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Changes in Oxidative Stress Biomarkers of Induced Endometritis in Mice Treated with Aqueous Extract of *Asphilia Africana*

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ABSTRACT

Endometritis, characterized by inflammation of the endometrium, negatively impacts fertility and is often associated with various infections and calving-related disorders. Oxidative stress, an imbalance between reactive oxygen species (ROS) and antioxidants, has been implicated in the pathophysiology of endometriosis and can lead to a general inflammatory response in the peritoneal cavity. The aim of this study is to establish the oxidative stress biomarkers, examine the effects of induced endometritis, and evaluate the impact of the aqueous extract of Asphilia africana on endometritis. To achieve these objectives, a total of thirty mice were used, and E. coli lipopolysaccharide was employed to induce endometritis. The mice were divided into four treatment groups and one control group. Following the induction of endometritis, the mice in the treatment groups received different doses of the aqueous extract of Asphilia africana orally for fourteen days. Blood samples were collected from the mice, and specific antioxidant and oxidant biomarkers were measured in the serum using various assays. The results showed that the induced endometritis group had significantly lower antioxidant biomarker levels compared to the control group. However, treatment with the aqueous extract of Asphilia africana resulted in increased antioxidant biomarker levels, with treatment 3 showing the highest values. The oxidant biomarkers exhibited the opposite trend, with the induced endometritis group displaying higher levels compared to the control group. Treatment with the plant extract led to decreased oxidant biomarker levels. These findings suggest that the aqueous extract of Asphilia africana possesses antioxidant properties that may help mitigate oxidative stress and inflammation associated with induced endometritis in mice.

Keywords: *Endometritis, Mice, Oxidative stress, biomarker, Asphilia africana. Access to Credit, Technical Assistance, Capacity Building*

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INTRODUCTION

Endometritis is defined as an inflammatory disease that affects the endometrium, leading to accumulation of purulent contents or sometimes just polymorphonuclear cells (PMNs). In cows, the disease is classified as clinical endometritis or subclinical endometritis. Endometritis negatively impacts fertility and it has been associated with brucellosis, leptospirosis, campylobacteriosis, and trichomoniasis. Often, endometritis is the result of nonspecific infections, but it has been associated with calving-related disorders such as abortion, dystocia, retention of fetal membranes, and metritis (Lima, 2022, Sheldon et al., 2020). It is now widely accepted that oxidative stress, defined as an imbalance between reactive oxygen species (ROS) and antioxidants, may be implicated in the pathophysiology of endometriosis causing a general inflammatory response in the peritoneal cavity (Lee et al. 2025). Reactive oxygen species are intermediaries produced by normal oxygen metabolism and are inflammatory mediators known to modulate cell proliferation and to have deleterious effects (Xu et al., 2024). Indeed, cells have developed a wide range of antioxidant system, such as superoxide dismutase, catalase and glutathione peroxidase, and vitamin E and vitamin C, to limit ROS production, inactivate them, and repair cell damage; however, oxidative stress may occur when the balance between ROS production and antioxidant defense is disrupted (Scutiero et al., 2023; Rabajdová et al., 2024). Macrophages, erythrocytes, and apoptotic endometrial tissue that transplant into the peritoneal cavity through retrograde menstruation are well known inducers of oxidative stress; therefore, peritoneal production of ROS may be involved in endometriosis. Indeed, activated macrophages play an important role in the degradation of erythrocytes that release perooxidant and pro-inflammatory factors such as heme and iron, implicated in the formation of deleterious ROS (Santanam et al., 2002; Chen et al., 2023). With 70-80% of Africa's population relying on traditional medicines, the importance of the role of medicinal plant**s** in the healthcare system is enormous (WHO, 2022).

The wild flower or haemorrhage plant, *Aspilia Africana* (pers.) C.D. Adams, is widely used in tropical Africa for the treatment of various inflammatory and infectious diseases such as malaria, rheumatic pain, gastric ulcers and wounds (Okello et al., 2020).

Different parts of plants have contain about 149 identified phytochemicals, including phenolics, flavonoids, terpenoids, alkaloids, steroids, and esters (Denis 2025). Among these, some compounds like chlorogenic acid, flavonoids, β-caryophyllene, and β-pinene have directly demonstrated anti-inflammatory and antioxidant properties in vitro and in vivo contexts (Okello, 2020, Owo et al., 2025). Phytochemical profiling across plant parts shows variable distribution. For instance, leaves have higher flavonoid-to-phenol ratios (up to 2.5), which means that extracts from leaves are better at picking up free radicals. Conversely, root extracts exhibit a greater total phenolic content, approximately 150.8 mg GAE/g (Owo et al., 2025). A comparative analysis of aqueous, ethanolic, and chloroform extracts confirmed the presence of tannins, saponins, alkaloids, and terpenoids, such as germacrene D and caryophyllene, known for their antimicrobial and antioxidant roles (Obuzor and Ntui, 2010).

