Antioxidant and Cytoprotective Properties of Leaf Extract of *Brillantaisia patula* on Uterus and Ovarian Function in Cyclophosphamide Model of Gonadal Toxicity in Rats

***Ogunro, O. B. and Odesola, A. F.**
[1]*Reproductive & Endocrinology, Toxicology, and Bioinformatics Research Laboratory, Department of Biological Sciences, KolaDaisi University, Ibadan. Nigeria*
***Correspondence – email:** *olalekanbukunmi@gmail.com*
Mobile phone: +2348069845995

Abstract
This study evaluated the antioxidant and cytoprotective properties of aqueous leaf extract of *Brillantaisia patula* on function indices of the uterus and ovary of cyclophosphamide-induced gonadal toxicity in female Wistar rats. A total of 36 female rats assigned randomly to six groups of six animals were treated for 21 days. Group 1 received only distilled water. Groups 2 – 6 were treated with a single dose (200 $mgkg^{-1}$ body weight) of cyclophosphamide intraperitoneally and in addition, received the same volume (0.5 ml) of distilled water, 18, 36, 72 $mgkg^{-1}$ body weight of the extract and 200 $mgkg^{-1}$ body weight of vitamin C orally. Findings indicated that cyclophosphamide significantly ($p < 0.05$) increased the blood concentrations of follicle stimulating hormone, luteinizing hormone, and prolactin but contrarily reduced (($p < 0.05$) the concentration of estrogen and progesterone. In addition, the uterine and ovarian activities/levels of acid and alkaline phosphatases, 3- β-hydroxysteroid dehydrogenase, 17-β-hydroxysteroid dehydrogenase, cholesterol, glucose; superoxide dismutase, catalase, glutathione reductase, and glutathione-S-transferase were significantly ($p < 0.05$) reduced by cyclophosphamide. Meanwhile, cyclophosphamide increased ($p < 0.05$) the activity of myeloperoxidase as well as the concentrations of TNF-α and interleukin 6. On the contrary, the extract reversed the action of cyclophosphamide to level that compared well with the control ($p < 0.05$). Furthermore, the treatment-related histoarchitectural changes in the ovary and uterus of the female rats caused by cyclophosphamide were reversed by the extract. Put together, leaf extract of *B. patula* demonstrated the ability to reverse damages to the ovaries and uterus and thus can be explored as a safe agent against toxic side effects of chemotherapy and fertility-related malady of the uterus and ovary.

Keywords: *Brillantaisia patula*, Reproduction toxicology, Functional toxicity, Sterility, Chemoprophylaxis, Cyclophosphamide

1 Introduction

Gonadal toxicity resulting from cancer therapy using drugs like cyclophosphamide have adverse effect on the ovaries (causing them to stop releasing eggs) and estrogen which are detrimental to fertility in females. For a long time, the protective capabilities of plants in herbal medicine have been relied on to mitigate infertility from cancer therapy. Humans, especially in the African countries, are known to explore herbal agents against toxicants [1,2]. Evaluation of medical plants as agents to extenuate the side effects from cancer therapy continue to gain ground due to desire or desperation of females to conceive [1].

Various reasons are responsible for why women (especially in African countries) have preference for herbal drugs over the orthodox drugs for mitigating side effects from cancer therapy. These reasons (not separable from treatment of infertility) may include the folkloric validation on the efficacy of herbal drugs, the choice herbal users have for natural treatments and the desires for alternative medicines. In addition, wrong belief about the superiority of herbal products to manufactured agents, discontentment with orthodox drugs and the mindset that herbs-derived drugs have better effectiveness in treating some diseases than conventional drugs are also part of the reasons. Other reasons include adverse effects and high cost of most conventional drugs; advances in the efficacy, quality, and safety of herbal drugs; the belief of patient that physicians may not have properly identified their problem, and the drive toward self-medication [3]. Furthermore, some women find it uncomfortable to discuss medical problems because they lack the confidence to handle their fertility information. The fear of possible misdiagnosis and wrong

treatment for patients that do not exhibit any specific symptoms may also be among the reasons. In addition, general unease and difficulty to see a physician are also reasons why the use of herbal drugs to boost fertility has been on the increase [4].

The potency of these herbal therapies from medicinal plants to mitigate gonadal toxicity have been substantiated by scientific findings over the years. For instance, experimental and clinical studies have demonstrated the effectiveness of these herbal ingredients in the regulation of gonadotropin-releasing hormone and concentrations of ovarian sex hormone for stimulating ovulation and speeding up the flow of blood to the ovaries alongside improving ovarian reserve [5]. However, many of them remain unevaluated for their efficacy. In this context, the best dosage, mode of action, side effects, possible contradictions, as well as the way of interaction with other orthodox drug is still a flux. It is, however, essential to be concerned about the safety of these herbal agents that are regularly used as remedy against gonadal toxicity. Most importantly, it is apparent that adequate information about the mode of action, side effects, and contradictions of most plant agents will promote safe and their rational use.

One of these plants with vital properties against gonadal toxicity but without scientific evidence on their efficacy is *Brillantaisia patula*. *B. patula*, of the family acanthaceae commonly known as *ewe ọwọ́* by the Yoruba ethnic group of Nigeria is a robust-shrubby plant that is 3 m high; it is common in the Southwestern part of Nigeria. The use of *B. patula* range from Togo to West Cameroons and across the Congo basin to Uganda and Angola. In Southwest of Nigeria, it is taken by barren females to ensure conception [6]. Other reported ethnomedicinal uses associated with *B. patula* are the treatment of yaws and rheumatism. The decocted leaves also ease childbirth, menstrual pains, and stomachache. In addition, the leaves are used for dressing of wounds and as disinfectant against circumcision infections when mixed with snail shell powder. *B. patula* leaves also serves as a remedy for anaemia and malnutrition (when eaten as a vegetable), stomach-trouble, chest-conditions, and infantile spleen infection. It also serves as a sedative in epilepsy and insanity when instilled into both eyes and nose. The sap alone or its mixture with other decocted leaves or dilution with palm-wine can improve abnormal rapid heartbeat [7]. Moreover, the nutritional evaluation of *B. patula* leaves shows that it is a good source of essential amino acids and major mineral elements [6] while the leaf oils were reported to be characterised by a dominance of alcohols [8]. The reported pharmacological activity includes antibacterial [7], anti-plasmodial and analgesic [9], and antiplasmodial [10]. Furthermore, glycosides, phenolics, saponins, alkaloids, flavonoids and tannins have been reported as the secondary metabolites in the leaf extract of *B. patula* [7].

The evaluations of protective capabilities of leaf extract of *B. patula* against a popular gonadal toxicant, cyclophosphamide is therefore necessary. The scope was on information from the hormonal balance [follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, progesterone, and estrogen], uterine and ovarian function indices [alkaline phosphatase (ALP), acid phosphatase (ACP), cholesterol, 3- β-hydroxysteroid dehydrogenase (3-β HSD), 17-β-hydroxysteroid dehydrogenase (17-β-HSD), and glucose], antioxidant and inflammation indices [superoxide dismutase (SOD), catalase, glutathione-S-transferase (GST), reduced glutathione (GSH); tumour necrosis factor-α (TNF-α), interleuken-6 (IL-6), and myeloperoxidase (MPO)] as well as the histopathological findings on the ovaries and uterus. Overall, the study is intended to provide more scientific insights on the antioxidant and cytoprotective properties of *B. patula* leaves in cyclophosphamide-exposed female rats.

