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Original Research Article

Structural and functional response of the genital tract to paraquat (PQ) dichloride exposure in female Wistar rats

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Abstract

Purpose: *To investigate the structural and functional response of genital tract to paraquat (PQ) exposure in female Wistar rats.*
Methods: *A total of 24 healthy female Wistar rats weighing between 150 - 180 g were grouped randomly into four equal groups. Group 1 served as control (received normal saline) while Groups 2, 3 and 4 were referred to as study groups and received 1, 5, and 10 mg/kg/bw PQ, respectively, once daily for 42 days. Blood samples (5 mL) were collected by cardiac puncture and centrifuged. Serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), estrogen, and progesterone were quantified. The animals were sacrificed by cervical dislocation and the ovary and uterus were excised and processed using H & E staining.*
Results: *Paraquat (PQ) disrupted the histoarchitecture of the ovary and uterus by inducing ovarian atrophy and chronic endometritis. Furthermore, PQ significantly increases serum levels of LH, FSH, estrogen, and progesterone compared to control ($p < 0.05$).*
Conclusion: *Paraquat (PQ) induces ovarian atrophy, chronic endometriosis, and increases levels of LH, FSH, estrogen, and progesterone. Further large-scale studies in higher animals may be needed to validate these findings and provide a regulatory framework for PQ administration.*

Keywords: *Paraquat, Genital tract, Histochemistry, Histoarchitecture*

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INTRODUCTION

Paraquat (PQ) is an herbicide used in weed control for agricultural and non-agricultural purposes. Due to its uniqueness and importance as an herbicide in crop production, it is produced and used worldwide [1]. It has been documented that PQ is highly toxic in humans and animals and may result in severe multiple pathologies such as neurotoxicity, mitochondrial disintegration, and altered DNA methylation in human neural stem cells [2].

Like most herbicides, Paraquat induces toxicity by undergoing redox-cycling *in vivo,* generating reactive oxygen species (ROS), with the tendency to damage internal organs [3]. These ROS, such as free radicals like hydrogen peroxide and superoxide anions, are highly toxic [4]. In a previous study, short-term exposure of PQ initiated retardation of leydig cells, thereby decreasing testosterone production and inhibiting spermatogenesis [5]. Studies on female reproduction showed that PQ causes failure of *in*

vitro fertilization (IVF), degeneration of follicles, and makes the corpus luteum occupy most of the parenchyma cells. Reports from a recent study revealed that exposure to PQ resulted in significant changes in the estrous cycle, hormonal levels, and several developing ovarian follicles in animal models [6].

Despite several studies on the effect of PQ on different organs, none have detailed the anatomic and functional outcome on the ovary and uterus. Furthermore, studies of varying doses of PQ on the ovaries and uterus alongside the hormonal assay patterns are scant and uncommon. Therefore, this study investigated the structural and functional response of the genital tract to paraquat (PQ) dichloride exposure in female Wistar rats.

EXPERIMENTAL

Materials

Paraquat dichloride (1, 1'-dimethyl-4, 4' bipyridinium, trade name: Paraforce, batch number: 2018022501, NAFDAC registration number: A5-0109, production date: 2018-02-25 and expiring date: 2021-02-25 (Jubaili Agrotec, Nigeria). A litre of the solution was properly sealed in an airtight, opaque plastic container and kept at room temperature for further use.

Animals

A total of 24 healthy adult female Wistar rats weighing between 150 - 180 g were obtained from the animal house unit of the College of Health Science at Delta State University of Abraka. Delta State, Nigeria. The rats were housed in clean iron cages and allowed to acclimatize for two weeks with unrestricted access to feed and clean water *ad libitum* under standard conditions (12 h light and 12 h cycle, temperature 28-31 °C, humidity 50-55 %) throughout the experimental period. The experiment lasted for a period of 42 days. Ethical approval was obtained from the Research and Ethics Committee of the Faculty of Basic Medical Science, Delta State University, Abraka (approval no. REC/FBMS/DELSU/21/84) and the study was conducted in accordance with the Guidelines for the Care and Use of Animals [7].

Study design and methodology

A total of 24 adult Wistar rats were weighed, sorted and grouped randomly into 4 groups comprising 6 rats each. Group 1 served as the control group while Groups 2, 3, and 4 were study groups classified based on the dose of PQ received (Table 1). Treatment was done once daily for 42 days.

