective effects in biological systems [40], respectively. The occurrence of terpenoids, glycosides, and steroids further supports the therapeutic potential of the plant, as these compounds have been linked to various pharmacological activities [41–43]. The diverse phytochemical profile observed in the present study may therefore account for the protective effects of the extract against BPA-induced ovarian toxicity [44].

4.2 Protective Effect of Aqueous Seed Extract of D. tripetala on Ovarian Biochemical Parameters in BPA-Induced Oxidative Stress

The present study demonstrated that exposure to Bisphenol A (BPA) induced significant oxidative stress in the ovaries of female Wistar rats, as evidenced by elevated malondialdehyde (MDA) levels and decreased concentrations of reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and total protein. These findings are consistent with previous reports indicating that BPA promotes excessive generation of reactive oxygen species (ROS), resulting in lipid peroxidation, cellular damage, and impairment of endogenous antioxidant defense systems [44, 45].

Treatment with aqueous seed extract of *D. tripetala* significantly attenuated these biochemical alterations in a dose-dependent manner. The observed reduction in MDA concentration suggests inhibition of lipid peroxidation and stabilization of cellular membranes, while the increases in GSH, SOD, and CAT indicate restoration of antioxidant defense mechanisms [4, 46. The highest extract dose produced effects comparable to those observed in the Vitamin C-treated group, suggesting strong antioxidant activity. These effects may be attributed to the abundance of flavonoids, phenolic compounds, alkaloids, and other phytochemicals present in *D. tripetala*, which are known to scavenge free radicals and enhance antioxidant enzyme activity [47]. Therefore, the extract appears to protect ovarian tissues against BPA-induced oxidative injury and preserve cellular homeostasis.

4.3 Protective Effect of Aqueous Seed Extract of *D. tripetala* on Reproductive Hormones in BPA-Induced Toxicity

Bisphenol A administration significantly reduced serum estradiol, progesterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) concentrations compared with the normal control group. These findings support previous studies demonstrating that BPA acts as an endocrine-disrupting chemical capable of interfering with the hypothalamic-pituitary-ovarian axis and impairing steroidogenesis. Reduced concentrations of these hormones may adversely affect follicular development, ovulation, and fertility [48–50].

Administration of aqueous seed extract of *D. tripetala* significantly restored hormone concentrations toward normal values, with the highest dose showing results comparable to those of the Vitamin C-treated group. The improvement in hormonal status may be associated with the antioxidant properties of the extract, which likely protected ovarian follicles, granulosa cells, and steroidogenic tissues from oxidative damage [51]. The preservation of these structures may facilitate normal hormone synthesis and secretion. Consequently, the findings suggest that *D. tripetala* possesses endocrine-supportive properties capable of mitigating BPA-induced reproductive dysfunction.

4.4 Protective Effect of Aqueous Seed Extract of *D. tripetala* on Ovarian Microbial Load

The present study revealed that BPA exposure increased ovarian microbial burden, as evidenced by elevated total viable count, coliform count, fungal count, and the presence of diverse bacterial and fungal isolates. Increased oxidative stress may compromise tissue integrity and local defense mechanisms, thereby creating conditions favorable for microbial colonization.

Treatment with aqueous seed extract of *D. tripetala* significantly reduced microbial counts and progressively eliminated microbial isolates in a dose-dependent manner. At the highest dose, no significant bacterial growth and no fungal isolates were observed. These findings are consistent with previous reports demonstrating the broad-spectrum antimicrobial activity of *D. tripetala* against several pathogenic microorganisms[37, 52, 53]. The antimicrobial effects may be attributed to phytochemicals such as tannins, flavonoids, alkaloids, and essential oils, which can disrupt microbial cell membranes, inhibit metabolic pathways, and suppress microbial proliferation. The ability of the extract to reduce microbial burden may contribute to the maintenance of a healthier ovarian microenvironment during oxidative stress conditions [52–54].

