Paradigm Academic Press
Journal of Innovations in Medical Research
ISSN 2788-7022
JUN. 2024 VOL.3, NO.2

Modulatory Effects of Aqueous *Moringa Olifera* Extract in Lead Mediated Endometriosis in Wistar Rats

Gabriel Idoko[1], Peter Onoja[1], Paul Beega[1], Vershima Kiekwe[1], Thaedeus Aende[1], Moses Mlumun[1], Paul Omirida[1], Gabriel Akunna[1] & Linus Saalu[1]

[1] Department of Anatomy, College of Health Sciences, Benue State University, Makurdi

Correspondence: Gabriel Idoko, Department of Anatomy, College of Health Sciences, Benue State University, Makurdi.

doi:10.56397/JIMR/2024.06.09

Abstract

Context: The study aims to evaluate the modulatory effect of an aqueous extract of *Moringa olifera* on lead-mediated endometriosis in Wistar rats. ***Settings and Design:*** Twenty-five female Wistar rats were used for the study. The rats were divided into five groups, each containing five rats, and were treated daily for fourteen days. Group I (Control) was administered 5ml/Kg of distilled water per oral (PO), Group II was given 50mg/Kg of Lead PO, Group III was given 50mg/Kg Lead and 100mg/Kg of aqueous *Moringa olifera* extract (AMOE), Group IV received 50mg/Kg Lead plus 200mg/Kg AMOE while Group V received 50mg Lead plus 300mg AMOE PO. ***Results:*** At the end of the experiment, there was decreased estrogen level across the groups with only group II being significant ($P<0.5$) relative to the control, Progesterone levels increased significantly in all the groups relative to the control, Inflammatory markers Interleukin-6 levels decreased significantly in all the treated groups relative to the control group($P<0.5$), however only groups II and III showed significant increases in Tissue necrotic factor. SOD levels decreased significantly in all the treated groups while MDA increased in groups II, III, and V ($P<0.5$). Histologically, Groups II-V showed varying degrees of loose cytoplasmic content, cell edema, and vascular degeneration with Group II also displaying extensive hemorrhage, the number of primordial follicles decreased significantly across groups II-V, primary follicles also decreased significantly in groups II and III, while secondary follicles increased significantly in Groups III and V ($P<0.5$). ***Conclusion:*** Results showed that Lead could induce endometriosis, especially through oxidative stress, AMOE, however, offers a modulatory effect, adjudged by it's effects on inflammatory markers, hormone concentration as well as oxidative stress markers.

Keywords: infertility, toxicity, oxidative stress, Lead, *Moringa olifera*, endometriosis

1. Introduction

Endometriosis is defined as the presence of endometrial glandular and stromal cells outside their usual place in the uterus. This can induce a chronic inflammatory reaction, resulting in the production of scar tissue (adhesions, fibroids) in the pelvis and other regions of the body (WHO, 2021). It primarily affects the abdominopelvic cavity, including the ovaries, cul-de-sac, and other sections of the pelvic peritoneum, colon, and diaphragm, with rare cases impacting extra-abdominal sites such as the pleura and pericardium (WHO, 2021). Endometriosis is a common benign gynecological condition that may occur in 10–15% of women (Smolarz *et al*., 2021). It may even affect up to 60% of women of reproductive age presenting with pelvic symptoms or impaired fertility (Giudice & Kao, 2004). Despite the common occurrence of this condition and its profound effect on reproduction, so much is still unknown about its etiology, and currently available medical therapies are still unsatisfactory, considering that the focus has rather been on treating the symptoms than addressing the causes. Aside, these treatment options cannot be sustained over a prolonged period owing to severe secondary side

effects elicited (Brown *et al.*, 2017). This emphasizes the need for the development of a new therapy that provides a specific and more efficient panacea for endometriotic lesions, preventing their recurrence and, in other words, preserving reproductive potentials, as research has revealed a concerning statistic regarding the prevalence of endometriosis in women between the reproductive ages of 30 to 40 years and even younger (Smolarz et al., 2021). Christ et al. (2021) report that 50% of these cases may result in infertility. Though endometriosis is a disease with no certain etiology (Smolarz *et al.,* 2021). Growing evidence points to the fact that endocrine-disrupting chemicals (EDCs) may be inducers of this condition (Smarr *et al.,* 2016). Some heavy metals from the environment have been shown to have endocrine-disrupting properties. These interfere with the hypothalamic–pituitary–ovarian (HPO) axis (Jackson *et al.*, 2008). A notable example of such heavy metal which has been implicated in reproductive toxicities is lead acetate. Studies on endometriosis and uterine myomas are scarce, despite the fact that numerous harmful reproductive effects associated with heavy metal exposure have been noted in toxicological and epidemiological investigations (Jackson et al., 2008; Rzymski et al., 2015). Because of the pitfalls encountered by the current treatment methods for endometriosis, recent thoughts are being shifted towards therapeutic sources of herbal origin which are known to contain a wide variety of active phytochemicals reported to have numerous disease-fighting and managing attributes (Demiray *et al.*, 2009). Among the numerous medicinal plants which have shown such remarkable potential is *Moringa oleifera*; commonly known as Moringa (Bosch, 2004). Moringa is a tropical tree that is popular for its versatility and boundless economic uses across many continents (Orwa *et al.,* 2009; Islam *et al.*, 2021; Ghimire *et al.,* 2021). Nicknamed the "Miracle tree" or "tree of life" *Moringa oleifera* has been a reported source of many pharmaceutical and industrial byproducts (Radovich, 2009; Toma & Deyno, 2014; Kashyap *et al.*, 2022).

Abdull et al. (2014) state that moringa contains high concentrations of naturally occurring antioxidants, including flavonoids that scavenge free radical-activating enzymes and prevent oxidation, as well as polyphenols (ellagic, chlorogenic, gallic, and ferulic acids). Though there is no literature or report of scientific findings that support the alleviating effect of *Moringa oleifera* leaf extract on endometriosis, its content of antioxidants can be inferred to possess a protective effect against endometriosis. It is thought that an imbalance between ROS and antioxidant levels may play an important role in the pathogenesis of endometriosis-associated infertility, hence the focus of this present study is to ascertain the effect of moringa leaf extract on Lead mediated endometriosis in rodent models.

2. Materials and Methodology

2.1 Materials

2.1.1 Chemicals

Lead was procured from World Corsica Company Limited Makurdi, Benue State. The metal (lead) was measured and dissolved in distilled water, and stored at optimum temperature.

2.1.2 Plant Material

Moringa leaves were collected from a private garden in Wurukum area of Makurdi local government area, Benue State, Nigeria.

2.2 Methods

2.2.1 Moringa Leaves Extraction Method

The Moringa leaves were air-dried at room temperature for two weeks and later pulverized. The powdered leaves were then weighed and 500ml of distilled water was added to it. The solution was left to stand for 48 hours at room temperature after which it was filtered and the chaff collected and dried.

The dried chaff was later weighed and the concentration of dissolved matter was determined by direct subtraction.

3. Experimental Animals

Twenty-five (25) matured female Wistar rats weighing between 150-200 g were used for the study. The animals were obtained from and were kept and maintained in the research laboratory at the Animal House of the College of Health Sciences, Benue State University, for the duration of the experiment. The animals were housed in polypropylene cages and kept in a well-ventilated room where they were acclimatized over two weeks. During this period the animals were subjected to standard room temperature (25±5˚C) and 12/12-hour light-dark cycle. They were also adequately fed with standard rat chow and allowed access to water *ad libitum.*

3.1 Animal Grouping and Treatment

The rats were randomly divided into five (5) groups each consisting of five (5) rats, and treated in the following order:

GROUP I: 5mls/kg body weight distilled water per oral, daily for 14 days.

GROUP II: 50mg/kg of body weight of Lead per oral daily for 14 days.

GROUP III: 50mg/kg body weight of Lead and 100mg/kg of body weight of aqueous *Moringa oleifera extract (AMOE)*, per oral daily for 14 days.

GROUP IV: 50mg/kg body weight of Lead and 200mg/kg body weight of *AMOE* per oral daily for 14 days.

GROUP V: 50mg/kg body weight of Lead and 300mg/kg body weight of *AMOE* per oral daily for 14 days.

3.2 Animal Sacrifice

At the end of the 14-day, all 25 rats were humanely sacrificed by cervical dislocation. The blood samples were collected in sterile bottles for biochemical analysis, and the ovaries were harvested and fixed in 10% formal saline for tissue processing and histological analysis.

3.3 Biochemical Analysis

3.3.1 Estimation of Oxidative Stress Enzymes

Oxidative stress makers analysis was carried out for the following: superoxide dismutase (SOD), Lipid Peroxidation (malondialdehyde), using auto-analyzer.