2 Materials and Methods

2.1 Chemicals

Standard laboratory grade chemicals and reagents were used in this research. ELISA kit for interleukin-6 (IL-6), and tumour necrosis factor- α (TNF-α) were a product of the UK's ABCAM Scientific Corporation. Alkaline phosphatase (ALP), acid phosphatase (ACP), and glucose assay kits were sold by the Randox Laboratory, Co-Atrim, United Kingdom. Assay kits from Diagnostics Laboratories, Freiburg, Germany were procured to assay for progesterone, prolactin, follicle stimulating hormone, estradiol, and luteinizing hormone.

2.2 Plant Sample and Preparation

Fresh leaves of *B. patula* were self-obtained from Ademilokun metropolis of Ibadan, Oyo state, Nigeria. The voucher number U.I.H 1079 was assigned to the plant specimen after it was authenticated at the Herbarium of Department of Plant Biology, University of Ilorin, Nigeria.

The leaves were freshly collected and rinsed in distilled water to remove dirt. The clean leaves were then oven-dried at 40 ^{o}C for four days until a constant weight of the leaves was gotten. The dried leaves were thereafter pulverized with an electric blender until a fine powder was obtained. The resulting powder (200 g) of the blended plant material was extracted in 200 mL of distilled water for 48 hours after which it was filtered using a Buchner funnel with Whatman's No 1 filter paper to obtain a filtrate. The resulting filtrate was concentrated to dryness in a lyophilizing machine to yield a powdered sample of 25.18 ± 3.4g. Calculated amount of the lyophilized sample was reconstituted in distilled water (used as the vehicle) to obtain the equivalent doses of 36 mg/kg body weight (based on ethnobotanical survey on how the leaves are used traditionally). A half dose (18 mg/kg body weight) and multiple dose (72 mg/kg body weight) of 36 mg/kg body weight were also used in the present study.

2.3 Animal Model, Study Design and Treatment

The average weight of the female Wistar rats used in this study was 150.5g. The animals were made to adjust to the new study environment for 14 days in plastic cages in a well-ventilated animal house (maintained on 43% humidity and 29°C temperature, with 12 hours dark and light cycle) prior to the commencement of the study. Care was given to the rats by following the guidelines of the National Academy of Science (NAS) in the eighth edition of "Guide for the Care and Use of Laboratory Animals". Furthermore, the animals were provided rat chows and clean tap water *ad libitium.* Approval for the study was granted from Ajayi Crowther University's Research Ethic Committee. The study was also done in conformity with the regulations on the care and the use of murine models in the laboratory.

A total of 36 female animals were allotted into 6 groups (n=6) each and treated with the specified doses between 8:00 am and 9:00 am as follows:

Group 1 served as the control and were treated with 0.5 ml distilled water. Animals in groups 2 – 6 were treated with a single dose (200 mgkg^{-1} body weight) of cyclophosphamide intraperitoneally and in addition, orally received the same volume of distilled water, 18 mgkg^{-1} body weight of the extract, 36 mgkg^{-1} body weight of the extract, 72 mgkg^{-1} body weight of the extract and 200 mgkg^{-1} body weight of vitamin C respectively for 21 days. Recorded weights of the animals before and after treatment were noted. The doses of cyclophosphamide and vitamin C were based on previous work of Raeeszadeh et al. [11].

2.4 Qualitative and Quantitative Analysis Secondary Metabolites in the Leaf Extract of *Brillantaisia patula*

The obtained extract was subjected to qualitative and quantitative analyses for secondary metabolites in the leaf of *B. patula*. The extract was analyzed for alkaloid [12], cardenolides and dienolides [13], cardiac glycoside [14], phlobatannins [15], steroids [16], flavonoids and phenolics [17], saponins [18], anthraquinones [19], and tannins/terpenes [20]. Standard methods were used for the quantitative analysis of flavonoids, anthraquinones, alkaloid, saponins, phlobatannins, phenolics, terpernoids, steroids and tannins.

2.5 Sacrifice and Tissue Preparation

The animals were sacrificed by cervical dislocation 24 hours after the last treatment dose. Blood from the animals was collected through retro-orbital venous plexus in to heparinized bottles. Centrifugation of the blood at 3,000 x *g* for 10 minutes yielded a clear plasma that were dispensed into Eppendorf microliter tubes. The separated plasma samples were thereafter kept below 4 °C in a freezer for subsequent determination of hormones. Furthermore, the ovaries and uterus were excised, rinsed off blood in frigid 0.15 % KCl, dried with blotting paper, weighed, and then processed for antioxidant assays in the present study.

2.6 Determination of Circulating Hormones and Reproductive Indices

The procedural steps as described in the assay kits according to the principles highlighted by Tietz [21], were adopted for the determination of plasma concentration of progesterone, follicle-stimulating hormone (FSH), luteinising hormone (LH), prolactin, and estrogen.

Determination of the activities of alkaline phosphatase (ALP) and acid phosphatase (ACP) were achieved by adopting the protocol proscribed by Roth et al. [22] and Lopez [23] respectively. 3β- and 17-β-hydroxysteroid dehydrogenase were determined utilizing the procedural steps of de Araujo et al. [24]. Glucose concentration was quantified using the protocol described by Barham and Trinder [25] while cholesterol concentration was evaluated by adopting the method of Corso et al. [26].

2.7 Studies of Antioxidant Status, Inflammation and Oxidative Stress

The method of Misra and Fridovich [27] was employed to determine the activity of superoxide dismutase (SOD) while catalase (CAT) activity was ascertained by the protocol of Habig [28]. Glutathione-S-transferase (GST) activity was determined utilizing the procedural steps of Habig and Jakoby [28]. Reduced glutathione (GSH) level was achieved adopting the protocol proscribed by Jollow [29]. The total protein concentration was evaluated using the procedural steps of Lowry et al. [30] but with minor adjustments.

TNF-α and IL-6 levels were quantified by ELISA kits. MPO activity in the tissues was determined by adopting the method of the method described by Granell et al. [31].

2.8 Histopathological Study for the Ovaries and Uterus

Uterine and ovarian tissues embedded in Bouin's solution were sectioned to thickness of 5 µm, deparaffinized, and stained with Haematoxylin and Eosin. Microanatomy of the tissues was examined using a light microscope (Olympus CH; Olympus, Tokyo, Japan). Photomicrographs were taken with a Samsung HMX-F90 Camcorder (Samsung, Vietnam) by expert pathologists who were dimmed to control and the respective treatment groups.

3 Statistical Analysis of Data

Results were expressed as Mean ± SEM. Significant changes among the respective treatment groups were ascertained with the use of One-way Analysis of variance (ANOVA), complemented with the Dunett's *posthoc* run. By using a graphPad Prism5.0, variations/differences were considered significant amongst entities with $p < 0.05$ of all the treatment groupings.

4 Results

4.1 *Brillantaisia patula* Leaf Extract Augmented the Circulating Hormonal Levels in Cyclophosphamide-treated Rats

Figure 1 shows the effect of the leaf extract of *Brillantaisia patula* on circulating hormones of female Wistar rats exposed to cyclophosphamide. By comparison to the control, cyclophosphamide significantly ($p < 0.05$) increased the blood concentrations of luteinizing hormone (Figure 1A), follicle stimulating hormone (Figure 1B), and prolactin (Figure 1E) but significantly ($p < 0.05$) decreased the concentration of progesterone (Figure 1C) and estrogen (Figure 1D). However, in the rats co-administered with cyclophosphamide and leaf extract of *Brillantaisia patula* (18 $mgkg^{-1}$, 36 $mgkg^{-1}$ and 72 $mgkg^{-1}$ body weight), there were significant decrease in the concentration of luteinizing hormone (Figure 1A), follicle stimulating hormone (Figure 1B), and prolactin (Figure 1E) but a significant ($p < 0.05$) increase in the concentration of progesterone (Figure 1C) and estrogen (Figure 1D) when compared with cyclophosphamide only treated rats ($p<0.05$). The same decrease trend ($p<0.05$) was demonstrated in rats co-administered with cyclophosphamide and vitamin C when compared with cyclophosphamide only treated rats.