4.5 Protective Effect of Aqueous Seed Extract of *D. tripetala* on Ovarian Histoarchitecture

Histopathological examination revealed marked ovarian damage in BPA-treated rats, characterized by follicular atresia, degeneration of granulosa cells, vascular congestion, inflammatory cell infiltration, and disruption of normal stromal organization. These structural alterations are characteristic features of oxidative stress-mediated ovarian injury and may contribute to impaired reproductive function.

Conversely, treatment with aqueous seed extract of *D. tripetala* produced dose-dependent improvements in ovarian histology. Animals receiving higher doses exhibited preservation of follicular architecture, intact granulosa and theca cell layers, normal stromal organization, and absence of significant inflammatory lesions. The histological appearance of the 800 mg/kg group was comparable to that of the normal control and Vitamin C-treated groups. These observations suggest that the extract effectively protected ovarian tissues from BPA-induced morphological damage.

The histopathological findings corroborate the biochemical and hormonal results obtained in the present study. The restoration of antioxidant enzyme activities and reduction of oxidative damage likely contributed to the preservation of ovarian cellular integrity and follicular development [55, 56]. Furthermore, the reduced microbial burden may have minimised inflammatory responses, thereby enhancing tissue recovery and maintaining normal ovarian function [57].

Overall, the findings demonstrate that aqueous seed extract of *Dennettia tripetala* exerts significant antioxidant, endocrine-modulating, antimicrobial, and tissue-protective effects against BPA-induced ovarian toxicity in female Wistar rats.

5. Conclusion

The present study successfully evaluated the protective effects of aqueous seed extract of *D. tripetala* against Bisphenol A (BPA)-induced ovarian toxicity in female Wistar rats and comprehensively determined its phytochemical composition. Qualitative phytochemical screening clearly revealed the presence of flavonoids, phenolic compounds, alkaloids, tannins, saponins, terpenoids, glycosides, and steroids, thereby demonstrating the rich phytochemical nature of the extract. BPA exposure significantly and adversely affected ovarian function by inducing oxidative stress, disrupting reproductive hormone concentrations, increasing microbial burden, and causing histopathological damage to ovarian tissues. Conversely, treatment with aqueous seed extract of *D. tripetala* effectively and progressively ameliorated these alterations in a dose-dependent manner. Notably, the extract markedly improved ovarian biochemical parameters, substantially restored reproductive hormone concentrations, significantly reduced microbial counts and isolates, and histologically preserved ovarian tissue architecture. Furthermore, the highest dose of the extract produced the most pronounced protective effects and performed comparably to Vitamin C treatment. Overall, the findings strongly suggest that aqueous seed extract of *D. tripetala* possesses potent antioxidant, antimicrobial, endocrine-supportive, and ovarian protective activities and may therefore serve as a promising natural therapeutic agent for the effective management of BPA-induced reproductive toxicity and other oxidative stress-related ovarian disorders.

Declarations

Ethical Approval

All experimental procedures involving animals were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals and were approved by the Animal Ethics Committee of the Department of Animal and Environmental Biology, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, Rivers State, Nigeria, prior to the commencement of the study (Approval No.: IAUE/FNAS/AEB/REC/2026/010).

Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

Owo, Gogo James conceived and designed the study. **Okwelle, Achinike Austin., Owo, Gogo James., and Enyindah, Kingsley Chimene** carried out the experimental work, collected and analyzed the data, and prepared the manuscript respectively. All authors read and approved the final manuscript.