3.3.2 Serum Hormonal Assay — Follicle Stimulating Hormone (FSH)

The assays were conducted in accordance with the modified protocol that Amballi established in 2007. The blood that was drawn and placed into simple containers was briefly left to coagulate. To achieve separation, each sample was centrifuged for 10 minutes at 1000 rpm. Each time, the collected serum was divided into aliquots, labeled, and kept in storage at –200C. The samples were analyzed for hormone estimation using enzyme immunoassay (EIA) in accordance with the World Health Organization (WHO) matched reagent program protocol (manual) for EIA kits (protocol/version of December 1998 for LH, FSH). One aliquot of each specimen was taken at a time to prevent repeated freezing and thawing. NIADDK — NIH provided the children (USA).

3.4 Analysis of Progesterone

3.4.1 Principle of the Assay

The serum progesterone concentration was measured using the Mouse/Rat Progesterone ELISA Kit. The foundation of this solid-phase enzyme-linked immunosorbent test (ELISA) is the idea of competitive binding. Progesterone conjugated to horseradish peroxidase and an unknown quantity of progesterone in the sample compete for the binding sites of progesterone antiserum coated to microplate wells. The microplate is cleaned four times after being incubated on a shaker. The progesterone concentration is inversely related to the observed optical density following the addition of the substrate solution.

3.4.2 Determination of the An-Inflammatory Cytokines (IL-1 β) and Tumor Necrosis Factor-Alpha (TNF-α)

Serum levels of interleukin 1 beta (IL-1 β), tumor necrosis factor-alpha (TNF-α), and interleukin 10 (IL-10) were determined by using ELISA kits according to the manufacturer's guidelines. The cytokines concentration was expressed in pg/ml.

3.5 Histological Analysis

The tissues were processed using a standard processing schedule for H&E.

3.5.1 Histomorphometry and Follicle Counts

Follicles were quantified in three sections per ovary, representing approximately 25%, 50%, and 75% of the tissue block, respectively. For each part, only follicles with a visible nucleus were counted and classified as follows: (1) primordial follicles (PF), with one layer of flattened pre-granulosa cells (GCs); (2) primary, with two or more cuboidal GCs up to one complete layer of cuboidal GCs; (3) secondary, with at least two layers of GCs but no antrum; and (4) tertiary, with visible antrum. Two blinded observers rated the same sections independently, and the findings were compared, revealing an inter-observer concordance greater than 90%. *Corpora lutea* (CL) were quantified in the same three sections per animal as an indirect measure of the ovulation rate.

The ovary was approximated to a prolate ellipsoid and the ovarian volumes (mm^3) extrapolated using the ellipsoid formula $4/3\ \pi\ a\ b^2$, where 'a' is the length of the entire ovary derived by multiplying the total number of sections (*n*) by 0.005 (representing the thickness of each section), and $\pi\ b^2$ is the Area of the middle section (A*m*) of the ovary. Therefore, ovarian volume (*V*) = 4/3 * A*m* * *n* * *0.005*.

3.6 Statistical Analysis

All values were calculated to determine the mean and standard error of the mean (S.E.M). The control and treated groups were compared using one way ANOVA and Duncan's multiple range tests. Differences were judged statistically significant at $p < 0.05$.

3.7 Ethical Consideration

Ethical approval was sought and obtained from the Research and Ethics Committee (REC), College of Health Science, Benue State University, Makurdi. All experimental procedures carried out were following the guidelines on animal experiments as prescribed by the Ethics Committee.

4. Results

4.1 Physical Observation/Body Weight

Figure 1 shows the mean body weight changes across groups compared to one-way ANOVA. For both initial body weight, final body weight, and body weight differences, no group showed a statistically significant difference in mean against the control group (group 1) when compared to one-way ANOVA.

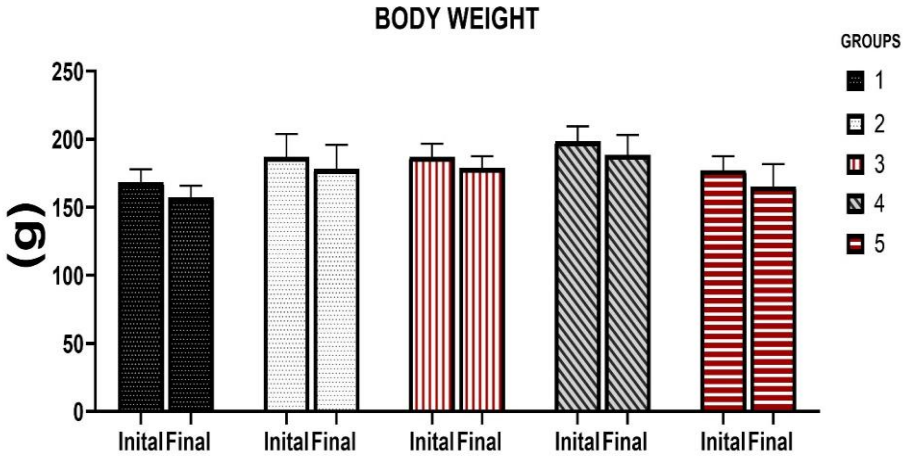


Figure 1. Showing the Mean Body Weight and Changes across Groups Compared to One-Way ANOVA

Note: N = 5; MEAN±SEM; SEM = Standard Error in Mean.

4.2 Reproductive Hormone Levels

Figure 2 shows the mean reproductive hormone levels across groups compared to one-way ANOVA. For estrogen; only group 2 showed a statistically significant difference (decrease) in mean when compared to the control group (group 1) while for progesterone; groups 2, 3, 4, and 5 all showed a statistically significant difference (increase) in mean when compared to the control group (group 1) on one-way ANOVA (* = statistically significant at P<0.05 when compared to group 1).

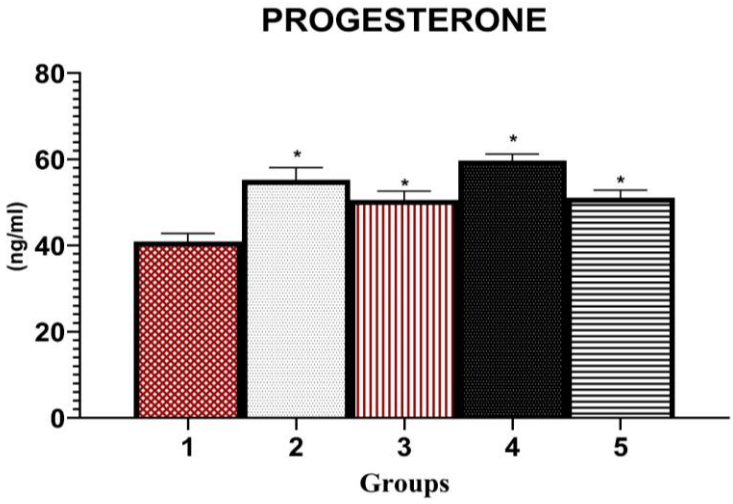


Figure 2.1 Showing the Mean Progesterone Levels across Groups

Note: N = 5; MEAN±SEM; SEM = Standard Error in Mean;

* = statistically significant at P<0.05 when compared to group 1.

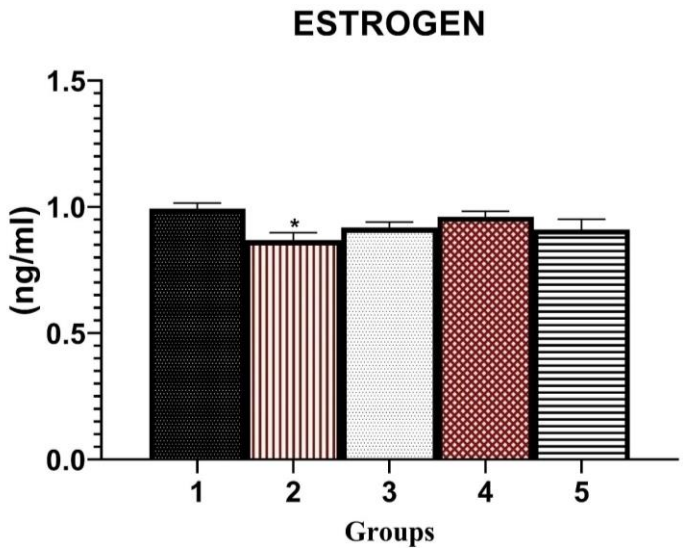


Figure 2.2 Showing the Mean Estrogen levels across the Groups

Note: N = 5; MEAN±SEM; SEM = Standard Error in Mean;

* = statistically significant at P<0.05 when compared to group 1.

4.3 Inflammatory Markers

Figure 3 shows the mean inflammatory markers across groups compared to one-way ANOVA. For interleukin-6; groups 2, 3, 4, and 5 all showed a statistically significant difference (decrease) in mean when compared to the control group (group 1) with a progressive increase from groups 2–5. For Tissue Necrotic Factor; groups 2 and 3 showed a statistically significant difference (increase) in mean when compared to the control group (group 1) on one-way ANOVA (* = statistically significant at P<0.05 when compared to group 1).