Serum hormonal levels

Blood samples (5 mL) were collected by cardiac puncture into EDTA bottles. The blood samples were centrifuged for 3 - 5 min at room temperature and 3500 rpm. Serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), estrogen and progesterone were determined with the accubind enzyme linked immunosorbent assay (ELISA) using the respective kit with product code for LH 625-300, FSH 425-300, estrogen 4925-300, and progesterone 4825-300 [8].

Histoarchitecture

The excised ovaries and uterus were dissected and fixed in 10 % formal saline and processed under standard histological tissue preparation and mounted on a slide for photomicrography [9].

Photomicrography

Prepared slides were viewed, and tissue images were captured using a digital microscope (CARL ZEISS, Primo Star) with an 8.3 megapixel camera connected to a computer. Obtained micrographs were interpreted to ascertain the histological and cytological effects of PQ on the ovary and uterus [10].

Statistical analysis

Data was analyzed using Statistical Packages for Social Sciences (SPSS, version 21.0, IBM, Armonk, NY, USA) and means compared using one-way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

Table 1: Details of experimental design and paraquat administration

Group	Number of animals	Designation	Treatment and duration
1	6	Control	0 mg/kg body weight (7, 21, 42 days)
2	6	Low dose	1 mg/kg body weight (7, 21, 42 days)
3	6	Moderate dose	5 mg/kg body weight (7, 21, 42 days)
4	6	High dose	10 mg/kg body weight (7, 21, 42 days)

RESULTS

Histoarchitecture of the ovary and uterus of control group

The various histoarchitecture of the ovary showed normal ovarian tissues characterized by the presence of primordial, primary, and growing follicles (ovary; Figure 1). Furthermore, the histoarchitecture of the uterus revealed an intact myometrium and perimetrium (uterus; Figure 2).

Histoarchitecture of the ovary and uterus of low-dose group

The various histoarchitecture of the ovary was characterized by mild ovarian atrophy at day 7 to severe ovarian atrophy at day 42 (ovary; Figure 3). Furthermore, features of the uterus revealed acute to chronic endometriosis (Figure 4).

Histoarchitecture of the ovary and uterus of moderate-dose group

The ovarian histoarchitecture was characterized by mild, moderate and severe ovarian atrophy at day 7, 21 and 42 respectively (Figure 5). Features of the uterus revealed acute to chronic endometritis (Figure 6).

Histoarchitecture of the ovary and uterus of high-dose group

Ovarian histoarchitecture was characterized by mild, moderate and severe ovarian atrophy at day 7, 21 and 42, respectively (Figure 7). Uterine histoarchitecture revealed acute to chronic endometritis (Figure 8).

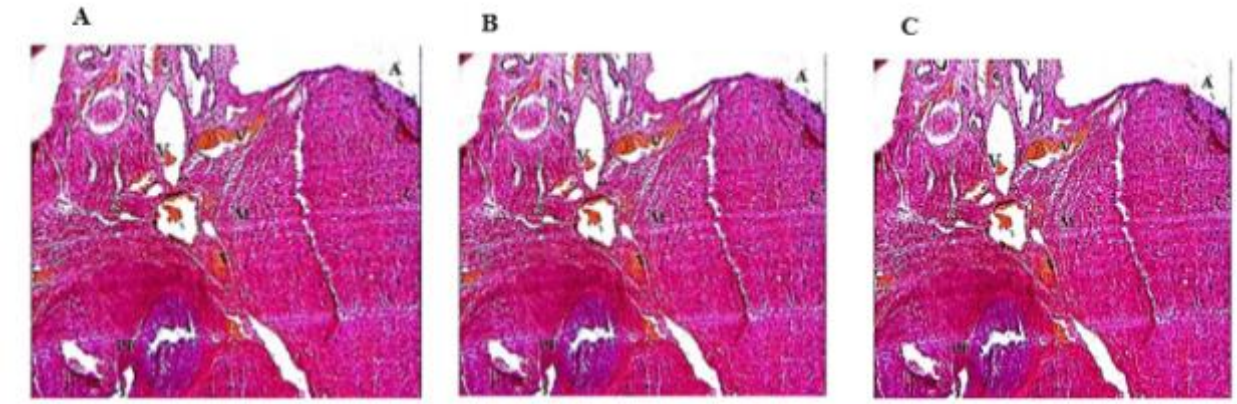

Figure 1: Ovarian histoarchitecture of control group. A (week 1). B (week 3). C (week 6). Section shows the ovary composed of a cortex with several luteal bodies of varying sizes. In the hilum is a medulla and several venous sinuses. Developing primordial and primary follicles are also present there. A few growing follicles are also present and extending to the cortex. Adipose tissue is also present in the pseudocortex. Features suggest normal histoarchitecture. H & E staining (magnification: x 400)