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References

1. El-Saadony MT, Saad AM, Mohammed DM, Korma SA, Alshahrani MY, Ahmed AE, Ibrahim EH, Salem HM, Alkafaas SS, Saif AM, Elkafas SS, Fahmy MA, El-Mageed A, Abady TA, Assal MM, El-Tarabily HY, Mathew MK, AbuQamar BT, El-Tarabily SF, K. A., Ibrahim SA. Medicinal plants: bioactive compounds, biological activities, combating multidrug-resistant microorganisms, and human health benefits - A comprehensive review. Front Immunol. 2025;16:1491777. https://doi.org/10.3389/fimmu.2025.1491.
2. Kpomah ED, Owo GJ. Therapeutic Potential of Methanolic Stem Extract of Costus afer on Serum Uric Acid, Tumor Necrosis Factor-alpha (TNF-α), and Interleukin-6 (IL-6) Levels in Monosodium Urate (MSU)-Induced. J Adv Med Pharm Sci. 2024;26(11):45–54. https://doi.org/10.9734/jamps/2024/v26i11724.
3. Owo GJ, Kpomah ED. Blood Urea Reducing Effects of Ethanolic Extracts of Yellow Monkey Kola Fruit (*Cola Lepidota*) Seeds on Ibuprofen-Induced Hyperuremia in Albino Rats. Iconic Res Eng Journals. 2023;6(12):323–7.
4. Okari KA, Owo GJ, Ezekwe AS, Sukuba A. Protective Effects of the Ethanolic Pulp Extract of *Lycium barbarum* (Goji Berry) on Carbon Tetrachloride–Induced Nephrotoxicity, Cardiac Dysfunction, and

Oxidative Stress in Wistar Rats. Asian J Res Biology. 2026;9(1):50–65. https://doi.org/10.56557/ajrib/2026/v9i166.

5. Owo GJ, Beresford SJ. Blood sugar lowering potentials of aqueous and ethanol extracts of the mixture of rinds of Citrullus vulgaris schrad (watermelon) and Chrysophyllum albidum G. (udara) fruits on alloxan-induced diabetic Wistar rats. J Pharm Res Int. 2020;32:86–90. https://doi.org/10.9734/JPRI/2020/v32i2130755.
6. Owo GJ. Alkaloid and Flavonoid Content of *Pterocarpus Santalinoides*: Effects on Pancreatic Biochemistry in Mice. Quantum J Med Health Sci. 2026;5(1):54–61.
7. Okwelle AA, Owo GJ, Kpomah ED. A Comparative approach to the effects of aqueous leaf and root extracts of *Moringa oleifera* on selected biochemical, haematological, and gut microbiota parameters of alloxan-induced diabetic Wistar rats. Cornous Biology. 2026;4(1):18–26. https://doi.org/10.37446/corbio/rsa/4.1.2026.18-26.
8. Kpomah ED, Owo GJ. Comparative In-vitro Analyses of the Anti-inflammatory, Antioxidant, and Antimicrobial Properties of Selected Soup Thickeners Commonly Used in the Niger Delta Region of Nigeria. Asian J Food Res Nutr. 2024;3(4):972–82.
9. Chandra M. Role of bioactive compounds in hormonal bioregulation. Bioactive components: A sustainable system for good health and well-being. Singapore: Springer Nature Singapore; 2022. pp. 323–42.
10. Rodríguez-Negrete EV, Morales-González Á, Madrigal-Santillán EO, Sánchez-Reyes K, Álvarez-González I, Madrigal-Bujaidar E, Valadez-Vega C, Chamorro-Cevallos G, Garcia-Melo LF, Morales-González JA. Phytochemicals and Their Usefulness in the Maintenance of Health. Plants. 2024;13(4):523. https://doi.org/10.3390/plants13040523.
11. Rodríguez-Yoldi MJ. Anti-Inflammatory and Antioxidant Properties of Plant Extracts. Antioxidants. 2021;10(6):921. https://doi.org/10.3390/antiox10060921.
12. Ezekwe SA, Chikezie PC. GC-MS analysis, hypoglycemic activity of aqueous root extract of Carica papaya and its effects on blood lipid profile and hepatorenal tissues biomarkers of diabetic rats. J Diabetes Metabolism. 2017;8(5):740–8. https://doi.org/10.4172/2155-6156.1000740.
13. Uro-Chukhu HC. Body Weight Changes, Histological Features and Anti-Hyperglycemic Effects of Cocoyam-Soya Bean-Bambara Groundnut Flour Blend-Fed Streptozotocin (STZ)-Induced Diabetic Rats. Lond J Med Health Res. 2024;24(8):9–25.
14. Kpomah ED, Owo GJ, Kpomah B. Aphrodisiac activity of ethanolic root extracts of *Acacia pycnantha* (Golden Wattle) in healthy male Wistar rats. J Res Appl Basic Med Sci. 2025;11(1):59–67.
15. Kpomah ED, Arhoghro EM. Investigation into the intake of a popular polyherbal drug (Jalin Herbal Mannex Liquid) on selected biochemical indices of male Wister rats. Afr J Biochem Res. 2022;16(4):55–62.
16. Owo GJ, Okari KA, Kpomah ED. Flavonoid-to-Phenol Ratios in Methanolic Extracts of *Aspilia africana*: Implications for Managing Oxidative Stress. Asian Basic Appl Res J. 2025;125–33. https://doi.org/10.56557/abaarj/2025/v7i1155.