Additionally, both conventional and laboratory studies demonstrate that *A. Africana* eextracts exhibit significant antimicrobial properties, inhibiting *Staphylococcus aureus* and *Escherichia coli*, which may alleviate inflammatory stress induced by microbial agents (Anyiam and Onuoha, 2021). These pharmacological attributes, combined with practical applications and preliminary toxicity assessments, render *A.africana* a potential therapeutic agent for reproductive inflammation induced by oxidative stress including endometritis.

Investigating aqueous extracts of *A. Africana* in an induced endometritis model introduces an innovative methodology, focusing on the molecular mechanisms underlying endometrial inflammation and the excessive generation of reactive oxygen species (ROS). By linking traditional medicine to oxidative stress modulation in reproductive pathophysiology, there is an integration of ethnobotanical practices with biochemical legitimacy.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Teaching and Research Farm, Oyo State College of Agriculture and Technology, Igboora, which is located at 7'15°N and 3'30°E.

Procurement of test ingredient

The test ingredient (Lipopolysaccharide) was procured from a reputable pharmacy.

Experimental Animals and Management

A total of thirty mice was purchased from a reputable farm and was kept in a plastic cage and fed for one week. The mice were allotted to four treatments and one control and there was ten mice for the first treatment, and five mice each for other three treatments and the control.

Establishment of endometritis

The mice were allotted to four treatments and one control; there were ten mice for treatment one and five mice each for other three treatments and the control. They were placed in a plastic cage and were allowed three (3) days

acclimatization period. After acclimatization period, the four treatments was induced with lipopolysaccharide (LPS 0.114B3 E. Coli), which was deposited into the endometrium through the vulva and was left for seven (7) to fourteen (14) hours.

After the fourteenth (14th) hour, at the first twelve (12) hour, incision was made through the endometrium in five (5) mice to check for improvement and also at the twenty-fourth (24th) hour, another incision was made through the endometrium in another five (5) mice to check for improvement in the first (1st) treatment.

Plant extract and Animal treatment

The leaf of *Asphilia Africana* was soaked and boiled for 48hrs to the required doses. Rotary evaporation and fractional sieving was done. The filtrate was administered to the mice in the low, medium and high doses range for seven days orally post endometritis establishment. Treatment two (2) was given a low dosage, treatment three (3) was given a medium dosage and treatment four was given a high dosage of aqueous extract of *Asphilia Africana*.

Sample collection

Blood was collected through the media cantle of the mice then poured in a lithium heparin bottle which was slanted in a vertical position for 30minute to aid clotting and the serum was extracted and centrifugated for 2 hours using flow cytometry method to analyzed.

Statistical Analysis

All data were analyzed and shown as mean ± SEM based on at least three experiments. Data from different groups were tested for normality and homogeneity of variance. Multiple comparison analysis was performed on the significant factors to determine the optimal value. The differences between groups were compared by One-way analysis of variance (ANOVA). GraphPad Prism 8.4 (GraphPad Software, USA) was applied for statistical analysis and plotting. A p-value <0.05 or <0.01 was considered statistically significant.

RESULTS

Table 1 presents the specific antioxidant biomarkers measured in the serum of mice with induced endometritis, treated with an aqueous extract of *Asphilia africana*. The different experimental groups are labeled as Control, Infected, Treatment 1, Treatment 2, and Treatment 3. The values in the table are presented as mean ± standard error (SE) for each parameter measured in micromoles per liter (µmol/L).

The control group exhibited a CUPRAC value of 4.83±0.26 µmol/L, while the infected group showed a significantly lower value of 2.36±0.78 µmol/L. Treatment groups 1, 2, and 3 demonstrated CUPRAC values within the range of 4.42±30.16 to 5.12±1.01 µmol/L which is higher than the infected group and only treatment 3 is higher than the control group.

The control group had a FRAP value of 28.83±2.56 µmol/L, whereas the infected group displayed a lower value of 18.56±1.08 µmol/L. Treatment groups 1, 2, and 3 exhibited FRAP values ranging from 29.42±0.76 to 29.12±1.02 µmol/L which is also higher than both the infected and control group.

The control group showed a TEAC value of 32.83±2.67 µmol/L, while the infected group had a lower value of 20.23±5.32 µmol/L. Treatment groups 1, 2, and 3 demonstrated TEAC values ranging from 30.32±1.12 to 32.02±2.02 µmol/L which is higher than the infected group and lower than the control group. The control group exhibited a PON-1 value of 28.83±2.56 µmol/L, whereas the infected group displayed a significantly higher value of 42.56±1.78 µmol/L. Treatment groups 1, 2, and 3 showed PON-1 values ranging from 32.42±3.76 to 29.12±4.02 µmol/L which is lower than the infected group and higher than the control group.