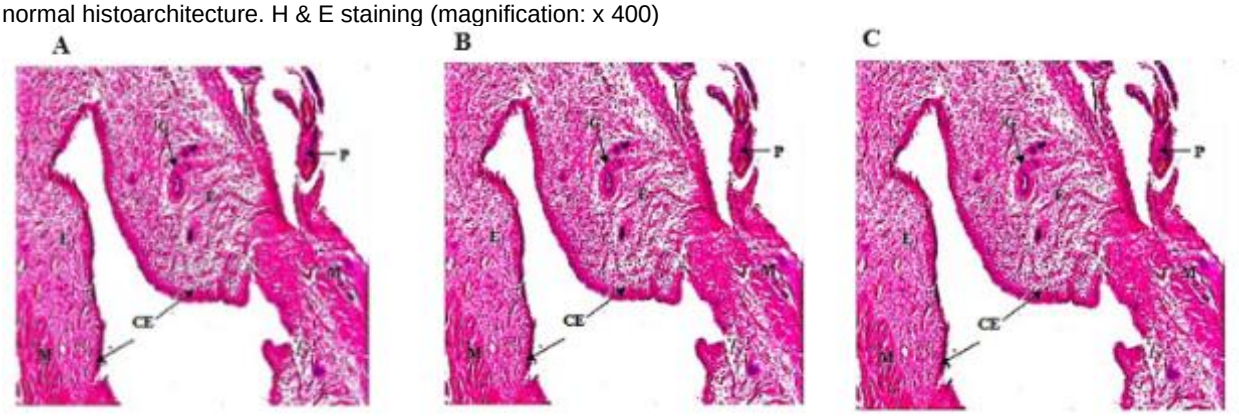

Figure 2: Uterine histoarchitecture of control group. A (week 1). B (week 3). C (week 6). Section shows the uterus composed of endometrium, myometrium and perimetrium. The underlined myometrium and perimetrium are intact, while the endometrium is folded and lined by columnar epithelium. Few glands and blood vessels are present in the circular endometrial stroma. These features suggest a normal uterus. H & E staining (magnification: x 400)

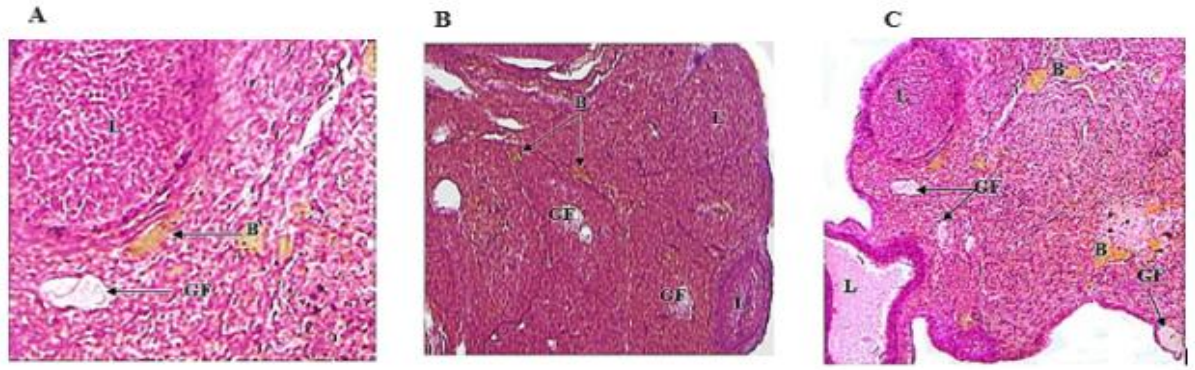

Figure 3: Ovarian histoarchitecture of low-dose group. A (week 1). B (week 3). C (week 6). **A**: Section shows the ovarian cortex with corpus luteum surrounding a hilum of blood vessels and few primary and growing primordial follicles. These microscopic features suggest mild ovarian atrophy. **B**: Section showed luteal bodies in the ovarian cortex and growing follicles interspersed in the ovarian stroma. Blood vessels are located within the medulla gland. Features suggest moderate ovarian atrophy. C: Section shows ovarian tissue composed of luteal bodies (disposed in the cortical area), growing follicles in the glandular periphery and blood vessels scattered within the hilum of the ovarian stroma. These features suggest severe ovarian atrophy. H & E staining (magnification: x 400)