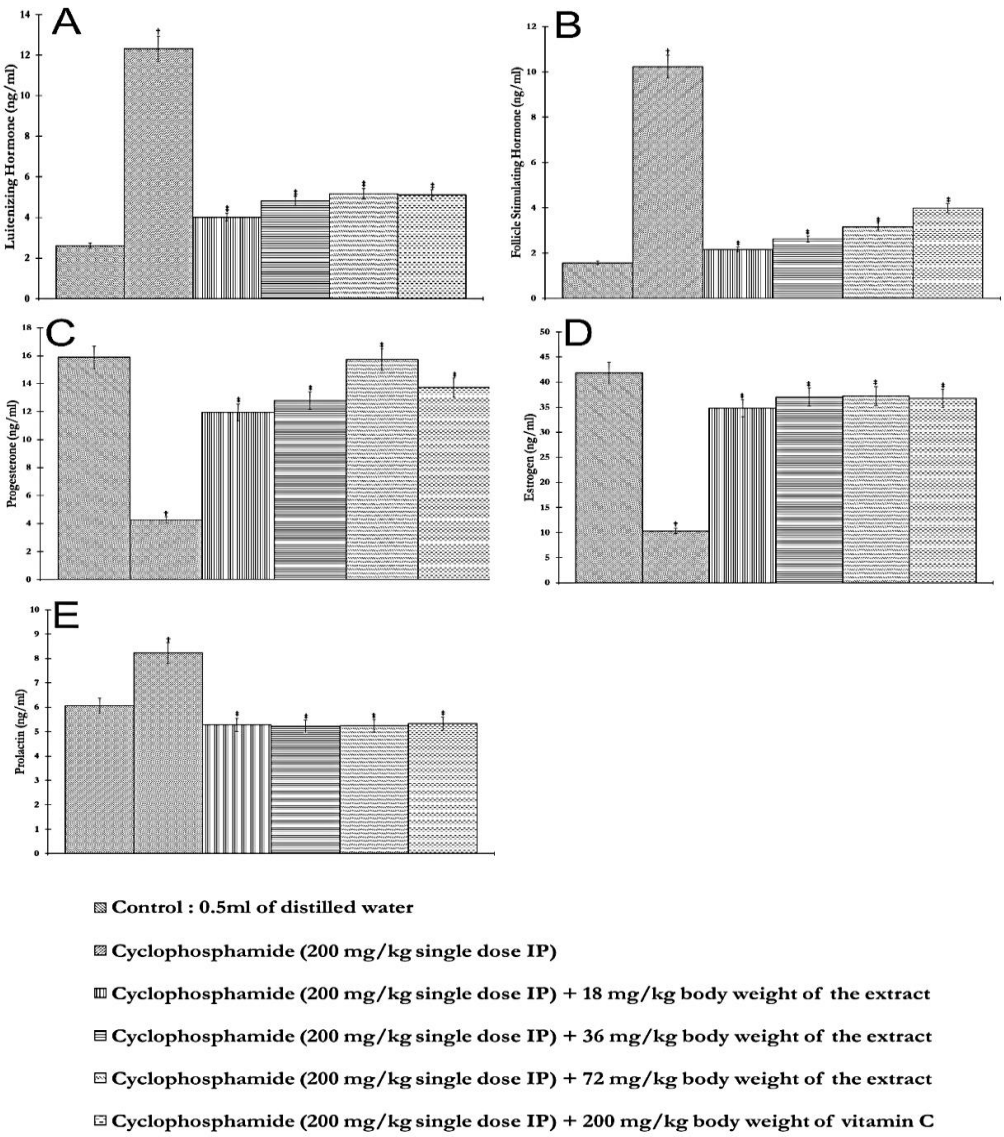

Figure 1: Impacts of leaf extract of *Brillantaisia patula* on circulating hormonal levels in cyclophosphamide-treated rats. **(A)** Luteinizing hormone, **(B)** Follicle Stimulating Hormone, **(C)** Progesterone, **(D)** Estrogen, and **(E)** Prolactin levels of rats after treatment with single dose of cyclophosphamide (200 mg/kg body weight) alone or co-administered with leaf extract of *Brillantaisia patula* (18, 36 and 72 mg/kg body weight) or Vitamin C (200 mg/kg body weight) for 21 days. Data are presented as Mean ± SEM of 6 rats per group. Values that differ significantly from the control at $p < 0.05$ are indicated by†; and from cyclophosphamide treated group by‡.

4.2 *Brillantaisia patula* Leaf Extract Preserved Uterine and Ovarian Parameters of Cyclophosphamide-treated Rats

The effects leaf extract of *Brillantaisia patula* elicited on the uterine and ovarian activities of ALP, ACP, 3-β-HSD, and 17-β-HSD, as well as the concentrations of glucose and cholesterol of rats exposed to cyclophosphamide is shown in Figure 2.

Administration of cyclophosphamide alone caused a significant ($p<0.05$) decrease in the uterine and ovarian activities of ALP (Figure 2A), ACP (Figure 2B), 3-β-HSD (Figure 2E), and 17-β-HSD (Figure 2F), and in the levels of cholesterol (Figure 2C), and glucose (Figure 2D) when compared with the control rats. Contrastingly, co-administration of leaf extract of *Brillantaisia patula* (18 $mgkg^{-1}$, 36 $mgkg^{-1}$ and 72 $mgkg^{-1}$ body

weight) with cyclophosphamide significantly abrogated the cyclophosphamide-mediated alteration in activities/levels of ALP (Figure 2A), ACP (Figure 2B), cholesterol (Figure 2C), glucose (Figure 2D), 3-β-HSD (Figure 2E), and 17-β-HSD (Figure 2F), when compared with the cyclophosphamide alone treated rats (p<0.05). In addition, co-administration of cyclophosphamide and vitamin C had similar treatment-related effect when compared with leaf extract of *Brillantaisia patula* and cyclophosphamide co-treated group.