17. Adewoyin M, Ibrahim M, Roszaman R, Md Isa ML, Mat Alewi NA, Rafa A, A. A., Anuar MN N. Male infertility: the effect of natural antioxidants and phytocompounds on seminal oxidative stress. Diseases. 2017;5(1):9.
18. Liu X, Zeng T, Zhang E, Bin C, Liu Q, Wu K, Wei S. Plant-based bioactives and oxidative stress in reproduction: Anti-inflammatory and metabolic protection mechanisms. Front Nutr. 2025;12:1650347.
19. Bhardwaj JK, Panchal H, Saraf P. Ameliorating effects of natural antioxidant compounds on female infertility: a review. Reproductive Sci. 2021;28(5):1227–56.
20. Orsi NM, Baskind NE, Cummings M. Anatomy, development, histology and normal function of the ovary. Pathology of the ovary, fallopian tube and peritoneum. Cham: Springer International Publishing; 2024. pp. 1–33.
21. Al-Suhaimi EA, Khan FA, Homeida AM. Regulation of male and female reproductive functions. Emerging concepts in endocrine structure and functions. Singapore: Springer Nature Singapore; 2022. pp. 287–347.
22. Das PK, Mukherjee J, Banerjee D. Female reproductive physiology. Textbook of veterinary physiology. Singapore: Springer Nature Singapore; 2023. pp. 513–68.
23. Kobayashi H, Yoshimoto C, Matsubara S, Shigetomi H, Imanaka S. Altered energy metabolism, mitochondrial dysfunction, and redox imbalance influencing reproductive performance in granulosa cells and oocyte during aging. Reproductive Sci. 2024;31(4):906–16.
24. Yan F, Zhao Q, Li Y, Zheng Z, Kong X, Shu C, Shi Y. The role of oxidative stress in ovarian aging: a review. J ovarian Res. 2022;15(1):100.
25. Shi YQ, Zhu XT, Zhang SN, Ma YF, Han YH, Jiang Y, Zhang YH. Premature ovarian insufficiency: a review on the role of oxidative stress and the application of antioxidants. Front Endocrinol. 2023;14:1172481.
26. Begum IA. Oxidative stress: Oocyte quality and infertility. Reprod Toxicol. 2025;137:109011. https://doi.org/10.1016/j.reprotox.2025.109011.
27. Das A, Roychoudhury S. Reactive oxygen species in the reproductive system: sources and physiological roles. Oxidative Stress and Toxicity in Reproductive Biology and Medicine: A Comprehensive Update on Male Infertility-Volume One. Cham: Springer International Publishing; 2022. pp. 9–40.
28. Zhu B, Tao Z, Edupuganti L, Serrano MG, Buck GA. Roles of the microbiota of the female reproductive tract in gynecological and reproductive health. Microbiol Mol Biol Rev. 2022;86(4):e00181–21.
29. Escorcia Mora P, Valbuena D, Diez-Juan A. The role of the gut microbiota in female reproductive and gynecological health: insights into endometrial signaling pathways. Life. 2025;15(5):762.
30. Li H, Zang Y, Wang C, Li H, Fan A, Han C, Xue F. The interaction between microorganisms, metabolites, and immune system in the female genital tract microenvironment. Front Cell Infect Microbiol. 2020;10:609488.