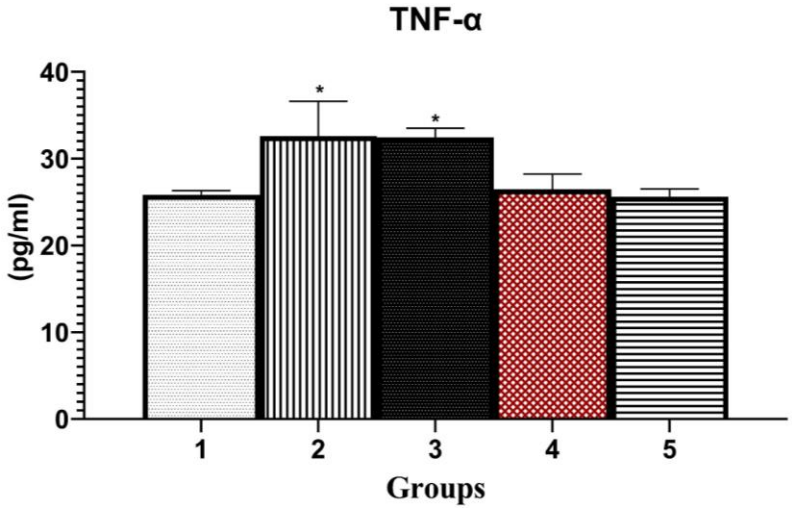


Figure 3.1 Showing the Mean Tissue Necrotic Factor value across Groups Compared on One-Way

Note: ANOVA. N = 5; MEAN±SEM; SEM = Standard Error in Mean;

* = statistically significant at P<0.05 when compared to group 1.

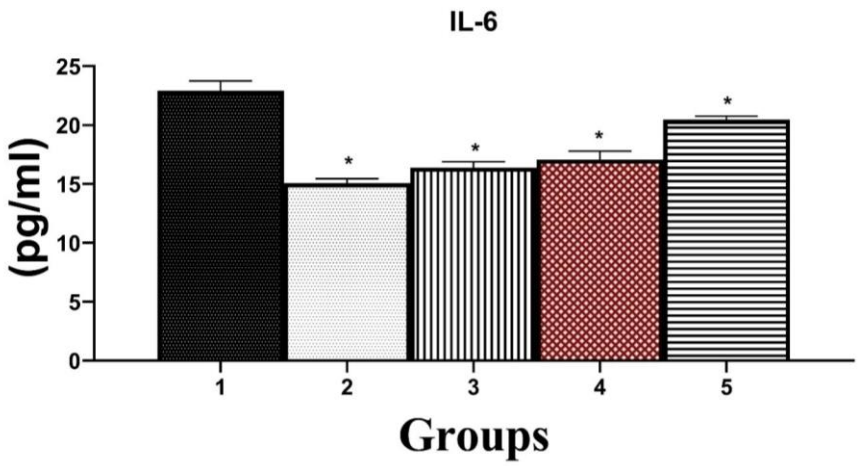


Figure 3.2 Showing the Mean interleukin-6 value across Groups Compared on One-Way

Note: ANOVA. N = 5; MEAN±SEM; SEM = Standard Error in Mean;

* = statistically significant at P<0.05 when compared to group 1.

4.4 Oxidative Stress Levels

Figure 4 shows the mean oxidative stress markers compared to one-way ANOVA. For SOD, groups 2, 3, 4, and 5 all showed a statistically significant difference (decrease) in mean when compared to the control group (group 1) on one-way ANOVA. For MDA, groups 2, 3, and 5 showed a statistically significant difference (increase) in mean when compared to the control group on one-way ANOVA (* = statistically significant at P<0.05 when compared to group 1).

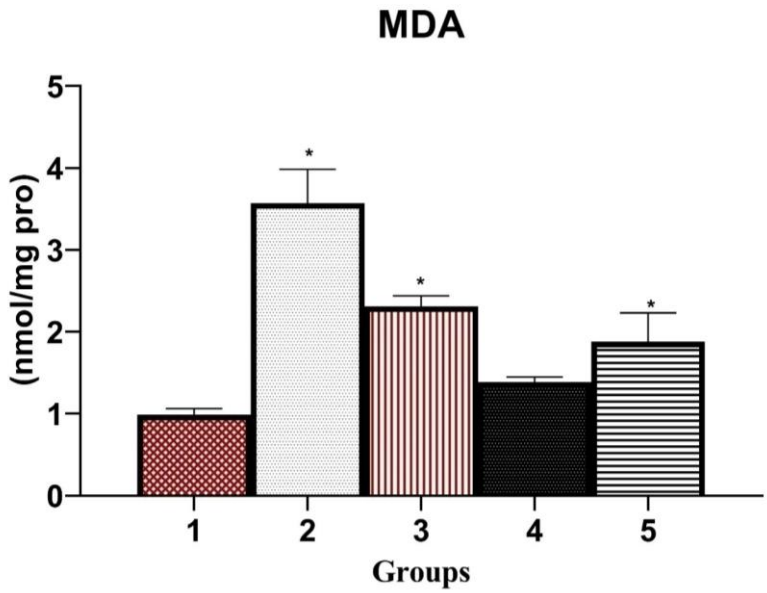


Figure 4.1 Showing the Mean Malondialdehyde (MDA) levels across Groups Compared on One-Way

Note: ANOVA. N = 5; MEAN±SEM; SEM = Standard Error in Mean;

* = statistically significant at P<0.05 when compared to group 1.

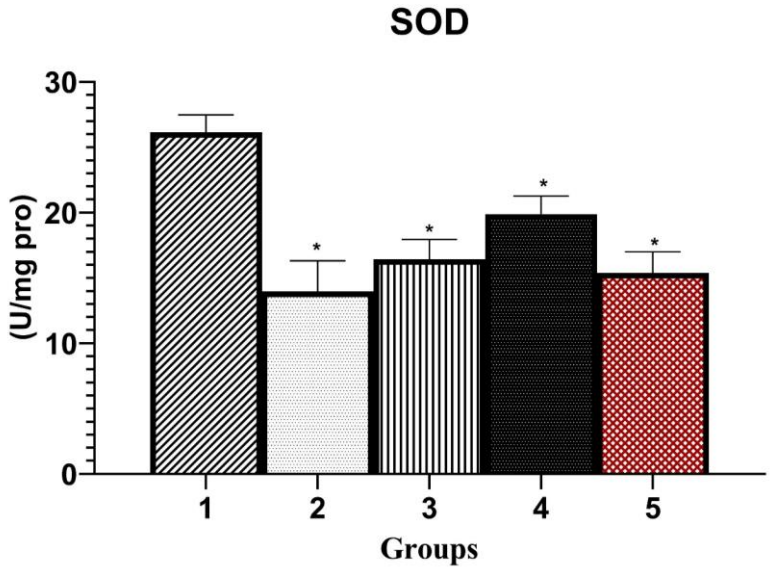


Figure 4.2 Showing the Mean Superoxide dismutase (SOD) levels across Groups Compared on One-Way

Note: ANOVA. N = 5; MEAN±SEM; SEM = Standard Error in Mean;

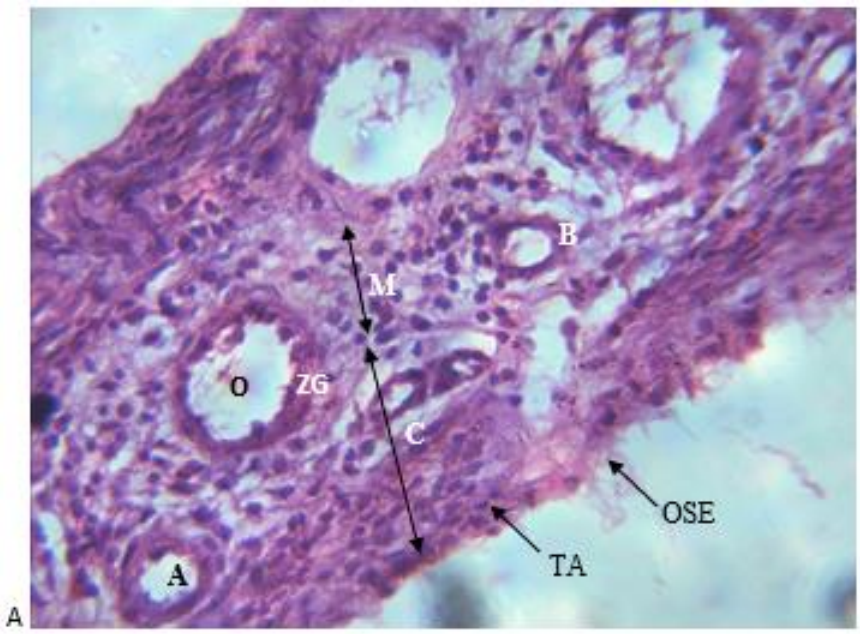
* = statistically significant at P<0.05 when compared to group 1.

4.5 Histomorphology

Compared with the control group, the endometrial tissue of groups 2-5 showed a certain degree of loose cytoplasmic, cell edema, vacuolar degeneration, and other changes in the lead group, under the light microscope. Group 2 rats treated with lead also showed extensive hemorrhage in the histologic slides.