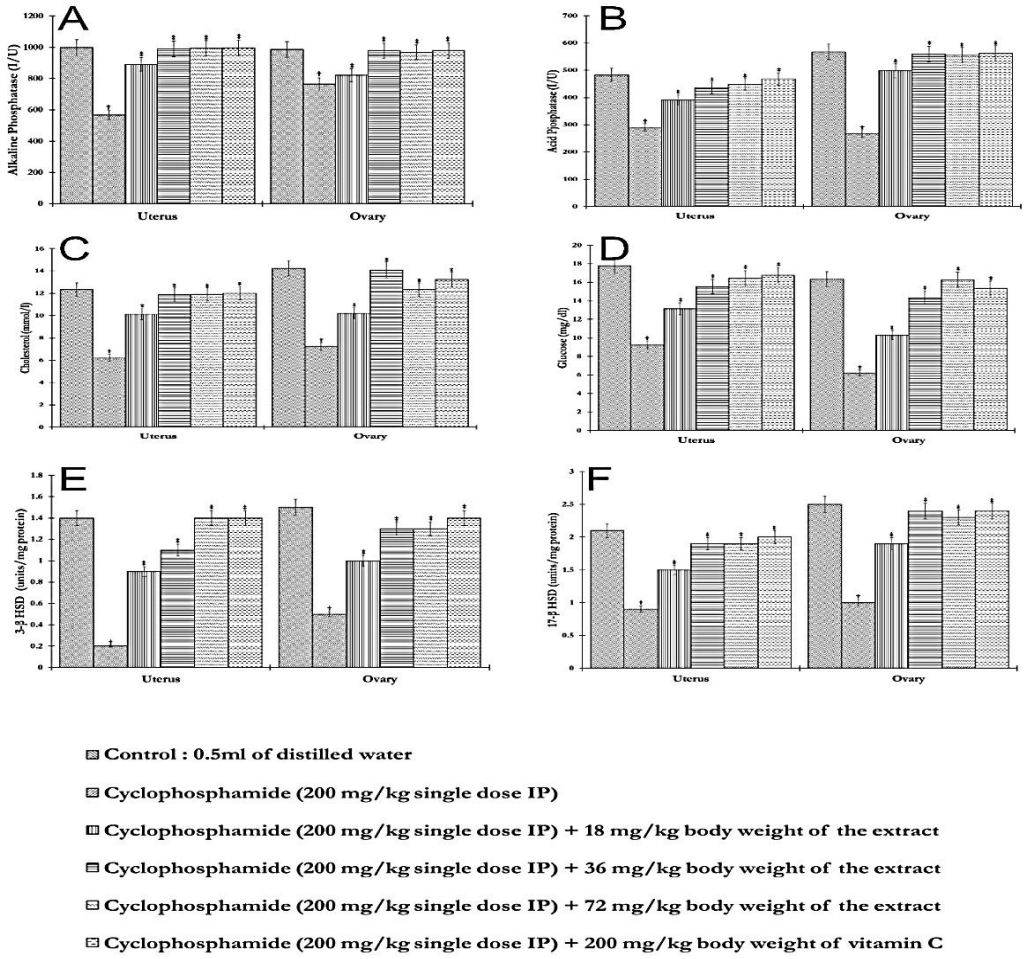

Figure 2: Impacts of leaf extract of *Brillantaisia patula* on uterine and ovarian function indices of cyclophosphamide-treated rats. **(A)** Alkaline phosphatase activity, **(B)** Acid phosphatase activity **(C)** Cholesterol level, **(D)** Glucose level, **(E)** 3-β Hydroxysteroid dehydrogenase activity, and **(F)** 3-β Hydroxysteroid dehydrogenase activity of rats after treatment with single dose of cyclophosphamide (200 mg/kg body weight) alone or co-administered with leaf extract of *Brillantaisia patula* (18, 36 and 72 mg/kg body weight) or Vitamin C (200 mg/kg body weight) for 21 days. Data are presented as Mean ± SEM of 6 rats per group. Values that differ significantly from the control at $p < 0.05$ are indicated by†; and from cyclophosphamide treated group by‡.

4.3 *Brillantaisia patula* Leaf Extract Abrogated the Extent of Oxidative Damages and Inflammation in the Uterus and Ovary of Cyclophosphamide-treated Rats

The impact of leaf extract of *Brillantaisia patula* on some antioxidant and inflammation biomarkers in the uterus and ovary of rats exposed to cyclophosphamide is depicted in Figure 3. Relative to the control group, there were significant (p<0.05) reduction in the uterine and ovarian activities/level of SOD (Figure 3A), catalase (Figure 3B), GSH (Figure 3C), and GST (Figure 3D) but with a corresponding increase in the levels of TNF-α (Figure 3E) and IL-6 (Figure

3F), as well as activity of MPO (Figure 3G) in the uterine and ovarian tissue of the animals treated with cyclophosphamide alone. Contrastingly, co-administration of cyclophosphamide with leaf extract of *Brillantaisia patula* (18 $mgkg^{-1}$, 36 $mgkg^{-1}$ and 72 $mgkg^{-1}$ body weight) or co-administration of cyclophosphamide and vitamin C significantly elevated the uterine and ovarian activities/level of SOD, catalase, GST, GSH; but correspondingly reduced the levels/activity of TNF-α, IL-6 (Figure 3F), and MPO when compared with cyclophosphamide only treated rats ($p<0.05$).