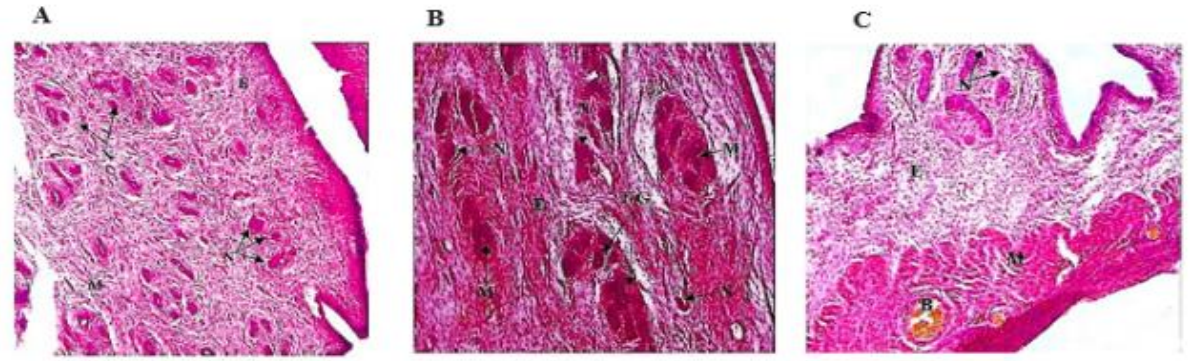

Figure 4: Uterine histoarchitecture of low-dose group. A (week 1). B (week 3). C (week 6). **A**: Section shows marked infiltrates of neutrophils into the endometrium stroma and myometrium. There is moderate endometrial oedema with glandular hyperplasia. Features suggest acute endometrial inflammation. **B**: Section shows an endometrium and myometrium with moderately extensive endometrial glandular hyperplasia, abundant neutrophils and some macrophages in the edematous endometrial stroma. Features suggest chronic endometritis. **C**: Section shows an endometrium and myometrium. There is extensive fibrosis with occasional edema of the endometrium and abundant neutrophils in the endometrial stroma. Features suggest acute uterine endometritis. H & E staining (magnification: x 400)

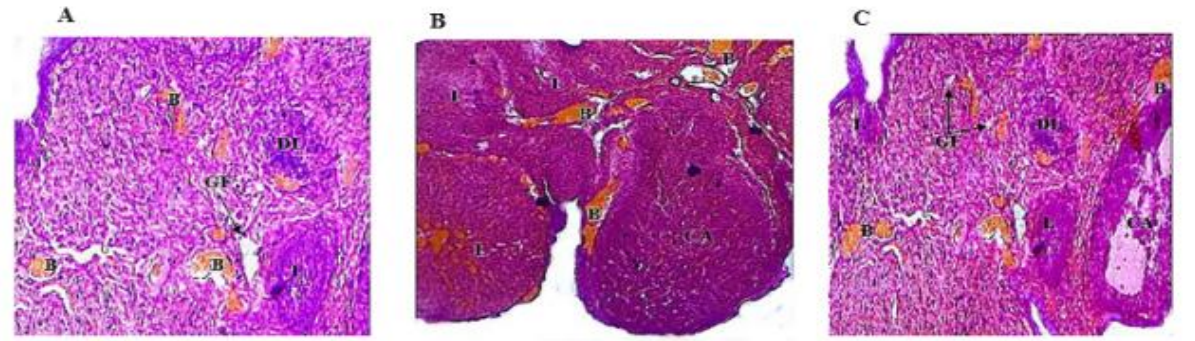

Figure 5: Ovarian histoarchitecture of moderate-dose group. A (week 1). B (week 3). C (week 6). **A**: Section shows luteal and degenerating luteal bodies in the ovarian cortex and growing follicles interspersed in the ovarian stroma with blood vessels located within the gland medulla. These features suggest mild ovarian atrophy. **B**: Section shows several cortical luteal bodies in the ovarian cortex with abundant blood vessels surrounding a hilum of sinosis, and a corpus albicans. These features suggest moderate ovarian atrophy. **C**: Section shows ovarian tissue composed of luteal bodies disposed to the cortical area of the gland, growing follicles in the glandular periphery, corpus albicans and blood vessels in the hilum. Features suggest severe ovarian atrophy. H & E staining (magnification: x 400)