4.6 Follicular Count

Plate 1 shows the mean follicular count across groups compared to one-way ANOVA. For primordial follicles; groups 2 and 5 showed a statistically significant difference (decrease) in mean compared to the control group (group 1). For primary follicle; groups 2 and 3 showed a statistically significant difference (decrease) in mean when compared to the control group while for secondary follicle, groups 3 and 5 showed a statistically significant difference (increase) in mean when compared to the control group (group 1) on one-way ANOVA (* = statistically significant at P<0.05 when compared to group 1).



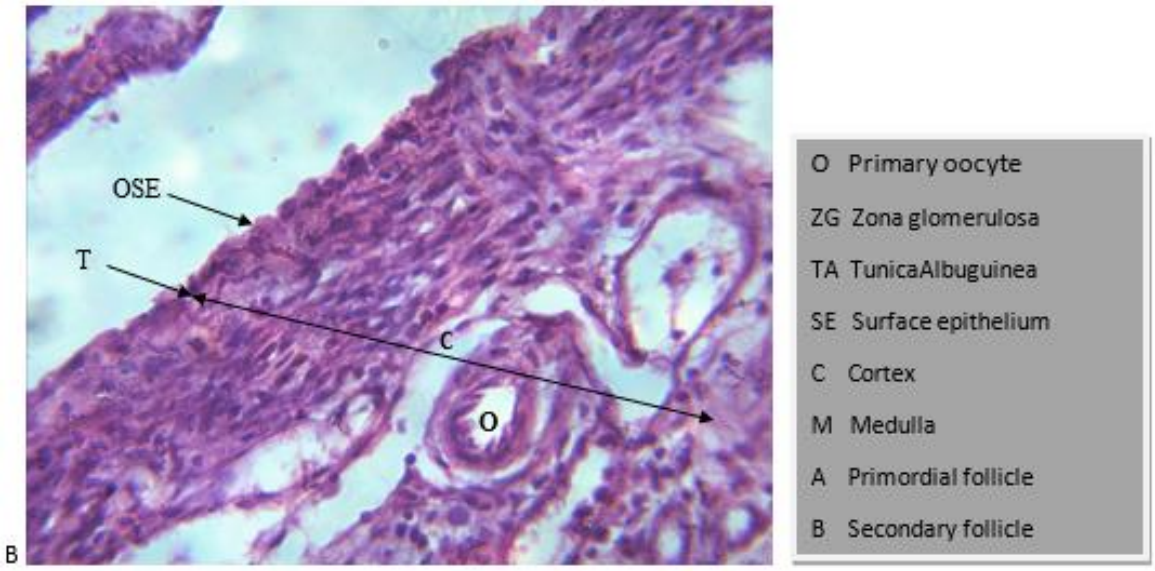


Plate 1: Histological slides of group 1 rats showing normal histology of the ovaries with prominent follicles and clear cytoplasm. H&E (×400).

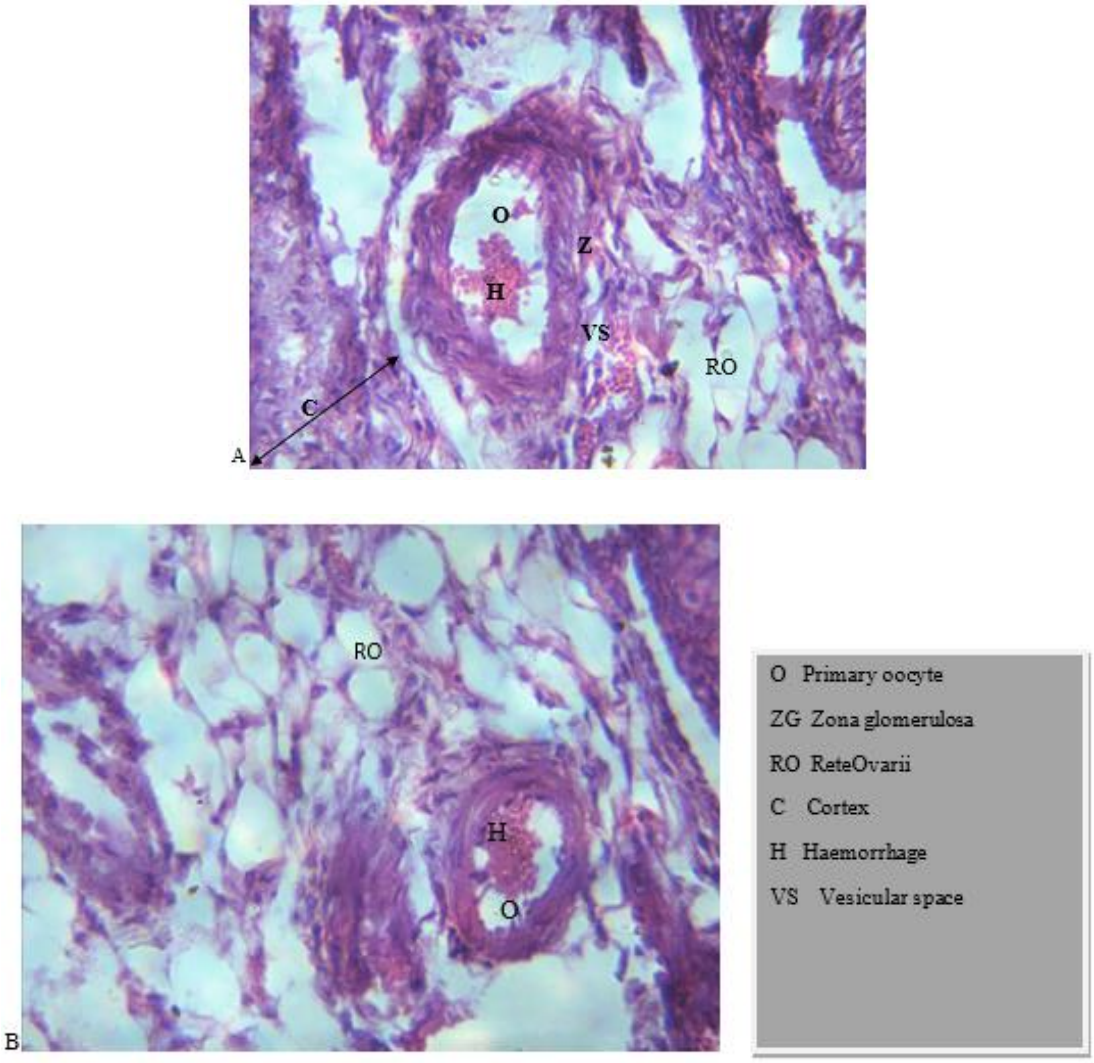


Plate 2: Histological slides of group 2 rats showing ruptured follicles and hemorrhage within the ovaries. H&E (×400).