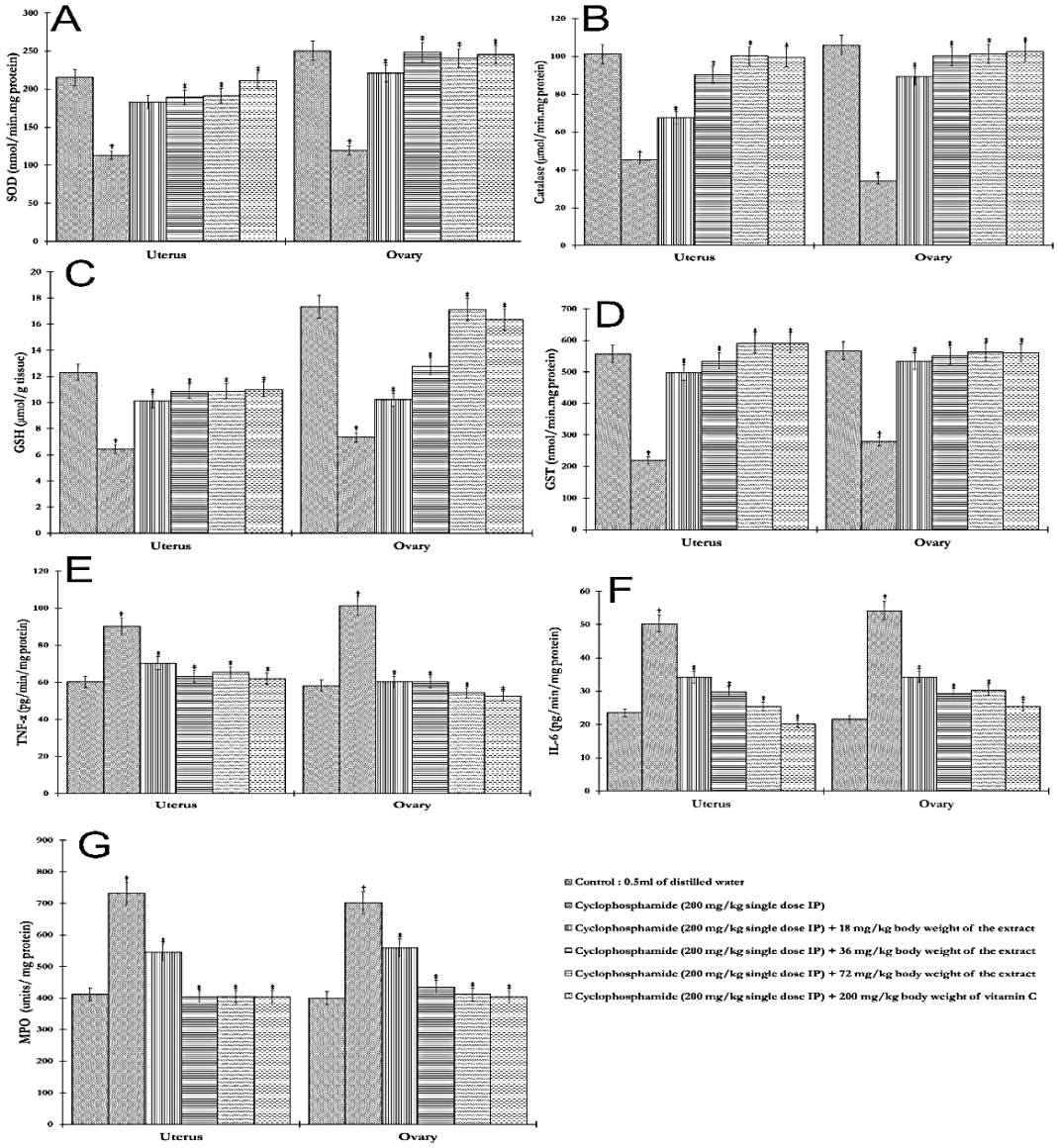

Figure 3: Impacts of leaf extract of *Brillantaisia patula* on oxidative damages and inflammation in the uterus and ovary of cyclophosphamide-treated rats. **(A)** Superoxide dismutase activity, **(B)** Catalase activity **(C)** Reduced glutathione level, **(D)** Glutathione S-transferase activity, **(E)** Tumour necrosis factor-α level, **(F)** Interleukin-6 level, and **(G)** Myeloperoxidase activity of rats after treatment with single dose of cyclophosphamide (200 mg/kg body weight) alone or co-administered with leaf extract of *Brillantaisia patula* (18, 36 and 72 mg/kg body weight) or Vitamin C (200 mg/kg body weight) for 21 days. Data are presented as Mean ± SEM of 6 rats per group. Values that differ significantly from the control at $p < 0.05$ are indicated by†; and from cyclophosphamide treated group by‡.

4.4 Effect of Leaf Extract of *B. patula* on Uterine and Ovarian Architecture of Rats exposed to Cyclophosphamide

The photomicrographs (x400; H & E) to demonstrate the impact of leaf extract of *Brillantaisia patula* on the uterus and ovary of female Wistar rats exposed to cyclophosphamide is given in Figures 4 and 5. The uterine histoarchitecture of the control animals treated distilled water were in normal proliferative phase (Figure 4A). Animals exposed to cyclophosphamide 200 $mgkg^{-1}$ body weight demonstrated altered architecture with short cuboidal lining the epithelium (Figure 4B). In addition, animals exposed to cyclophosphamide and the extract (at 18 $mgkg^{-1}$, 36 $mgkg^{-1}$ and 72 $mgkg^{-1}$ body weight) or vitamin C demonstrated normal architecture and normal epithelium lining (Figure 4C- F).

Furthermore, the control animals that received distilled only demonstrated developing follicles devoid of recent gestation in the ovary (Figures 5A) while the ovary of the rats exposed to cyclophosphamide 200 $mgkg^{-1}$ body weight demonstrated altered architecture with short cuboidal lining the epithelium (Figures 5B). In addition, animals exposed to cyclophosphamide and the extract (at 18 $mgkg^{-1}$, 36 $mgkg^{-1}$ and 72 $mgkg^{-1}$ body weight) or vitamin C demonstrated normal to moderate architecture with developing ovarian follicle ((Figure 5C- F).

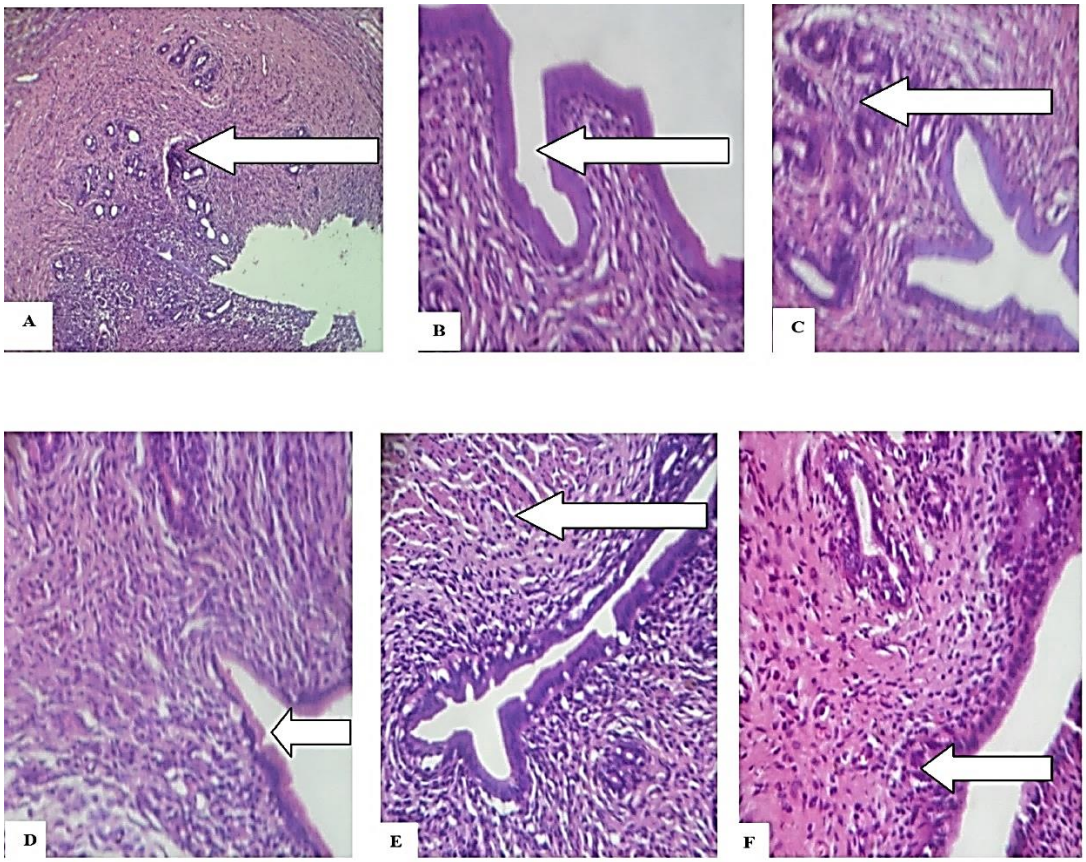

Figure 4: Histopathological slides of the effect of leaf extract of *B. patula* on uterine architecture of rats exposed to cyclophosphamide. **A:** Control animals treated with 0.5 mL of distilled water demonstrated normal architecture, endometerium in proliferative phase, and normal epithelium lining; **B:** Animals exposed to cyclophosphamide 200 $mgkg^{-1}$ body weight demonstrated altered architecture with short cuboidal lining the epithelium; **C:** Animals exposed to cyclophosphamide and treated with 18 $mgkg^{-1}$ body weight of the leaf extract with normal architecture and improved epithelium lining; **D:** Animals exposed to cyclophosphamide and treated with 36 $mgkg^{-1}$ body weight of the leaf extract showed normal architecture with normal lining of the epithelium; **E:** Animals exposed to cyclophosphamide and treated with 72 $mgkg^{-1}$ body weight of the leaf extract demonstrated normal architecture and normal epithelium lining. **F:** Animals exposed to cyclophosphamide and treated with 200 $mgkg^{-1}$ body weight of vitamin C demonstrated normal architecture and normal epithelium lining.