31. Qi X, Yun C, Pang Y, Qiao J. The impact of the gut microbiota on the reproductive and metabolic endocrine system. Gut Microbes. 2021;13(1):1894070.
32. Manzoor MF, Tariq T, Fatima B, Sahar A, Tariq F, Munir S, Ibrahim SA. An insight into bisphenol A, food exposure and its adverse effects on health: A review. Front Nutr. 2022;9:1047827.
33. Cimmino I, Fiory F, Perruolo G, Miele C, Beguinot F, Formisano P, Oriente F. Potential mechanisms of bisphenol A (BPA) contributing to human disease. Int J Mol Sci. 2020;21(16):5761.
34. Sharma M, Sharma R, Gupta P, Srivastava S. Bisphenol-A induced oxidative stress and its fertility aspects. Int J Pharm Sci Res. 2019;10:3519–31.
35. Okunlola BM. *Dennettia tripetala* (Pepper Fruit), a review of its ethno-medicinal use, phyto-constituents, and biological properties. GSC Adv Res Reviews. 2021. https://doi.org/10.30574/gscarr.2021.6.3.0024.
36. Nwosu RA, Suleiman MM, Makun HJ, Ameh MP, Shetshak MA, Akefe IO. In vitro anthelmintic activity of *Dennettia tripetala* G. Baker (Annonaceae) Fruits against *Haemonchus contortus*. J Parasitic Dis. 2022;46(1):220–9.
37. Nwanze P, Onyenwuchi KSC, Amaralam EC, Opara UL. Phytochemical Analysis and Antimicrobial Activities of *Dennettia Trpetala* Leaf and Seed Extracts against *Escherichia coli* and *Staphylococcus aureus*. J Res Pharm Sci. 2021;7(10):05–10.
38. Okari KA, Owo GJ, Kpomah ED. Proximate Composition, Phytochemical Composition, and Hypolipidemic Activities of Methanolic Extracts of Selected Medicinal Plants Used in Traditional Medicine in Southern Nigeria. J Res Appl Basic Med Sci. 2025;11(3):244–53. http://dx.doi.org/10.61882/rabms.11.3.244.
39. Huang J, Zaynab M, Sharif Y, Khan J, Al-Yahyai R, Sadder M, Li S. Tannins as antimicrobial agents: Understanding toxic effects on pathogens. Toxicon. 2024;247:107812.
40. Li H, Zhai B, Sun J, Fan Y, Zou J, Cheng J, Guo D. (2021). Antioxidant, anti-aging and organ protective effects of total saponins from Aralia taibaiensis. Drug Des Devel Ther, 4025–42.
41. Velu G, Palanichamy V, Rajan AP. Phytochemical and pharmacological importance of plant secondary metabolites in modern medicine. Bioorganic phase in natural food: an overview. Cham: Springer international publishing; 2018. pp. 135–56.
42. Riaz M, Khalid R, Afzal M, Anjum F, Fatima H, Zia S, Aslam MA. Phytobioactive compounds as therapeutic agents for human diseases: A review. Food Sci Nutr. 2023;11(6):2500–29.
43. Kaushik B, Sharma J, Kumar P, Shourie A. Phytochemical properties and pharmacological role of plants: secondary metabolites. Biosci Biotechnol Res Asia. 2021;18(1):23.
44. Meli R, Monnolo A, Annunziata C, Pirozzi C, Ferrante MC. Oxidative stress and BPA toxicity: an antioxidant approach for male and female reproductive dysfunction. Antioxidants. 2020;9(5):405.
45. Kourouma A, Quan C, Duan P, Qi S, Yu T, Wang Y, Yang K. Bisphenol A induces apoptosis in liver cells through induction of ROS. Adv Toxicol. 2015;2015(1):901983.