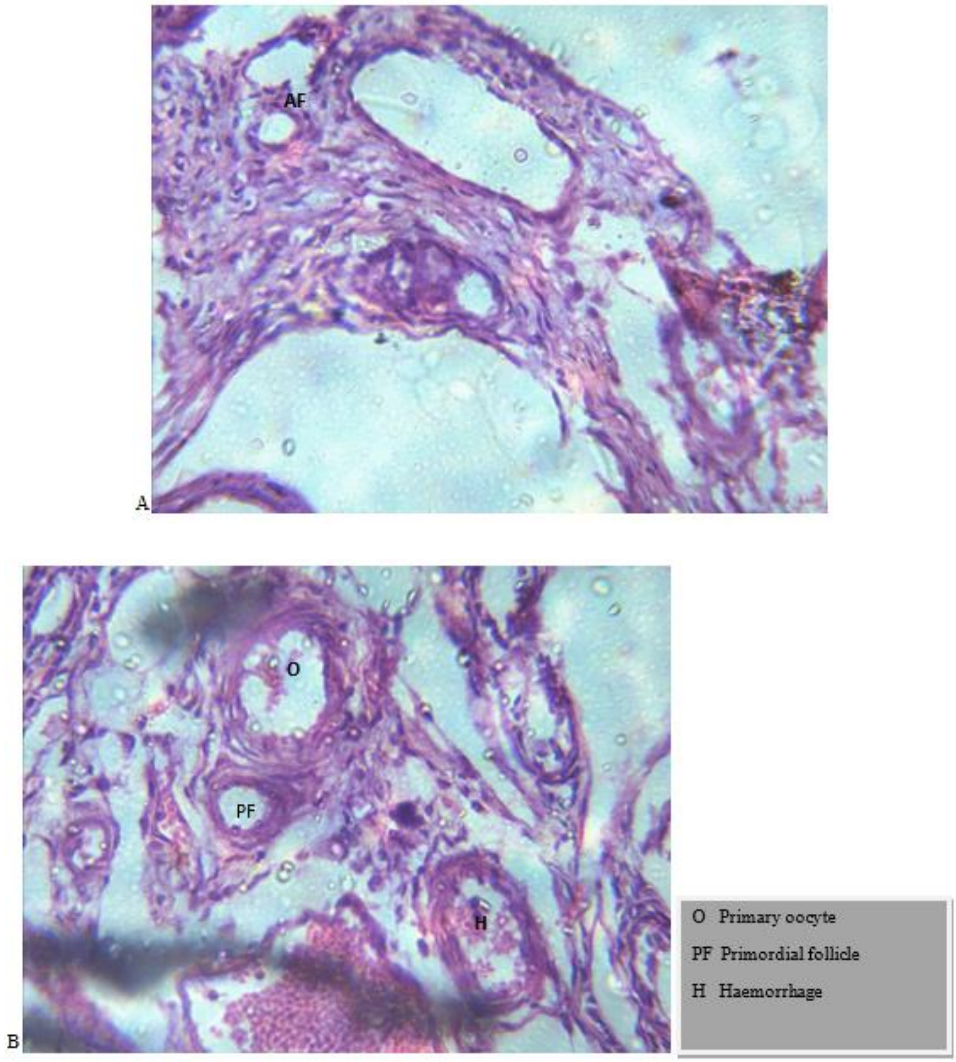
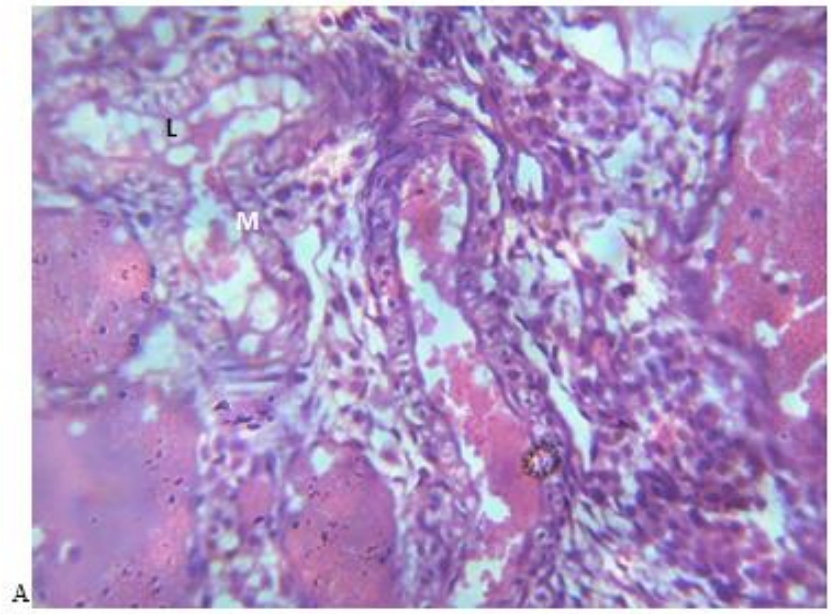


Plate 3: Histological slides of group 3 rats showing slight hemorrhage, compared to lead-treated rats. H&E (×400).



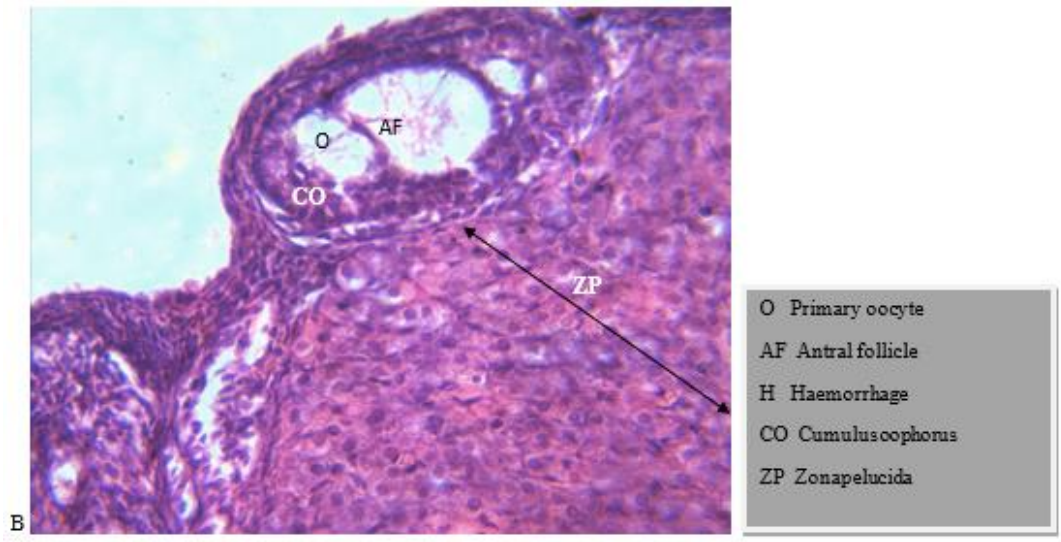


Plate 4: Histological slides of group 4 rats' clear follicles and less hemorrhage compared to groups 2 and 3. H&E (×400).

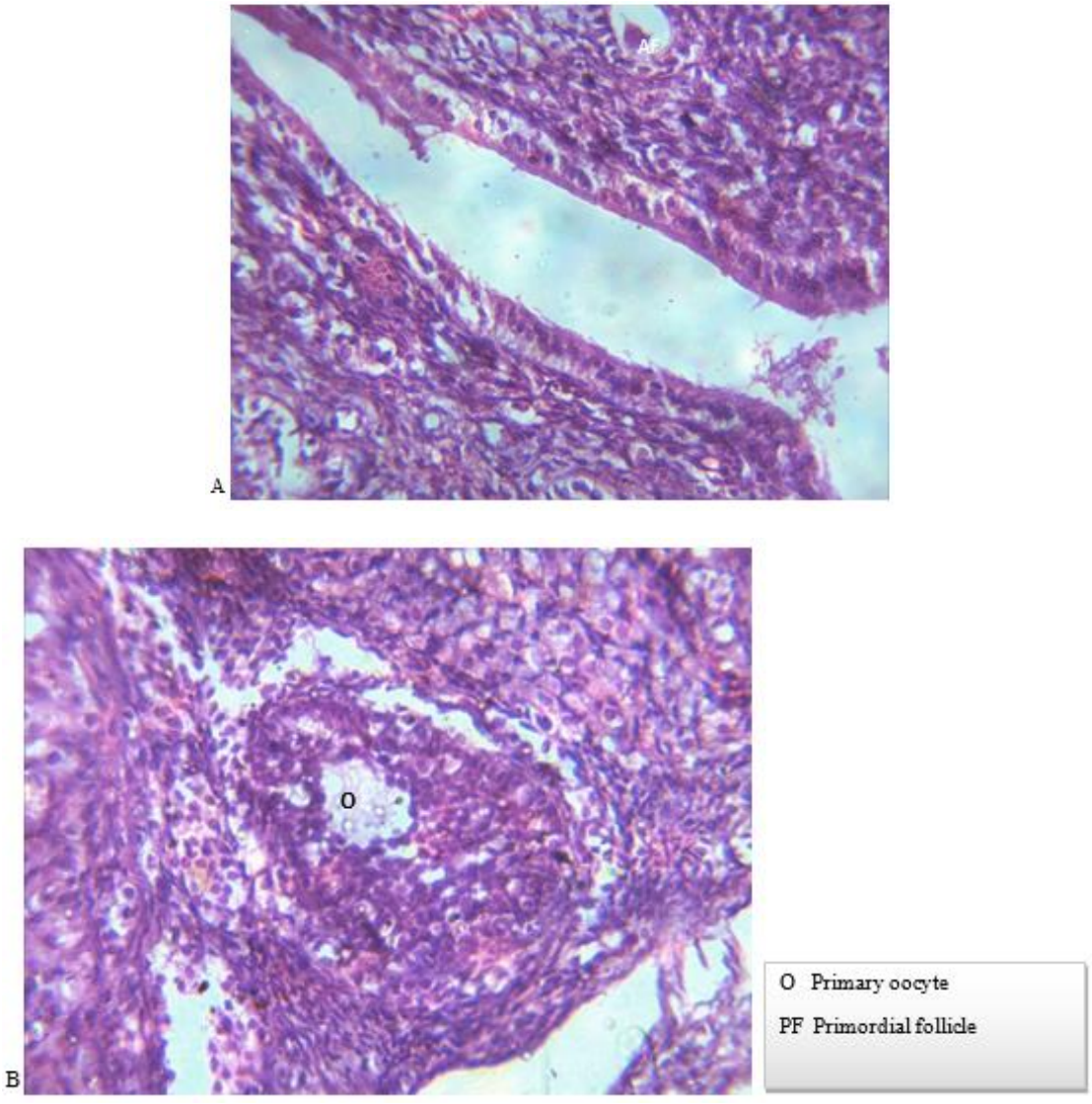


Plate 5: Histological slides of group 5 rats showing less prominent follicles compared to group 4. H&E (×400)

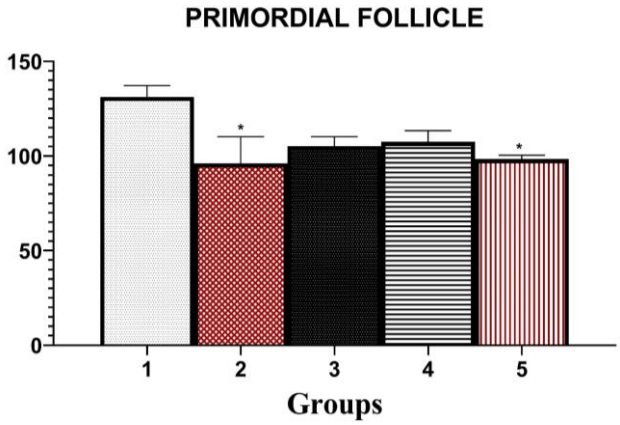


Figure 5.1 Showing the Mean Primordial Follicular Count across Groups Compared to One-Way ANOVA

Note: N = 5; MEAN±SEM; SEM = Standard Error in Mean;

* = statistically significant at P<0.05 when compared to group 1.