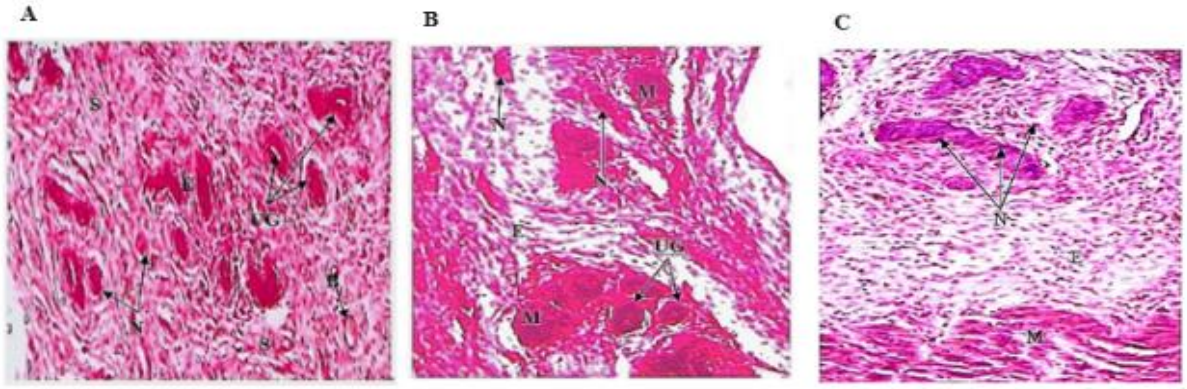

Figure 6: Uterine histoarchitecture of moderate-dose group. A (week 1). B (week 3). C (week 6). **A**: Uterine section shows marked infiltration of neutrophils into the endometrium stroma and myometrium, and abundant uterine glands interspersed within the tissue stroma. There is moderate endometrial oedema with stroma and glandular hyperplasia. Features suggest endometrial inflammation. **B**: Uterine section consists of an endometrium with moderately extensive endometrial glandular hyperplasia, abundant neutrophils and some macrophages present in the edematous endometrial stroma. Features suggest chronic endometritis. **C**: Uterine section shows an endometrium and myometrium with extensive fibrosis, endometrial edema, and abundant neutrophils in the endometrial stroma. Features suggest acute endometritis. H & E staining (magnification: x 400)

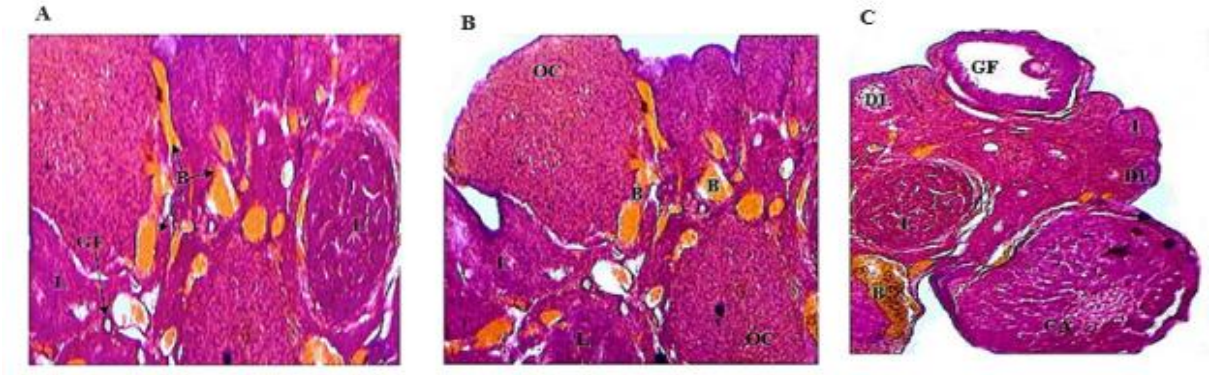

Figure 7: Ovarian histoarchitecture of high-dose group. A (week 1). B (week 3). C (week 6). **A:** Ovarian section shows several cortical luteal cells interspersed within the ovarian cortex, abundant blood vessels and scanty growing follicles within the ovarian stroma. Features suggest mild ovarian atrophy. **B**: Section shows several cortical luteal cells interspersed within the ovarian cortex, and abundant blood vessels within the ovarian stroma. Features suggest moderate ovarian atrophy. **C**: Section shows several cortical luteal and degenerating luteal bodies surrounding a hilum, graffian follicle in the periphery of the gland and a corpus albicans. Features suggest severe ovarian atrophy. H & E staining (magnification: x 400)

Biochemical effects of paraquat on the female gonadotropins

Effect on luteinizing hormone (LH)

Study group showed significantly higher serum LH levels compared to control group ($p < 0.05$). Furthermore, there was a dose-dependent decrease in serum LH levels in study groups (Table 2).