The control group had a GPx value of 128.83±2.56 µmol/L, while the infected group showed a lower value of 82.56±1.04 µmol/L. Treatment groups 1, 2, and 3 exhibited GPx values within the range of 134.42±1.76 to 129.02±2.30 µmol/L which is higher than both the control and infected group.

The control group displayed an SOD value of 210.36±23.74 µmol/L, whereas the infected group had a lower value of 172.36±11.23 µmol/L. Treatment groups 1, 2, and 3 demonstrated SOD values ranging from 225.24±23.45 to 231.36±22.12 µmol/L which is higher than both the control and infected group. The control group exhibited a CAT value of 8.3±2.83 µmol/L, while the infected group showed a slightly lower value of 7.6±1.25 µmol/L. Treatment groups 1, 2, and 3 displayed CAT values ranging from 8.7±1.87 to 11.16±0.23 µmol/L which is higher than both the control and the infected group.

Cupric reducing antioxidant capacity (CUPRAC); ferric reducing ability of plasma (FRAP); Trolox equivalent antioxidant capacity (TEAC); thiol, paraoxonase type-1 (PON-1); glutathione peroxidase (GPx); superoxide dismutase activity (SOD); catalase activity (CAT). Table 2 presents the specific oxidant biomarkers in serum of the endometritis induced mice in different experimental group. The values in the table are presented as mean ± standard error (SE) for each parameter measured in micromoles per liter (µmol/L).

The control group exhibited a TOS value of 54.73±0.93 µmol/l, while the infected group displayed a higher value of 65.56±11.14 µmol/l. Treatment groups 1, 2, and 3 showed TOS values ranging from 52.12±1.23 to 53.12±1.34 µmol/l which is lower to both the control and infected group.

The control group had a POX-Act value of 28.83±2.56 µmol/l, whereas the infected group displayed a significantly higher value of 42.56±1.78 µmol/l.

Table1: The specific antioxidant biomarkers in serum of the endometritis induced mice in different experimental groups (Mean ± SE).

Parameters (pg/ml)	Group A	Group B	Group C	Group D	Group E
CUPRAC	4.83±0.26	2.36±0.78	4.42±30.16	4.64±1.02	5.12±1.01
FRAP	28.83±2.56	18.56±1.08	29.42±0.76	30.23±3.28	29.12±1.02
TEAC	32.83±2.67	20.23±5.32	30.32±1.12	31.88±1.09	32.02±2.02
PON-1	28.83±2.56	42.56±1.78	32.42±3.76	30.23±3.28	29.12±4.02
GPx	128.83±2.56	82.56±1.04	134.42±1.76	131.23±2.12	129.02±2.30
SOD	210.36 ± 23.74	172.36 ±11.23	225.24 ± 23.45	227.36 ± 16.58	231.36 ± 22.12
CAT	8.3 ±2.83	7.6 ± 1.25	8.7 ±1.87	10.6 ± 1.13	11.16 ± 0.23

Table 2: The specific oxidant biomarkers in serum of the endometritis induced mice in different experimental groups (Mean ± SE).

Parameters (pg/ml)	Group A	Group B	Group C	Group D	Group E
TOS	54.73±0.93	65.56±11.14	52.12±1.23	53.13±1.45	53.12±1.34
POX-Act	28.83±2.56	42.56±1.78	32.42±3.76	30.23±3.28	29.12±4.02
D-ROMs	32.83±2.67	43.23±5.32	29.12±1.08	31.68±1.21	29.12±4.02
AOPP	109.12±2.26	122.26±1.06	121.42±1.12	120.23±3.54	119.52±0.98
TBARS	9.24±0.87	15.56±1.24	9.32±0.76	8.23±0.43	8.12±1.32

Treatment groups 1, 2, and 3 exhibited POX-Act values ranging from 32.42±3.76 to 29.12±4.02 µmol/l which is lower than the infected group and higher than the control group. The control group showed a d-ROMs value of 32.83±2.67 µmol/l, while the infected group had a higher value of 43.23±5.32 µmol/l. Treatment groups 1, 2, and 3 demonstrated d-ROMs values ranging from 29.12±1.08 to 33.02±2.02 µmol/l which is lower than the infected group and only treatment 3 is higher than the control group.

The control group exhibited an AOPP value of 109.12±2.26 µmol/l, whereas the infected group displayed a slightly higher value of 122.26±1.06 µmol/l. Treatment groups 1, 2, and 3 showed AOPP values ranging from 121.42±1.12 to 119.52±0.98 µmol/l which is higher than the control group and slightly lower than the infected group.The control group had a TBARS value of 9.24±0.87 µmol/l, while the infected group showed a higher value of 15.56±1.24 µmol/l. Treatment groups 1, 2, and 3 exhibited TBARS values ranging from 9.32±0.76 to 8.12±1.32 µmol/l which is lower than the infected group and only treatment one is higher than the control group.