46. Mursal M, Hasan I, Tiwari B, Srivastava RK, Yadav G, Fatima G. Disruptions in nitric oxide homeostasis, lipid peroxidation-derived oxidative stress, and antioxidant defense mechanisms in spinal cord injury: elucidating biomolecular correlates of disease severity. Mol Biol Rep. 2025;52(1):969.
47. Katiyar D, Saxena R, Kumar A, Bansal P, Prakash S, Ghosh D, Nagarajan K. A comprehensive review of the protective effects of herbals against toxicity of Bisphenol-A. Toxin Reviews. 2024;43(3):329–57.
48. Shoorei H, Seify M, Talebi SF, Majidpoor J, Koohestanidehaghi Y, Shokoohi M. (2023). Different types of bisphenols alter ovarian steroidogenesis: special attention to BPA. Heliyon, *9*(6).
49. Maniradhan M, Calivarathan L. Bisphenol a-induced endocrine dysfunction and its associated metabolic disorders. *Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune*. Endocr Metabolic Disorders). 2023;23(4):515–29.
50. Jozkowiak M, Piotrowska-Kempisty H, Kobylarek D, Gorska N, Mozdziak P, Kempisty B, Spaczynski RZ. Endocrine disrupting chemicals in polycystic ovary syndrome: the relevant role of the theca and granulosa cells in the pathogenesis of the ovarian dysfunction. Cells. 2022;12(1):174.
51. Kirabo NK. Oxidative Stress, Hormonal Response, and the Reproductive System: Therapeutic Prospects of Antioxidant-Rich Plant Extracts. Int Netw Organ Sci Res. 2025;15(3):10–5.
52. Ekandem JJ, Udourioh GA, Obi LK, Solomon MM. Chemical Compositions, Antimicrobial and Therapeutic Properties of Essential Oils of Dennettia tripetala. Curr Anal Chem. 2025;21(4):335–44.
53. Oluwasina OO, Ezenwosu IV, Ogidi CO, Oyetayo VO. Antimicrobial potential of toothpaste formulated from extracts of Syzygium aromaticum, Dennettia tripetala and Jatropha curcas latex against some oral pathogenic microorganisms. AMB Express. 2019;9(1):20.
54. Hochma E, Yarmolinsky L, Khalfin B, Nisnevitch M, Ben-Shabat S, Nakonechny F. (2021). Antimicrobial Effect of Phytochemicals from Edible Plants. *Processes*, *9*(11), 2089. https://doi.org/10.3390/pr9112089
55. Xie B, Zhang K, Lin J, Cheng H, Huang X. Female reproductive capacity preservation: antioxidant strategies in combating ovarian aging and cryopreservation challenges. Front Endocrinol. 2025;16:1711016.
56. Wang S, He G, Chen M, Zuo T, Xu W, Liu X. The role of antioxidant enzymes in the ovaries. Oxidative Med Cell Longev. 2017;2017(1):4371714.
57. Ma S, Zheng L, Zhuang X, Wang M, Zou Y. Pathogenic mechanisms and therapeutic potential of the microbiome in premature ovarian insufficiency. Front Immunol. 2025;16:1734367.

Figures



Figure 1

Dennettia tripetala (pepper fruit).