Effect on follicle-stimulating hormone (FSH)

Study group showed a significant increase in serum FSH levels from week 1 to week 6 compared to control group ($p < 0.05$; Table 3).

Effect on estrogen level

Study group showed significant increase in estrogen level at week 1 compared to control group ($p < 0.05$). However, estrogen level decreased from week 1 to week 6 at the same dose level (Table 4).

Effect on progesterone level

Study group showed significant increase in serum progesterone levels compared to control group ($p < 0.05$), with higher levels obtained at 5 mg/kg/bw (Table 5).

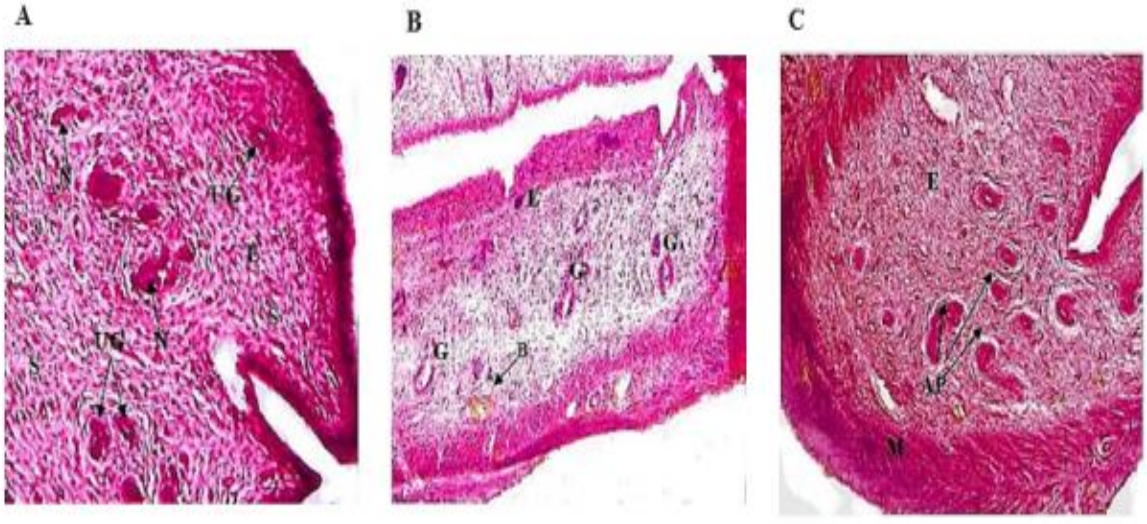

Figure 8: Uterine histoarchitecture of the ovary of high-dose group. A (week 1). B (week 3). C (week 6). **A**: Uterine section shows marked infiltration of neutrophils into the endometrial stroma and myometrium, with few uterine glands scattered within the tissue stroma. There is moderate endometrial oedema and glandular hyperplasia. Features suggest endometrial inflammation. **B**: Section shows scanty glands and blood vessels in the endometrium with extensive fibrosis. Features suggest extensive endometrial stromal fibrosis. **C**: Uterine section consists of an endometrium, which appeared to be intact with muscularis propria. There is extensive endometrial stroma fibrosis with few atrophic glands. Features suggest chronic endometritis and uterine glanular atrophy. ***Keys: (ovaries):*** *A- Adipose tissue, B- Blood vessels, BW- Body Weight, C- Cortex, CA- Corpus albicans, DL- Degenerating luteal bodies, GF- Primary and growing primordial follicles, GF- Graffian follicle, L: Luteal bodies, M- Medulla, OC- Ovarian cortex, PF- Primordial and primary follicles, PQ- Paraquat, V- Venous sinuses.* ***(uterus):*** *B- Blood vessels, BW- Body weight, C- Columnar epithelium, E- Endometrium, G- Glands, M- Macrophages, M- Myometrium, MP- Muscularis Propria, N- Neutrophils, P- Perimetrium, PQ-Paraquat, S- Stroma, UG- Uterine Glands*