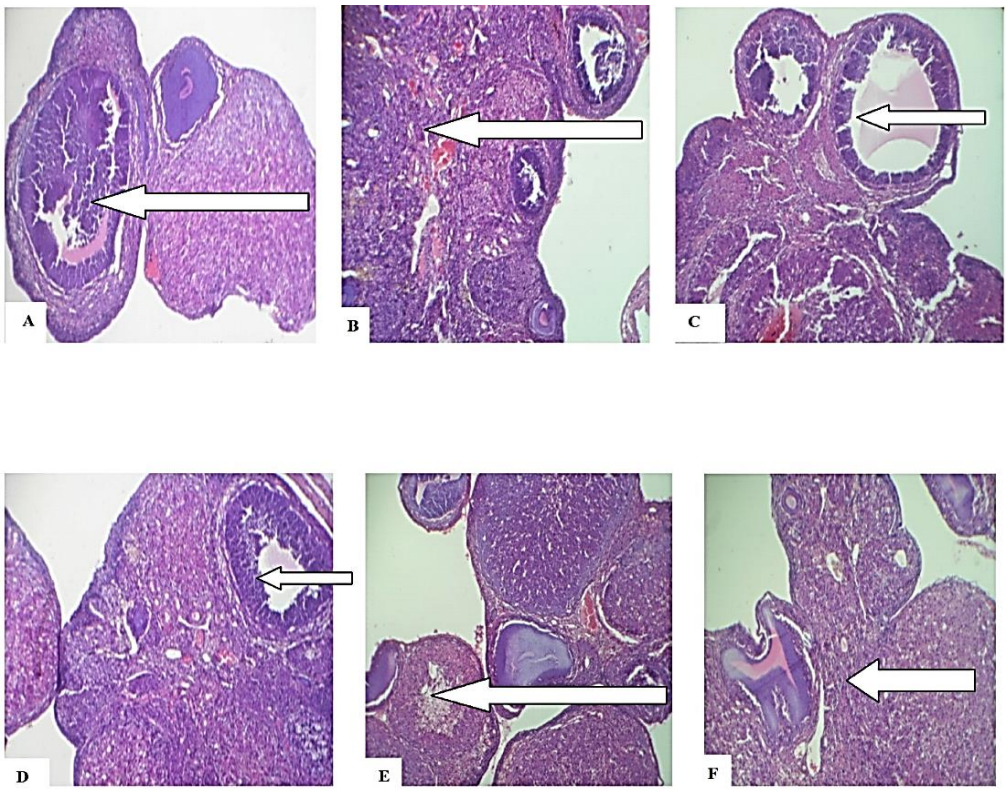

Figure 5: Histopathological slides of the effect of leaf extract of *B. patula* on ovarian architecture of rats exposed to cyclophosphamide. **A:** Control animals treated with 0.5 mL of distilled water demonstrated normal histoarchitecture characterized by the ovarian cortex with developing follicles devoid of recent gestation; **B:** Animals exposed to cyclophosphamide 200 $mgkg^{-1}$ body weight demonstrated poor architecture, no ovarian developing; **C:** Animals exposed to cyclophosphamide and treated with 18 $mgkg^{-1}$ body weight of the leaf extract showed moderate histoarchitecture with reduced ovarian developing follicles; **D:** Animals exposed to cyclophosphamide and treated with 36 $mgkg^{-1}$ body weight of the leaf extract with preserved histoarchitecture with developing Corpus luteum; **E:** Animals exposed to cyclophosphamide and treated with 72 $mgkg^{-1}$ body weight of the leaf extract demonstrated normal architecture with developing ovarian follicle; **F:** Animals exposed to cyclophosphamide and treated with 200 $mgkg^{-1}$ body weight of vitamin C demonstrated ovarian cortex showing developing follicles.

4.4 Secondary Metabolites in the Leaf Extract of *Brillantaisia patula*

Secondary metabolites present in *B. patula* leaves in different concentrations are saponin, flavonoids, phenols, tannins, and alkaloids (Table 1).

Table 1: Secondary Metabolites in the Extract of *Brillantaisia patula* Leaves

Secondary metabolites	Concentration (mg/ml)
Saponins	20.30 ± 0.01
Flavonoids	11.30 ± 0.00
Phenols	0.80 ± 0.02
Tannins	1.40 ± 0.03
Alkaloids	7.20 ± 0.01
Antraquinones	Not detected
Cardenolides and dienolides	Not detected
Steroids	Not detected
Cardiac glycoside	Not detected
Terpenes	Not detected

5 Discussion

In the female, herbal agents have effects on the molecular mechanism with resultant action in the prevention of estrogen-dependent endometrial hyperplasia favouring ovarian dysfunction, ovarian follicle and increased endometrial receptivity [32]. In addition, herbal drug with potent activity on the hypothalamic–pituitary–gonadal axis may influence reproductive physiology and serve as remedy to certain infertility problems that may arise from cancer therapy or other means. Several plants extract have gonadotrophic-like effects by being able to increase the weight of the ovary and uterus, induce ovulation, increase level of estradiol, progesterone or protein, and as well decrease the level of cholesterol [32].

The effect of medicinal plant commonly consumed for their therapeutic roles in mitigating the side effect of cancer therapy and indirectly in aiding fertility or conception can be examined on related functional test in the organs of the body to ascertain their efficacy. The ovary and uterus were assessed in the present study to ascertain the functional capability of these organs following treatment with the aqueous leaf extract of *B. patula* in cyclophosphamide-induced gonadal toxicity model of female Wistar rats.

Cyclophosphamide is among the popularly used alkylating agents. Because follicles of the ovaries are extremely sensitive to their effects, cyclophosphamide causes premature ovarian insufficiency and infertility. Little information is available about the mechanism of cyclophosphamide-induced ovarian damage, but its toxicity is associated with oxidative stress, inflammation, and apoptosis. The use of compounds with antioxidant and cytoprotective properties to protect ovarian function from deleterious effects during chemotherapy would be a significant advantage [33]. The uterus and ovary are unique organ of reproduction in female. Therefore, parameters assayed on this organ may be more of accessing the mitigating capability of the plant extract against cancer drugs like cyclophosphamide.

In the present study, the treatment of rats with cyclophosphamide increased circulatory levels of LH, FSH, and prolactin while it reduced the progesterone and estrogen level. Follicle stimulating hormone, luteinizing hormone, estrogen, progesterone, and prolactin are key hormones of mammalian reproduction [34], and their measurement can be used for the assessment of gonadal function. There is normally a physiological threshold point for these hormones for proper expression of their reproductive roles in females. At normal physiological concentrations, progesterone induces synthesis of egg white proteins, estrogen induces creatine kinase [35]. High concentrations of these hormones causes adverse effects of profiling the uterus cells and embryonic defects [36], autoimmune disease [37], morphogenetic alteration of uterine tissues [38]. Therefore, the increase in the concentrations of prolactin, FSH and LH by cyclophosphamide as well as the decrease in progesterone and estrogen demonstrated adverse effects to the uterus and the ovaries. The ability of the leaf extract of *B. patula* to reverse these hormones give more credence to the ability to reverse the adverse effect on the gonads.

In addition, the cyclophosphamide-induced increases in concentration of TNF-α, IL-6 and activity of MPO in the uterine and ovarain tissues were prevented in the animals co-treated with leaf extract of *B. patula*. MPO acts as a peroxidase when it converts chloride ions and hydrogen peroxide to hypochlorous acid, thereby taking an active part in cellular defense. Cyclophosphamide-induced increase in TNF-α and IL-6 levels showed that the expression of pro-inflammatory mediators in the rats [39]. The cyclophosphamide-induced elevation in the activity of MPO in the uterus and ovary of the experimental animals suggests that the accumulation of hydrogen peroxide (most measured ROS due to its stability when compared with superoxide and hydroxyl radicals) due to cyclophosphamide intoxication in the reproductive tissues of rats [40]. This suggests a harmful effect to the uterus and ovary because cyclophosphamide readily crosses their membranes to induce toxicity by raising the level of hydrogen peroxide. Interestingly, leaf extract of *B. patula* was able to reverse MPO activity and concentrations of TNF-α and IL-6 in these organs when co-administered with cyclophosphamide, which suggest this extract as a possible anti-inflammatory agent against diseases related to the uterus or ovary that are instigated through inflammation.