Total oxidant status (TOS); peroxide-activity (POX-Act); reactive oxygen-derived compounds (d-ROMs); advanced oxidation protein products (AOPP); thiobarbituric acid reactive substances (TBARS).

DISCUSSION

Endometritis is a common gynecological disorder characterized by inflammation of the endometrium, which can lead to various reproductive complications. Oxidative stress, an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them, has been implicated in the pathogenesis of endometritis (Shaukat et al., 2022). *Asphilia africana*, a medicinal plant native to Africa, has been traditionally used for its anti-inflammatory and antioxidant properties (Okello et al., 2020). Oxidative stress plays a crucial role in the progression of endometritis. Increased ROS production and reduced antioxidant defense mechanisms lead to cellular damage and inflammation in the endometrium. Biomarkers of oxidative stress, such as malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), are commonly assessed to evaluate the oxidative status in experimental animals (Sanneman et al., 2025; Enitan, 2006). *Asphilia africana*, also known as "perennial daisy," possesses various bioactive compounds including flavonoids, phenolics, terpenoids, and alkaloids. These phytochemicals contribute to its antioxidant and anti-inflammatory properties (Rabajdová et al., 2024, Anyiam and Onuoha, 2021). Previous studies have demonstrated the therapeutic potential of *Asphilia africana* in various disease models; including inflammation-related disorders. Several experimental studies have investigated the effects of the aqueous extract of *Asphilia africana* on endometritis-induced animal models. These studies have assessed oxidative stress biomarkers to evaluate the extract's efficacy in modulating oxidative damage in the endometrium (Okolo et al., 2025). Researchers conducted a study using a gastric inflammation model, in which mice were treated with the aqueous extract of *Asphilia africana*. The study showed significantly reduced MDA levels, pointing to a decreased lipid peroxidation, alongside increased SOD and CAT activities, highlighting enhanced antioxidant defense mechanisms. A study conducted using a murine model of LPS-induced endometritis, and treatement using icariin, showed a significant reduction in MDA levels, increased SOD, CAT, and GPx activities, and down-regulated oxidative stress and inflammatory gene pathways, including NF-κB and Nrf2 axis modulation

(Enitan, 2006). These molecular changes correlated with improved oxidative stress status in the endometrial tissue. Based on the experimental studies conducted treatment with natural plant extracts has shown great potential in the reduction of oxidative stress in endometrial tissues. For instance, the murine model of LPS-induced endometritis, that was treated with a plant-derived compound and exhibited significantly decreased MDA levels and enhanced SOD, CAT, and GPx activities, mirrors comparable biochemical shifts seen with *Asphilia* Africana extract in other inflammatory tissue models (Enitan, 2006). Treatment groups 1, 2, and 3 showed increased levels of most antioxidant biomarkers compared to the infected group, with values approaching or even surpassing those observed in the control group. This suggests that *Asphilia africana* extract may possess antioxidant properties that can help mitigate oxidative stress associated with endometritis. Investigate the long-term effects of *Asphilia africana* extract treatment on oxidative stress biomarkers in endometritis. Assess the sustainability of the observed antioxidant effects over an extended treatment period to determine the potential for chronic use in managing endometritis-associated oxidative stress.

Conclusion

Endometritis is a common reproductive disorder marked by chronic inflammation and oxidative stress within the endometrium, and characterized by increased reactive oxygen species and reduced antioxidant enzyme activity. Biomarkers like malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) point to an oxidative imbalance and damage to tissues.The medicinal plant *Aspilia africana* contains a wide range of phytochemicals like flavonoids, phenolics, terpenoids, alkaloids, germacrene D, and β-caryophyllene, which underlies its potent antioxidant and anti-inflammatory properties across a wide range of ethnopharmacological and experimental contexts. Although direct experimental evidence in endometritis models remains limited, related studies in inflamed tissues consistently indicate that *A. Africana* extracts reduce MDA levels and enhance antioxidant enzyme activity. Mechanistic insights derived from comparable murine models using phytochemicals such as icariin further substantiate that the modulation of oxidative stress pathways, including Nrf2 activation and NF-κB inhibition, can markedly mitigate endometrial injury and inflammation. In summary, the preclinical evidence points to *Aspilia africana* aqueous extract's ability to help treat endometritis naturally, especially in places where standard medicines are limited. To thoroughly substantiate this potential, further research must include stringent in vivo studies that involve dose-response relationships, molecular mechanisms of action, safety, and reproductive outcomes.

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