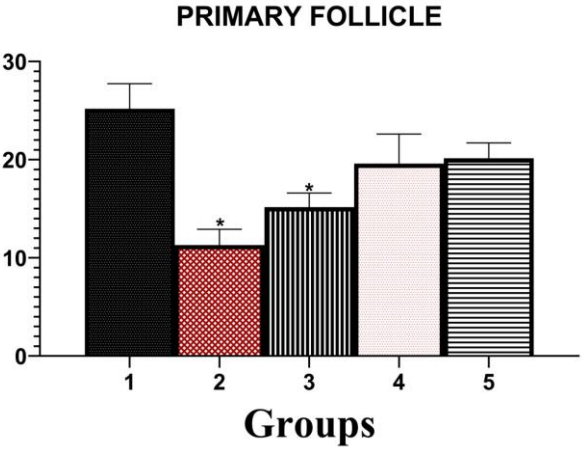


Figure 5.2 Showing the Mean Primary Follicular Count across Groups Compared to One-Way ANOVA

Note: N = 5; MEAN±SEM; SEM = Standard Error in Mean;

* = statistically significant at P<0.05 when compared to group 1.

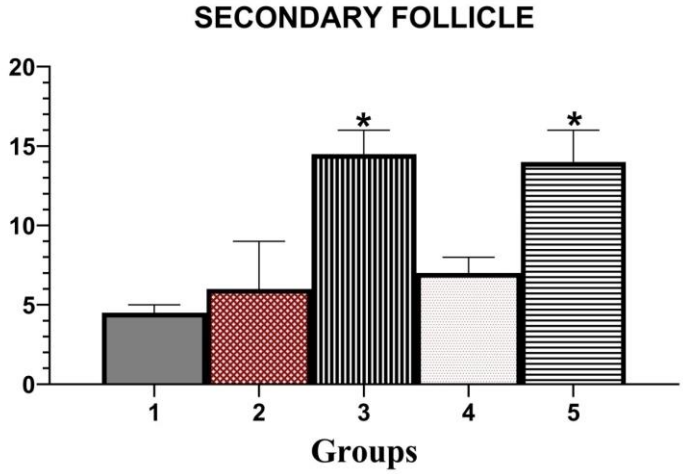


Figure 5.3 Showing the Mean Secondary Follicular Count across Groups Compared to One-Way ANOVA

Note: N = 5; MEAN±SEM; SEM = Standard Error in Mean;

* = statistically significant at P<0.05 when compared to group 1.

4.7 Physical Observation

The observation in Figure 1 showed a decrease in body weight across groups. However, the decrease in body weight may not be attributed to either lead or the extract, since group 1 (control group) which received only normal saline, also showed an equivalent decrease in body weight.

In a contrary report by Nwamarah and colleagues (2015), a significant increase in body weights of male Wistar rats was recorded in rats administered with *Moringa oleifera* extract. This could be due to the increased feeding rate of the male rats as well as the stage of development. However, the female rats showed a dose-dependent weight pattern as they showed less weight increase at lower doses and more weight gain at higher doses.

The decrease in body weight might be due to chance, stress during administration, physical activity, nutrition, or other uninvestigated factors.

4.8 Biochemical Analysis

Ovarian endometrioma, just like every other type of endometriosis, remains evasive with next to nothing known about its non-invasive diagnosis. Worse still is the fact that it is asymptomatic.

Although there is no clinically accepted biomarker for the diagnosis of endometriosis, in the meantime, Cancer Antigen 125 (CA-125) remains the most frequently studied and used marker (Gupta *et al*., 2006). However, the use of oxidative stress markers, inflammatory cytokines, and sex hormone levels have also been reported as possible pointers to the diagnosis of endometriosis, irrespective of its site (Clower *et al*., 2022).

4.9 Reproductive Hormones

Hormones play an important role in the etiopathogenesis of unexplained infertility. In endometriosis, medical treatments focus on either lowering estrogen or increasing progesterone to alter hormonal environments that promote endometriosis (Johnson & Hummelshoj, 2013). This might suggest the possible role of estrogen in the pathophysiology of endometriosis, hence it can be used as a marker alongside the levels of progesterone.

Lead has been reported to induce hypothalamic or pituitary disturbance when longer periods of exposure take place (Doumouchtsis *et al*., 2009). This affects the feedback mechanism of the HPG-Axis, hence the levels of sex hormones.

From this research, group 2 administered with Lead only, showed a statistically significant decline in the level of estrogen while progesterone level increased significantly compared to the control group. This is contrary to the report of Udeyraj *et al.* (2015) who reported a decline in progesterone levels of Lead treated rats, though the reduction was non-significant. While the decline in estrogen may not indicate possible endometriosis, a significant rise in levels of progesterone compared to the control group may indicate the infertility-causing ability of Lead. This is because high progesterone levels have been associated with infertility as it favors the shedding of the endometrium as occurs during menstruation, hence preventing implantation. The low estrogen level in Lead treated group has been reported earlier by Franks *et al.* (1989), though in prepubertal rats. A similar finding was also reported by Kim *et al*. (2013).

An explanation for the low estrogen and high progesterone levels recorded in this research could be due to the shorter period of the experiment compared to that of other researchers such as Udeyraj *et al. (*2015). Also, the age of the rats as when they were exposed to Lead could have contributed to the decline in estrogen and consequent rise in progesterone level.

Similarly, a significant increase in the level of progesterone was also observed in the group treated with *Moringa oleifera* extract only, though there was no statistically significant difference in the level of estrogen compared to the control groups. Several works have recorded a similar rise in the concentration of models treated with *moringa oleifera* extract. The results observed in this research agree with that of Ogunsola *et al*. (2017) who observed that *Moringa oleifera* increased progesterone levels in a dose-dependent manner.

However, there is a decrease in the concentration of progesterone in group 3 compared to group 2 treated with Lead only.

Also, groups 4 and 5 treated with increasing doses of the extract simultaneously with Lead, showed no significant difference in levels of estrogen compared to the control, though there was a statistically significant increase in levels of progesterone in both groups with the highest concentration in the low dose group 4. A similar increase in the level of progesterone was reported by Ajuogu *et al*. (2019) who observed a significant increase at the high dose of moringa powder in New Zealand rabbits. The significant increase in progesterone levels could be a protective mechanism against the surge of estrogen. It shows that *Moringa oleifera* leaves are protective against ovarian endometriosis which is usually marked with a rise in estrogen and a decline in progesterone.

When compared with group 2, there is a decrease in levels of progesterone in group 5, the high-dose group,

compared to Lead only group. This could indicate the possible progesterone-reducing potential of Moringa.

While it is possible to attribute the significant increase in levels of progesterone in groups 3, 4, and 5 to the effect of Moringa, a more logical explanation could be that the rats were in their post-ovulatory period at the time of sacrifice, hence the statistically significant increase in levels of progesterone across all levels. Therefore, it can be inferred that Moringa extract shows some levels of protective and curative effects on the ovaries as indicated by no significant difference in levels of estrogen when compared to the control group, especially at low doses.

4.10 Inflammatory Markers

From Figure 4, it is observed that the test substance(s) showed a significant decreasing effect on the interleukin-6 levels of groups 2, 3, 4, and 5 with the interleukin level showing a gradual, progressive increase from groups 2-5. For the Tissue Necrotic Factor, the result showed a significant increasing effect in groups 2 and 3.

Endometriosis causes chronic inflammatory reactions (Lindsey *et al.*, 2018) which may result in the formation of scar tissues in the pelvis as well as other parts of the body (Johnson *et al.*, 2017; WHO, 2018). This implies that the presence of inflammation markers or inducers such as TNF-α and IL-6 can both be used as possible biomarkers of endometriosis (Kokot *et al.*, 2021).

It may be deduced from this research that both Lead and *Moringa oleifera* extracts show inflammatory properties as marked by a significant increase in levels of Tissue Necrotic Factor-alpha (TNF-α) in group 2 (Lead only) and group 3 (*Moringa oleifera* extract only).