Table 2: Effects of paraquat dichloride on the levels of LH

Group	Concentration (IU/L)		
	Week 1	***Week 3***	***Week 6***
1 (Normal Saline)	3.80±0.003[a]	4.80±0.06[a]	5.60±0.02[a]
2 (1 mg/kg/bw of PQ)	7.21±0.006[b]	5.90±0.05[b]	8.20±0.01[b]
3 (5 mg/kg/bw of PQ)	6.20±0.060[b]	5.40±0.08[b]	7.00±0.02[b]
4 (10 mg/kg/bw of PQ)	5.30±0.005[b]	4.20±0.10[b]	6.10±0.10[b]

Values are expressed as mean ± SEM (n = 3). [b]*P* < 0.05 vs control group. BW: Body weight, PQ: Paraquat

Table 3: Effects of paraquat dichloride on the levels of FSH

Group	Concentration (mIU/mL)		
	Week1	***Week 3***	***Week 6***
1 (Normal Saline)	4.80±0.05[a]	6.30±0.05[a]	7.70±0.08[a]
2 (1 mg/kg/bw of PQ)	6.20±0.10[b]	6.20±0.06[a]	9.20±0.06[b]
3 (5 mg/kg/bw of PQ)	7.40±0.06[b]	6.50±0.10[b]	10.60±0.05[b]
4 (10 mg/kg/bw of PQ)	6.70±0.06[b]	5.80±0.06[b]	9.80±0.10[b]

Values are expressed as mean ± SEM (n = 3). [b]*P* < 0.05 vs control group; BW: Body weight, PQ: Paraquat

Table 4: Effects of paraquat on estrogen levels

Group	Concentration (pg/mL)		
	Week1	***Week 3***	***Week 6***
1 (Normal Saline)	11.07±0.55[a]	11.04±0.23[a]	17.03±0.03[a]
2 (1 mg/kg/bw of PQ)	26.70±0.35[b]	19.30±0.52[b]	14.70±0.17[b]
3 (5 mg/kg/bw of PQ)	24.70±0.35[b]	20.01±0.58[b]	12.30±0.64[b]
4 (10 mg/kg/bw of PQ)	22.60±0.29[b]	13.30±0.89[b]	11.50±0.29[b]

Values are expressed as mean ± SEM (n = 3). [b]*P* < 0.05 vs control group, BW: body weight, PQ: paraquat

Table 5: Effects of paraquat dichloride on progesterone levels

Group	Concentration (ng/mL)		
	Week1	***Week 3***	***Week 6***
1 (Normal Saline)	10.89±0.02[a]	11.21±0.010[a]	12.72±0.01[a]
2 (1 mg/kg/bw of PQ)	25.31±0.01[b]	12.80±0.010[b]	16.70±0.35[b]
3 (5 mg/kg/bw of PQ)	26.90±0.11[b]	26.20±0.003[b]	27.81±0.01[b]
4 (10 mg/kg/bw of PQ)	24.41±0.01[b]	18.34±0.030[b]	23.12±0.01[b]

Values are expressed as mean ± SEM for (n = 3). [b]$P < 0.05$ vs control group. BW: body weight, PQ: paraquat

DISCUSSION

Paraquat is a bipyridylium liquid herbicide dark gren in colour [11,12]. Due to its unique characteristics, it is produced and consumed worldwide. The chemical formula is N, N'-dimethyl-4, 4'-bipyridinium dichloride. This herbicide is highly toxic for humans and animals, mainly due to mitochondrial and microsomal oxidation and reduction systems [13]. Mechanism of PQ toxicity is due to the production of superoxide anions, reactive oxygen species (ROS), such as hydrogen peroxide which are major free radicals implicated in paraquat toxicity [11]. In a recent study, PQ modulated the hypothalamic–pituitary–gonadal (HPG) axis to affect reproductive functions in Japanese quails [14]. Also, PQ reduces *invitro* fertilization (IVF) outcomes arising from oxidative stress, changes oestrous cycle and induces ovarian lesions [15].

This study investigated the structural and functional changes of the ovary and uterus as well as hormonal levels following PQ exposure. Oral administration of PQ at lower dose induced ovarian atrophy which became severe at higher doses. Paraquat has been reported to disrupt the reproductive system both by exerting cytotoxic and genotoxic effect due to oxidative stress or through endocrine disruption [16]. Oxidative stress induced by herbicides has been reported to play vital roles in several pathologic conditions due to the generation of free radicals which initiate cellular alterations leading to loss of oocyte and follicular cell functions [17].