Also in the present study, cyclophosphamide decreased the activities of SOD, catalase, and GST as well as the GSH level in the ovary and uterus of rats. Excessive free radicals generation give room for oxidative stress which in turn reduce the rate of antioxidant enzymes like SOD, GST, and catalase as well as non-enzymatic antioxidants like GSH, vitamins C and E [41,42]. These antioxidants normally should help to suppress the impact of ROS/NOS in the reproductive tract thereby preserving the biochemical and physiological roles in fertilization. The fall in GST activity can be attributed to the inhibition of the enzyme by cyclophosphamide metabolites. This is in tandem with diminution in GSH diminution in the level of GSH in this study. This is because GSH (a non-enzymatic antioxidant and a key cofactor for

antioxidant enzymes) help in the conjugation of electrophilic metabolites of cyclophosphamide [43]. Thus, the cyclophosphamide-induced decrease in the level of GSH suggests oxidative stress in uterus and ovary of the experimental rats. The ability of the leaf extract of *B. patula* to normalize cyclophosphamide-induced inhibition of SOD, catalase, GST and, GSH in the ovary and uterus of rats further confirms its potential in reversing gonadal toxicity mediated by oxidative stress or free radicals.

Certain enzymes and metabolites play active role in stepwise process or entire process of conception in females. For instance, acid phosphatase is a lysosomal enzyme which contributes to various ovarian metabolic functions while alkaline phosphatase is a sialoglycoprotein and may play certain role in the attachment of blastocyst to the endometrium. Glucose is involved in providing energy for ovulation and survival of eggs, cholesterol derived from various sources is a precursor for steroidogenesis of ovarian endocrine tissues and are required for ovulation and folliculogenesis [44]. 3β- and 17-β-hydroxysteroid are needed in the pathway of cholesterol metabolism. They are essential in steroidogenesis and steroid degradation, making them key androgenic enzyme. Cholesterol is therefore a precursor in steroid hormone synthesis and its requirement for steroidogenesis has been well established which signifies its importance in fertility [45]. Steroidogenic cells make use of cholesterol as precursor to synthesize steroid hormones. Recent indications suggest that a disorder of cholesterol homeostasis contributes to the development of a chronic inflammatory condition [46]. The cyclophosphamide-induced reduction in uterine glucose, glycogen content, ALP, and ACP in this study may be an indicate obstruction to various processes like ovulation, transportation and survival of eggs and implantation. Such decrease in ovarian activity of ACP suggests that physiological role (oocyte maturation, mitotic divisions, germinal vesicle breakdown and ovulation) of the ovaries were being compromised. In the same way, the decrease in the ovarian and uterine content of cholesterol may suggest that inhibition of steroidogenesis in ovarian endocrine tissue because of inadequate cholesterol as a precursor [47]. This can further be corroborated by the decrease in the activities Δ5, 3β-HSD and 17β-HSD. However, the reversal in the level of these biomolecules by leaf extract of *B. patula* against the action of cyclophosphamide lend credence the traditional against toxicants.

Moreover, the secondary metabolites present in the leaf extract of *B. patula* are most likely to be responsible for the overall effects of functional capability on organs. Therefore, the saponins, flavonoids, phenols, tannins, and alkaloids constituent of *B. patula* might have acted individually or synergistically to elicit the various biological activities in this study by suppressing inflammation and boosting the antioxidant system.

Some drugs and chemicals adversely affect the uterus and related structures while some secondary metabolites in plants may contain toxic bioactive agents of plant extracts that can affect the regulation reproductive hormones. Histopathological studies are often done to determine possible morphological changes of tissues due to derangement, damage, or toxicity. The results obtained from enzymic and non-enzymic parameters of toxicity could be corroborated by the results obtained from histological studies. The uterus of the animals demonstrated normal architecture and normal epithelium lining which signifies a non-deleterious effect of the extract to the womb. The histological examination in the ovaries of the rats at different dosage of the extract also revealed a preserved ovarian tissue architecture.

Conclusion

Overall, the leaf extract of *Brillantaisia patula* reversed the cyclophosphamide-induced oxidative impairment and inflammation in the uterus and ovary of the experimental animals and offered protection to these tissues as an antioxidant, anti-inflammatory and antiapoptotic agent that can be explored as a remedy against sterility conditions. This will be of significance for policy maker, fertility experts, and gynaecologist and provide the heads up for cancer patients with fertility-related issues.

Conflict of Interest

The author declares no conflict of interest.

References

[1] Okaiyeto K, Oguntibeju OO. African herbal medicines: Adverse effects and cytotoxic potentials with different therapeutic applications. International Journal of Environmental Research and Public Health 2021; 18.

[2] Ogunro OB, Oyeyinka BO, Gyebi GA, Batiha GE-S. Nutritional benefits, ethnomedicinal uses, phytochemistry, pharmacological properties and toxicity of Spondias mombin Linn: a comprehensive review. The Journal of Pharmacy and Pharmacology 2023.

[3] Ofosu-Budu D, Hänninen V. Herbalists' explanations of infertility: The case of Northern and Southern Ghana. African Journal of Reproductive Health 2022; 26:96–106.

[4] Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Frontiers in Neurology 2014; 4 JAN:1–10.

[5] Lunenfeld B, Bilger W, Longobardi S, Alam V, D'Hooghe T, Sunkara SK. The development of gonadotropins for clinical use in the treatment of infertility. Frontiers in Endocrinology 2019; 10.

[6] Akinsola AF, Olatunde OC, Osasona I, Sekayo OF, Omotayo FO. Nutritional Evaluation of Brillantaisia patula Leaves. Asian Plant Research Journal 2021; 8:63–73.

[7] F. Faparusi, Bello-Akinosho MM, Oyede RT, Adewole A, Bankole PO, Ali FF. Research Journal of. Research Journal of Phytochemistry 2012; 6:9–12.

[8] Moronkola OD, Zaki FU, Adeleke O. Esse ntial oils of stem and leaf from nigerian brillantaisia patula t. and. var. Journal of Essential Oil-Bearing Plants 2009; 12:569–573.

[9] Makambila-Koubemba M-C, Mbatchi B, Ardid D, Gelot A, Henrion C, Janisson R, Abena AA, Banzouzi J-T. Pharmacological studies of ten medicinal plants used for analgesic purposes in congo Brazzaville. International Journal of Pharmacology 2011; 7:608–615.

[10] Mbatchi SF, Mbatchi B, Banzouzi JT, Bansimba T, Nsonde Ntandou GF, Ouamba JM, Berry A, Benoit-Vical F. In vitro antiplasmodial activity of 18 plants used in Congo Brazzaville traditional medicine. Journal of Ethnopharmacology 2006; 104:168–174.

[11] Raeeszadeh M, Saleh Hosseini SM, Amiri AA. Impact of Co-Administration of N-Acetylcysteine and Vitamin e on Cyclophosphamide-Induced Ovarian Toxicity in Female Rats. Journal of Toxicology 2022; 2022.

[12] Harborne JB. Phytochemical Methods: A guide to modern techniques of plant analysis. vol. 15. 2nd ed. London: Chapman & Hall; 1984.