This study also shows a non-significant difference in mean levels of TNF-α in group 4 (*Moringa oleifera* extract, low dose) and group 5 (high dose extract) compared to the group 1 rats. This indicates the possible anti-inflammatory effect of *Moringa oleifera* extract on the ovaries of adult female Wistar rats.

However, according to Mu *et al.* (2018), there are no overall associations between the plasma concentrations of TNF-α and IL-6, and are both insufficient pieces of evidence to draw meaningful conclusions for possible endometriosis.

4.11 Oxidative Stress

Oxidative stress refers to the imbalance between the formation of reactive oxygen species (ROS) and the antioxidant defense mechanism (Pizzino *et al.*, 2017). The significant role of oxidative stress in the inflammatory responses of many diseases, including endometriosis has been demonstrated (Cacciottola *et al*., 2021). The possible source of reactive oxygen species found in the serum may be a result of the activation of immune cells such as granulocytes and macrophages by proinflammatory cytokines, which are known to be capable of ROS production. This is usually secondary to the migration of endometrial cells and desquamated menstrual cells into the peritoneal cavity through retrograde menstruation, which causes inflammatory responses (Carvalho *et al.*, 2012).

From Figure 4.2, Lead showed a significantly decreasing effect on the SOD level of rats in group 2 and a significantly increasing effect on the MDA level of rats in group 2. This implies that lead causes oxidative stress. Lead acetate has been reported to induce oxidative stress causing the generation of high levels of reactive oxygen species (ROS) with consequent decline in levels of antioxidant activities (Oyem *et al*., 2021).

Many studies have shown that lead induces oxidative damage, which induces the formation of reactive oxygen species as well as lipid peroxidation and interferes with the antioxidant defense system, which includes the enzymes glutathione peroxidase, superoxidase, and catalase (Singh *et al.*, 2013).

Though scientists are still investigating the role of oxidative stress in the pathogenesis of endometriosis, according to Lai *et al.* (2017), lower superoxide dismutase activity is associated with the persistence of endometriosis. This, therefore, implies that oxidative stress could be a cause of endometriosis.

However, according to Amreen *et al*. (2019), there is no correlation between levels of oxidative stress markers and endometriosis.

This research also indicated that group 3 (extract only) showed oxidative stress as indicated by the significant decrease in SOD and the significant increase in MDA, a product of lipid peroxidation, compared to group one rats. This implies that both lead and *Moringa oleifera* extract may generate possible reactive oxygen species leading to oxidative stress when administered separately and alone. The generation of reactive oxygen in the extract-treated group may be due to the pro-oxidant contents, methanol, and quercetin, of the extract (Chang *et al.*, 2006). However, the production of reactive oxygen species by moringa could be beneficial as it targets selective cancer cells and therefore stimulates the production of antioxidants to help the body's defense system (Touriño *et al.*, 2008).

However, compared to groups 2 and 3, rats in groups 4 and 5 showed a decline in serum concentration of MDA

and increased concentration of SOD. This may imply that *Moringa oleifera* extract has antioxidant effects. A similar decrease in MDA was observed in mice administered with Moringa extract, as reported by Monraz-Mendez *et al*. (2022). This may be used as a cure for ovarian endometriosis as one of its possible etiology is the generation of reactive oxygen species.

4.12 Histomorphology

Compared with the control group, the endometrial tissue of groups 2-5 showed a certain degree of loose cytoplasmic, cell edema, vacuolar degeneration, and other changes in the lead group, under the light microscope. The lead-treated group also showed widespread hemorrhage within the ovary as well as ruptured oocytes. However, the ovaries of groups 3-5 showed less damage as marked by less hemorrhage and vacuolar degeneration.

This also substantiates the ameliorative effect of the extract against lead-induced ovarian endometrioma.

4.13 Follicular Count

From the observation in Figure 5, the Lead-treated group showed a significant decreasing effect on the primordial follicular count of rats in groups 2 and 5. For primary follicle, the test substance(s) showed a significant decreasing effect in groups 2 and 3 while for secondary follicular count, there was a significant increasing effect in groups 3 and 5.

In this study, the lead-treated group showed a statistically significant decrease in the number of primordial and primary follicles compared to the group one rats, though there was no significant difference in the number of secondary follicles. This indicates a poor ovarian reserve, a chief indicator of possible female infertility. It can therefore be inferred from the follicular count that Lead causes ovarian endometrioma which in turn manifests as destruction of follicles, hence the ovarian reserve. Intraperitoneal administration of 0.3 mg/kg BW/Day (low dose) of Lead acetate for 14 days revealed a non-significant decrease in the number of follicles of adult female Swiss-Webster mice (Ibtisam, 2017). Lead induces a reduction in the number of primordial follicles and increases the number of atretic follicles in the ovaries of mice, while it also damages the endometrium (Sharma *et al*., 2016).

However, in group 3 administered with *Moringa oleifera* leaf extract only, there was a marked increase in a mean number of primordial, primary, and secondary follicles compared to group 2. It can be inferred from this result that the extract could boost fertility as seen in the significant increase in the mean secondary follicle in the group compared to both groups one and 2. *Moringa oleifera* extract has been shown to increase the concentration of FSH at low doses (Nwamarah *et al*., 2015). FSH stimulates the growth and recruitment of immature ovarian follicles in the ovaries (Fowler *et al.*, 2003). The ability of *Moringa oleifera* to increase FSH concentration may help restore fertility as well as attenuate the follicle-destroying properties of Lead. This could show that moringa's ability to keep increased or constant FSH in the presence of Lead could be protective. However, in the same study, a decline in FSH concentration was observed at higher doses when compared to the control group (Low number of follicles could be due to a decline in levels of FSH at the low dose, so if the result shows that there is decreased follicles in treated groups, it could be that Lead inhibited it through its effect on the HPG or it could be that high dose of Moringa could have contributed, hence the possibility of its efficacy only at low dose).

However, Siahaan *et al.* (2022) have reported that the extract showed no significant increase in ovarian follicles in *Rattus norvegicus*, while Siahaan *et al.* (2022) also reported significantly lower follicle count in the *Moringa oleifera* 500 mg/kg BW treatment group compared to the PCOS control group.

Also, the extract showed a curative effect against Lead-induced endometrioma marked by significant decrease in follicles. This is evidenced in the increased ovarian reserve compared to the lead-treated group could cause infertility at high doses due to its progesterone-secreting ability. The efficacy of the extract in maintaining ovarian reserve could be said to be most visible at low doses.

5. Conclusion

This study shows that Lead could induce ovarian endometriosis, especially through its generation of pro-oxidants, however, its effect on hormone levels is not noticeable in this experiment. *Moringa oleifera* extract on its own does not show any remarkable effect on ovarian parameters as depicted by the results seen in groups where it was administered alone, however, seems to have curative effects due to its antioxidant effect, progesterone synthesis stimulation as well as decreasing pro-inflammatory cytokines concentration. The efficacy of the extract in protecting the ovaries against Lead-induced ovarian endometrioma is maximum at low doses as marked by levels of inflammatory markers, hormone concentration as well as oxidative stress markers whose values bore a similarity to those of the control group.

6. Recommendations

We recommend that a more detailed study be conducted to understand the pathogenesis of Lead-induced ovarian endometrioma. We also, recommend a longer duration of study and the use of a larger animal sample size to determine the efficacy of aqueous *Moringa oleifera* extract in the treatment of ovarian endometrioma.

Conflict of Interest

We hereby declare that there was no conflict of interest in the course of this research.

References

Abdull Razis, A. F., Ibrahim, M. D., & Kntayya, S. B., (2014). Health benefits of Moringa oleifera. *Asian Pacific journal of cancer prevention: APJCP*, *15*(20), 8571–8576.

Amreen, S., Kumar, P., Gupta, P. & Rao, P., (2019). Evaluation of Oxidative Stress and Severity of Endometriosis. *J. Hum. Reprod. Sci.*, *12*, 40–46.

Bosch, C.H., (2004). Moringa oleifera Lam. In: Grubben GJH, Denton OA. (Eds). *PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale)*, Wageningen, Netherlands.

Cacciottola, L., Donnez, J. & Dolmans, M. M., (2021). Can Endometriosis-Related Oxidative Stress Pave the Way for New Treatment Targets? *International journal of molecular sciences*, *22*(13), 7138.

Carvalho, L.F., Samadder, A.N., Agarwal, A., Fernandes, L.F. & Abrão, M.S., (2012). Oxidative stress biomarkers in patients with endometriosis: A systemic review. *Arch. Gynecol. Obstet.*, *286*, 1033–1040.

Chang, Y.F, Chi, C.W & Wang, J.J., (2006). Reactive oxygen species production is involved in quercetin-induced apoptosis in human hepatoma cells. *Nutrition and cancer*, *55*, 201-9.

Christ, J. P., Yu, O., Schulze-Rath, R., Grafton, J., Hansen, K. & Reed, S. D., (2021). Incidence, prevalence, and trends in endometriosis diagnosis: a United States population-based study from 2006 to 2015. *American journal of obstetrics and gynecology*, *225*(5), 500.e1–500.e9.