This study also revealed that PQ exposure led to endometrial inflammation and chronic uterine endometritis characterized by abundant neutrophils and massive glandular hyperplasia, which were exacerbated at higher doses. Several studies have indicated that exposure to herbicides induced female reproductive abnormalities, including cycle alterations, reduction in number of pregnancies and pregnancy losses, as well as endometritis [18]. These effects may be a result of a hypo-estrogenic state and anti-estrogenic activity of PQ. Also, this study revealed varying histological distortions in the uteri of experimental animals following PQ administration. This distortion initiated endometritis upshot. Hormonal levels also play key roles in maintaining integrity of the female genital tract. The hormones include LH, FSH, estrogen, and progesterone. This study revealed that PQ significantly increased the LH level compared to control group.

Reports from a previous study revealed that high LH level may indicate infertility and dysfunctional ovaries [19] confirming PQ as an anti-fertility agent. Also, LH significant increased at week 6 indicating ovarian degenerative changes. This correlates with the finding of the ovarian histology in which PQ induced ovarian atrophy in study groups compared to control group. This agrees with results from previous studies, which reported that PQ altered levels of LH [11]. These alterations were attributed to increased amount of gonadotropin-releasing hormone (GnRH), which increases serum level of LH [11]. Also, serum level of FSH was investigated following PQ exposure.

Study groups showed significant increase in FSH level compared to control group. The decline in FSH levels in high-dose group may have occurred due to immune system responses. Findings from this study is in tandem with Elham *et al.* [11], which showed significantly higher FSH levels in study group compared to control group. Therefore, this current study supports the evidence that PQ may be regarded as an endocrine disrupting chemicals (EDCs), due to its ability to interfere with the synthesis, secretion, transport, binding, action or elimination of reproductive sex hormones.

Estrogens, a class of steroid hormones, regulate the growth, development, and physiology of the human reproductive system. Estrogens are also involved in the neuroendocrine, skeletal, adipogenesis, and cardiovascular systems [20]. Following PQ exposure, there was a significant increase in estrogen level in study groups compared to control group which is in tandem with the findings of Elham *et al.* [11]. However, estrogen levels gradually reduced at week 3 and week 6. Lower estrogen level is associated with increased secretion of LH and FSH by the

pituitary gland, and possibly ovarian degenerative changes.

Although no study has reported the exact mechanism of PQ administration on estrogen receptors, research has demonstrated that other chemical pesticides including fenarimol, imidazole and prochloraz suppressed biosynthesis of estrogen through CYP19 aromatase inhibition *invitro* [21]. However, this present finding on the effect of PQ on estrogen level provides evidence that a distorted ovarian tissue will be faced with lower estrogen secretion resulting in lower blood levels. Progesterone is an endogenous steroid hormone commonly produced by the adrenal cortex as well as the gonads (ovaries and testes). Progesterone is also secreted by the ovarian corpus luteum during the first ten weeks of pregnancy, followed by the placenta in later phase of pregnancy [22].

Insufficient progesterone is associated with decreased fertility, increased endometrial hyperplasia, and subsequent risk of endometrial neoplasia. This study revealed that PQ exposure led to significant increase in progesterone level in study groups compared to control group reaching maximum level at 5mg/kg/bw for 6 weeks. Paraquat (PQ) is an endocrine-disrupting chemical interfering with critical hormonal systems by altering synthesis, secretion, transportation, binding, action or elimination of normal hormones. This disrupts development, behaviour, fertility and homeostasis [23]. The current study revealed that PQ induced rapid increase in progesterone level. This increase in serum progesterone may lead to an increase in myometrial contractility coupled with a decrease in immunologic defense, ultimately leading to higher risk of miscarriage and early delivery of the fetus. Moreso, some studies have reported decline in progesterone levels following PQ administration [11]. This contrasting result may be due to intrinsic physiological changes occurring within the animals which were not accounted for, differences in PQ dosages and route of administration.

CONCLUSION

Paraquat (PQ) induces ovarian atrophy, chronic endometritis, and increases levels of LH, FSH, estrogen, and progesterone. This study recommends that farmers and communities at large should be properly educated on the adverse effects of PQ. Furthermore, other methods of weed control with less adverse health effects should be pursued. Further large-scale studies in higher animals may be needed to validate these findings and provide a regulatory framework for PQ administration.

DECLARATIONS

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The authors are grateful to those researchers whose articles were cited in this study.

Availability of data and materials

The datasets used and/or analyzed in this study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article would be borne by the authors.

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