Source: Adapted from The Guardian Nigeria (accessed June 2026).https://guardian.ng/features/how-pepper-fruit-others-stop-prostate-cancer/

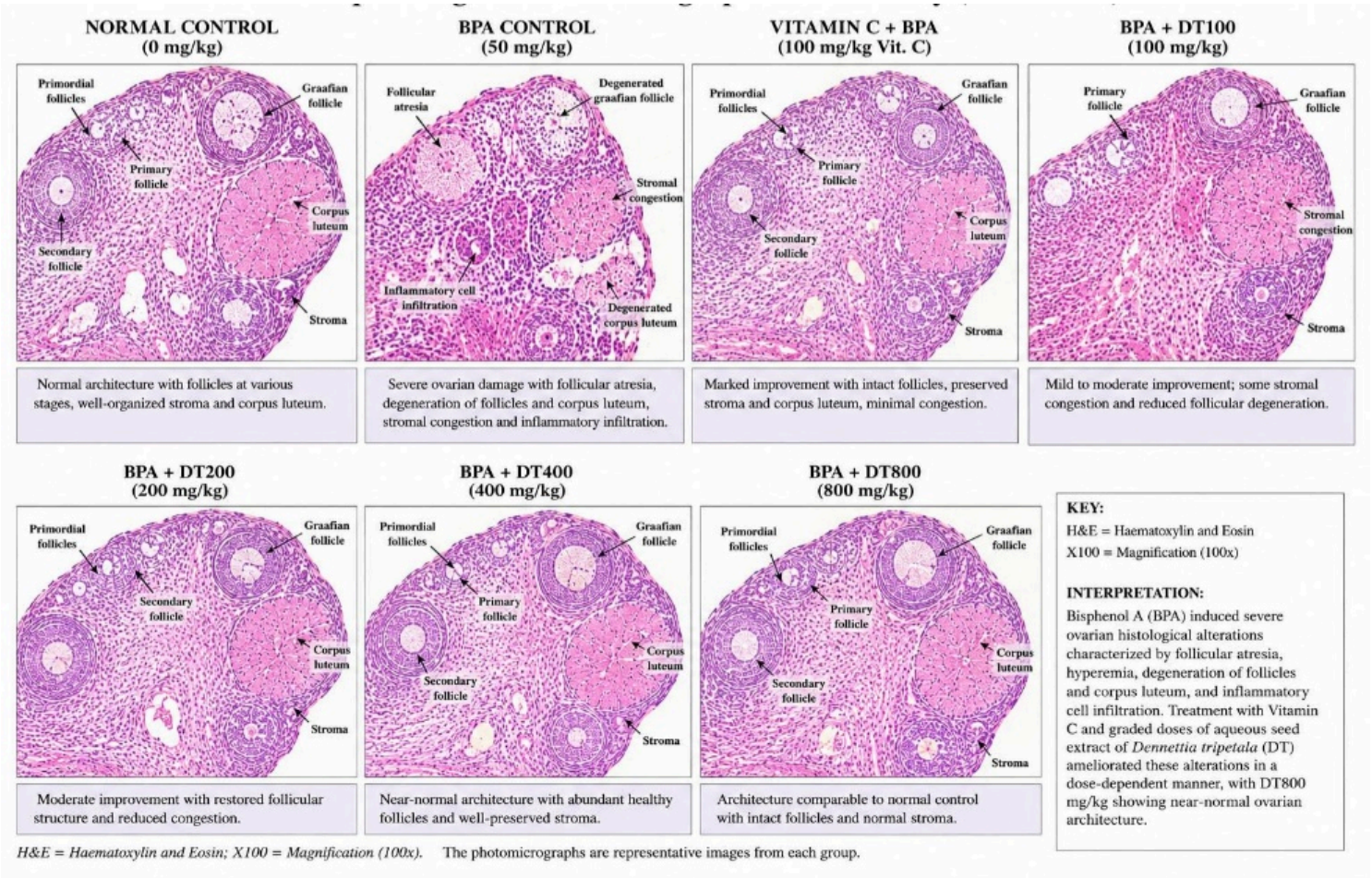


Figure 2

Histopathological photomicrographs of ovarian tissues from female Wistar rats exposed to Bisphenol A (BPA) and treated with graded doses of aqueous seed extract of *D. tripetala* (H&E, ×100).

Key: H&E = Hematoxylin and Eosin stain; ×100 = Magnification (100×); BPA = Bisphenol A; DT = Dennettia tripetala aqueous seed extract.