Clower, L., Fleshman, T., Geldenhuys, W. J. & Santanam, N., (2022). Targeting Oxidative Stress Involved in Endometriosis and Its Pain. *Biomolecules*, *12*(8), 1055.

Demiray, S., Pintado, M.E., & Castro, P.M.L., (2009). Evaluation of phenolic profiles and antioxidant activities of Turkish medicinal plants: Tiliaargentea, Crataegi folium leaves and Polygonumbistorta roots. *World Acad. Sci. Eng. Technol*., *54*, 312-317.

Doumouchtsis, K. K., Doumouchtsis, S. K., Doumouchtsis, E. K. & Perrea, D. N., (2009). The effect of lead intoxication on endocrine functions. *Pubmed*, *32*(2), 175-83.

Fowler, B., Bellinger, D. & Chisolm, J., (1993). *Measuring Lead exposure in infants, children, and other sensitive populations*. Washington, DC: National Academy Press.

Franks, P.A., Laughlin, N.K., Dierschke, D.J., Bowman, R.E. & Meller, P.A., (1989). Effects of lead on luteal function in rhesus monkeys. *Biol Reprod.*, 41, 1055–62.

Ghimire, S., Subedi, L., Acharya, N., & Gaire, B. P., (2021). *Moringa oleifera*: A Tree of Life as a Promising Medicinal Plant for Neurodegenerative Diseases. *Journal of agricultural and food chemistry*, *69*(48), 14358–14371.

Gupta S., Agarwal, A., Krajcir, N., & Alvarez, J. G., (2006). "Role of oxidative stress in endometriosis." *Reproductive BioMedicine Online*, *13*(1), 2291, 126–134.

Ibtisam, J. S., (2017). Study the adverse effects of exposure to lead acetate on mice ovarian tissue. *Int. J. Adv. Res*., *5*(5), 727-735.

Islam, Z., Islam, S. M. R., Hossen, F., Mahtab-Ul-Islam, K., Hasan, M. R., & Karim, R., (2021). *Moringa oleifera* is a Prominent Source of Nutrients with Potential Health Benefits. *International journal of food science*, 6627265.

Jackson L.W., Zullo M.D. & Goldberg J.M., (2008). The association between heavy metals, endometriosis and uterine myomas among premenopausal women: National Health and Nutrition Examination Survey 1999–2002. *Human Reproduction*, *23*(3), 679–687.

Jackson, L. W., Howards, P. P., Wactawski-Wende, J. & Schisterman, E. F., (2011). The association between cadmium, lead and mercury blood levels and reproductive hormones among healthy, premenopausal women. *Human reproduction*, *26*(10), 2887–2895.

Jackson, L.W., (2008). The association between heavy metals, endometriosis and uterine myomas among premenopausal women: National Health and Nutrition Examination Survey 1999–2002. *Human*

Reproduction, *23*(3), 679–687.

Johnson, N.P. & Hummelshoj, L., (2013). World Endometriosis Society Montpellier Consortium. Consensus on current management of endometriosis. *Human Reprod*uction, *28*(6), 1552–68.

Johnson, N.P., Hummelshoj, L. & Adamson, G.D., (2017). World Endometriosis Society consensus on the classification of endometriosis. *Human Reproduction*, *32*(2), 315–24.

Kashyap, P., Kumar, S., Riar, C. S., Jindal, N., Baniwal, P., Guiné, R. P. F., Correia, P. M. R., Mehra, R., & Kumar, H., (2022). Recent Advances in Drumstick (*Moringa oleifera*) Leaves Bioactive Compounds: Composition, Health Benefits, Bioaccessibility, and Dietary Applications. *Antioxidants (Basel, Switzerland)*, *11*(2), 402.

Kim, J. J. Kurita, T. & Bulun, S. E., (2013). "Progesterone action in endometrial cancer, endometriosis, uterine fibroids, and breast cancer." *Endocrine Reviews*, *34*, 130–162.

Kokot, I., Piwowar, A., Jędryka, M., Sołkiewicz, K. & Kratz, E. M., (2021). Diagnostic Significance of Selected Serum Inflammatory Markers in Women with Advanced Endometriosis. *International journal of molecular sciences*, *22*(5), 2295.

Lai, G.L., Yeh, C.C., Yeh, C.Y., Chen, R.Y., Fu, C.L., Chen, C.H. & Tzeng, C.R., (2017). Decreased zinc and increased lead blood levels are associated with endometriosis in Asian Women. *Reprod. Toxicol*., *74*, 77–84.

Monraz-Méndez, A., Escutia-Gutiérrez, R., Rodríguez-Sanabria, J.S. & Sánchez-Orozco, L., Armendáriz-Borunda, J. & Sandoval-Rodríguez, A., (2022). Moringa oleifera decreases biomarkers of oxidative stress in a murine NASH model. *Annals of Hepatology*., *27*, 100859.

Mu, F., Harris, H. R., Rich-Edwards, J. W., Hankinson, S. E., Rimm, E. B., Spiegelman, D. & Missmer, S. A., (2018). A Prospective Study of Inflammatory Markers and Risk of Endometriosis. *American journal of epidemiology*, *187*(3), 515–522.

Nwamarah, J.U., Otitoju, O. & Otitoju, G.T., (2015). Effects of Moringa oleifera Lam. aqueous leaf extracts on follicle stimulating hormone and serum cholesterol in Wistar rats. *African Journal of Biotechnology*, *14*(3), 181–186.

Ogunsola, A., Owolabi, J., Fabiyi, S., Nwobi, N.I. & Akinbola A.S., (2017). Moringa Plant Parts Consumption Had Effects on Reproductive Functions in Male and Female Rat Models. *IOSR Journal of Dental and Medical Sciences*, *16*, 82-86.

Orwa C & Mutua, A., (2009). Agroforestry Database: a tree reference and selection guide version 4.0. World Agroforestry Centre, Kenya.

Oyem, J. C., Chris-Ozoko, L. E., Enaohwo, M. T., Otabor, F. O., Okudayo, V. A., & Udi, O. A., (2021). Antioxidative properties of Ocimum gratissimum alters Lead acetate induced oxidative damage in lymphoid tissues and hematological parameters of adult Wistar rats. *Toxicology reports*, *8*, 215–222.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D. & Bitto, A., (2017). Oxidative Stress: Harms and Benefits for Human Health. *Oxidative medicine and cellular longevity*, *2017*, 8416763.

Radovich, T., (2009). Farm and forestry production and marketing profile for Moringa. In: Elevitch, C.R. (Ed.) *Specialty Crops for Pacific Island Agroforestry*. Permanent Agriculture Resources (PAR), Holualoa, Hawai.

Rzymski, P., Tomczyk, K., Rzymski, P., Poniedziałek, B., Opala, T. & Wilczak, M., (2015). Impact of heavy metals on the female reproductive system. *Annals of agricultural and environmental medicine AAEM*, *22*(2), 259–264.

Sharma, S., Singh, P. & Agarwal, M.K., (2016). The Effect of Chronic Lead Exposure on Fertility in female Rats via Hypothalamic — Pituitary and Ovarian Axis. *International Journal of Fauna and Biological Studies*. *3*(3), 91-2.

Siahaan, S. C. P. T., Santoso, B. & Widjiati, (2022). Effectiveness of Moringa oleifera Leaves on TNF-α Expression, Insulin Levels, Glucose Levels and Follicle Count in Rattus norvegicus PCOS Model. *Diabetes, metabolic syndrome and obesity: targets and therapy*, *15*, 3255–3270.

Singh, A.K., Chattopadhyay, R., Chakravarty, B. & Chaudhury, K., (2013). Markers of oxidative stress in follicular fluid of women with endometriosis and tubal infertility undergoing IVF. *Reprod. Toxicol*., *42*, 116–124.

Smarr, M.M., Kannan, K. & Buck Louis, G.M., (2016). Endocrine disrupting chemicals and endometriosis. *Fertil. Steril*., *106*, 959–966.

Symons, L.K., Miller, J. E., Kay, V. R., Marks, R.M., Liblik, K., Koti, M & Tayade, C., (2018). The Immunopathophysiology of Endometriosis. *Trends in Mol Med*, *24*(9), 748-764.

Toma, A & Deyno, S., (2014). Phytochemistry and pharmacological activities of Moringa oleifera. *International Journal of Pharmacognosy*, *1*, 222-231.

Touriño, S. Lizárraga, D. & Carreras A., (2008). Antioxidant/prooxidant effects of bioactive polyphenolics. *Electron J Environ Agric Food Chem*, *7*, 3348-52.

Udayraj, P. N., Satish, K. G., Abhishek, S., Soumen C., Rajkumar, S. Y., Kuldeep G. & Naresh, S., (2015). Lead-induced adverse effects on the reproductive system of rats with particular reference to histopathological changes in uterus. *Indian J Pharmacol*., *47*(1), 22–26.

WHO, (2018). International Classification of Diseases, 11th Revision (ICD11) Geneva.