[13] Martin RA, Lynch SP, Schmitz FJ, Pordesimo EO, Toth S, Horton RY. CARDENOLIDES FROM ASCLEPZAS ASPERULA SUBSP. CAPRZCORNU AND A. VZRZDZS. Phytochemistry 1991; 30:3935–3939.

[14] Solich P, Sedliaková V, Karlíček R. Spectrophotometric determination of cardiac glycosides by flow-injection analysis. Analytica Chimica Acta 1992; 269:199–203.

[15] Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology 2005; 4:685–688.

[16] Dinan L, Harmatha J, Lafont R. Chromatographic procedures for the isolation of plant steroids. Journal of Chromatography A 2001; 935:105–123.

[17] Hossain MA, Shah MD, Gnanaraj C, Iqbal M. In vitro total phenolics, flavonoids contents and antioxidant activity of essential oil, various organic extracts from the leaves of tropical medicinal plant Tetrastigma from Sabah. Asian Pacific Journal of Tropical Medicine 2011; 4:717–721.

[18] Wall ME, Krider MM, Krewson CF, Eddy CR, Willaman JJ, Corell DS, Gentry HS. Steroidal sapogenins. VII. Survey of plants for steroidal sapogenins and other constituents. Journal of the American Pharmaceutical Association American Pharmaceutical Association 1954; 43:1–7.

[19] Ben Ammar R, Miyamoto T, Chekir-Ghedira L, Ghedira K, Lacaille-Dubois MA. Isolation and identification of new anthraquinones from Rhamnus alaternus L and evaluation of their free radical scavenging activity. Natural Product Research 2019; 33:280–286.

[20] Tadesse G, Reneela P, Dekebo A. Isolation and characterization of natural products from Helinus mystachnus (Rhamnaceae). Journal of Chemical and Pharmaceutical Research 2012; 4:1756–1762.

[21] Tietz NW. Clinical Guide to Laboratory Tests. 3rd Editio. Philadelphia: 1995.

[22] Revathi P, Vani B, Sarathchandiran I, Kadalmani B, Shyam KP, Palnivel K. Reproductive toxicity of Capparis aphylla (Roth.) in male albino rats. International Journal of Pharmaceutical and Biomedical Research 2010; 1:102–112.

[23] Lopez J. Carl A. Burtis, Edward R. Ashwood and David E. Bruns (eds): Tietz Textbook of Clinical Chemistry and Molecular Diagnosis (5th edition). Indian Journal of Clinical Biochemistry 2013; 28:104–105.

[24] de Araújo VGB, de Oliveira RS, Gameleira KPD, Cruz CB, Lofrano-Porto A. Deficiência de 3β-hidroxiesteroide desidrogenase tipo 2 em teste de triagem neonatal. Arquivos Brasileiros de Endocrinologia e Metabologia 2014; 58:650–655.

[25] Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. The Analyst 1972; 97:142–145.

[26] Corso G, Papagni F, Gelzo M, Gallo M, Barone R, Graf M, Scarpato N, Dello Russo A. Development and Validation of an Enzymatic Method for Total Cholesterol Analysis Using Whole Blood Spot. Journal of Clinical Laboratory Analysis 2016; 30:517–523.

[27] Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry 1972; 247:3170–3175.

[28] Habig WH, Jakoby WB. Assays for Differentiation of Glutathione S-Transferases. Methods in Enzymology 1981; 77:398–405.

[29] Jollow DJ, Mitchell JR, Jampaglione N, Gillette JR. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology 1974; 11:151–169.

[30] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. The Journal of Biological Chemistry 1951; 193:265–275.

[31] Granell S, Gironella M, Bulbena O, Panés J, Mauri M, Sabater L, Aparisi L, Gelpi E, Closa D. Heparin mobilizes xanthine oxidase and induces lung inflammation in acute pancreatitis. Critical Care Medicine 2003; 31:525–530.

[32] Noh S, Go A, Kim D Bin, Park M, Jeon HW, Kim B. Role of antioxidant natural products in management of infertility: A review of their medicinal potential. Antioxidants 2020; 9:1–65.

[33] Spears N, Lopes F, Stefansdottir A, Rossi V, De Felici M, Anderson RA, Klinger FG. Ovarian damage from chemotherapy and current approaches to its protection. Human Reproduction Update 2019; 25:673–693.

[34] Okafor IA, Nnamah US, Nnaka J. The fertility assessment of normal cyclic Wistar rats following the administration of methanolic extract of Portulaca oleracea: an experimental study. Middle East Fertility Society Journal 2021; 26.

[35] Wohlgemuth KJ, Arieta LR, Brewer GJ, Hoselton AL, Gould LM, Smith-Ryan AE. Sex differences and considerations for female specific nutritional strategies: a narrative review. Journal of the International Society of Sports Nutrition 2021; 18.

[36] Kumar P, Magon N. Hormones in pregnancy. Nigerian Medical Journal 2012; 53:179.

[37] Desai MK, Brinton RD. Autoimmune disease in women: Endocrine transition and risk across the lifespan. Frontiers in Endocrinology 2019; 10.

[38] Vannuccini S, Clifton VL, Fraser IS, Taylor HS, Critchley H, Giudice LC, Petraglia F. Infertility and reproductive disorders: Impact of hormonal and inflammatory mechanisms on pregnancy outcome. Human Reproduction Update 2016; 22:104–115.

[39] Anatoliotakis N, Deftereos S, Bouras G, Giannopoulos G, Tsounis D, Angelidis C, Kaoukis A, Stefanadis C. Send Orders of Reprints at reprints@benthamscience.net Myeloperoxidase: Expressing Inflammation and Oxidative Stress in Cardiovascular Disease. Current Topics in Medicinal Chemistry 2013; 13:115–138.

[40] Omole JG, Ayoka OA, Alabi QK, Adefisayo MA, Asafa MA, Olubunmi BO, Fadeyi BA. Protective Effect of Kolaviron on Cyclophosphamide-Induced Cardiac Toxicity in Rats. Journal of Evidence-Based Integrative Medicine 2018; 23:1–11.

[41] Walczak-Jedrzejowska R, Wolski JK, Slowikowska-Hilczer J. The role of oxidative stress and antioxidants in male fertility. Central European Journal of Urology 2013; 66:60–67.

[42] Kurkowska W, Bogacz A, Janiszewska M, Gabryś E, Tiszler M, Bellanti F, Kasperczyk S, Machoń-Grecka A, Dobrakowski M, Kasperczyk A. Oxidative Stress is Associated with Reduced Sperm Motility in Normal Semen. American Journal of Men's Health 2020; 14.

[43] Cengiz M, Ayhancı A, Heybeli N, Kulcanay Sahin İ. Myelosuppression and Oxidative stress Induced by Cyclophosphamide in Rats: The Protective Role of Selenium. Adıyaman University Journal of Science 2019; 9.

[44] Acconcia F, Marino M. Steroid Hormones: Synthesis, Secretion, and Transport. 2018.

[45] Turcu AF, Auchus RJ. Adrenal Steroidogenesis and Congenital Adrenal Hyperplasia. Endocrinology and Metabolism Clinics of North America 2015; 44:275–296.

[46] Hu J, Zhang Z, Shen WJ, Azhar S. Cellular cholesterol delivery, intracellular processing and utilization for biosynthesis of steroid hormones. Nutrition and Metabolism 2010; 7:7–9.

[47] Da Broi MG, Giorgi VSI, Wang F, Keefe DL, Albertini D, Navarro PA. Influence of follicular fluid and cumulus cells on oocyte quality: Clinical implications. Journal of Assisted Reproduction and Genetics 2018; 35:735–751.