

Dietary supplementation with Powdered Ginger root ameliorates Lead Carbonate-induced Ovarian Injuries in Adult Wistar Rats

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Abstract

Lead contamination has remained a persistent global health issue; with lead being known to disrupt biological systems such as the reproductive system by inducing oxidative stress and ovarian damage. Plants containing antioxidant molecules such as ginger (*Zingiber officinale*) are promising remedies due to their antioxidant and anti-inflammatory properties. This study investigated the protective effects of powdered ginger root against lead-induced ovarian toxicity in female Wistar rats. Twenty-four female Wistar rats were randomly assigned to four experimental groups of six (6) rats each: Animals in Group A served as the healthy control and received standard diet plus distilled water, animals in Group B (lead control) received standard diet and lead carbonate (30 mg/kg) via gavage. Groups C and D received lead carbonate (30 mg/kg) and 1% and 5% ginger supplemented diets respectively. The experiment lasted 28 days, after which the animals were euthanised, and blood and ovarian tissue samples were collected. Biochemical assays were conducted to evaluate oxidative stress markers, including malondialdehyde (MDA), total antioxidant capacity (TAC), Tumor Necrosis Factor-alpha (TNF- α), and interleukin-6 (IL-6). Histological analysis of ovarian tissues was performed to assess morphological changes. Statistical analysis was conducted using ANOVA with significance set at $p < 0.05$. Lead exposure significantly increased MDA levels and pro-inflammatory cytokines (TNF- α and IL-6) while reducing TAC compared to the healthy control group ($p < 0.05$). Rats whose diet were supplemented with ginger had a dose-dependent reduction in MDA and inflammatory cytokines; alongside an increase in TAC. Histological examination of the ovaries of Lead-Carbonate treated rats revealed degenerative changes, including follicular atresia and stromal damage, which were mitigated in ginger-treated groups, particularly at the 5% supplementation level. Ginger supplementation protected against lead-induced ovarian toxicity by reducing oxidative stress and inflammation, while preserving ovarian morphology. These findings suggest that ginger may serve as a natural therapeutic agent for mitigating heavy metal-induced reproductive toxicity. Further studies are recommended to elucidate the molecular mechanisms underlying its protective properties.

Keywords: Gonadotoxicity, Food supplements, Plumbum, Environmental Toxicants, Oxidative stress

1. Introduction

Over the past four decades, increasing industrialisation and urbanisation have significantly worsened environmental lead contamination, thereby posing a significant public health concern [1]. Lead, a pervasive heavy metal, is widely utilised in various industries, including ceramics, shipbuilding, cosmetics, paint production, and battery manufacturing [2]. Despite regulatory efforts to curb lead exposure, it remains a significant environmental pollutant, particularly in developing countries

where occupational and residential exposures are prevalent [3]. Lead contaminates air, soil, and water; with human exposure increasing through inhalation, ingestion, and dermal absorption [4].

Lead is documented to have systemic pathological effects in both humans and experimental animals [5]. It disrupts multiple biological systems, including the haematopoietic [6], hepatic [7,8], renal [8,9], and reproductive systems [10,11]. Specifically, lead exposure is linked to reproductive toxicity, impairing both male and female reproductive organs. In

females, lead accumulation in ovarian tissues leads to oxidative stress, hormonal imbalances, follicular atresia, and reduced fertility [12]. The primary mechanism underlying lead-induced ovarian toxicity involves the generation of reactive oxygen species (ROS), depletion of cellular antioxidants such as glutathione, and inhibition of sulfhydryl-dependent enzymes, leading to lipid peroxidation and apoptosis of ovarian cells [13,14].

Given the adverse reproductive consequences of lead toxicity and the fact that we have not yet succeeded in totally avoiding human contact with lead; there is a growing need for safe and effective agents that help with detoxification and reduction in the deleterious impact of lead on the body. In this regards, natural sources of such agents are valuable due to potential ease of access. Generally, naturally-occurring antioxidants, particularly phytochemicals, have gained considerable attention as potential therapeutic agents for mitigating heavy metal toxicity [15]. Among these, ginger (*Zingiber officinale*), a widely consumed medicinal plant, has demonstrated promising pharmacological properties [16]. Traditionally used in Ayurvedic, Chinese, and Unani medicine, ginger possesses potent anti-inflammatory, antioxidant, and detoxifying properties [17,18,19].

Recent studies have highlighted the protective effects of ginger against various toxic insults, including alcohol-induced hepatotoxicity [20], fungicide-induced liver damage [21], and heavy metal toxicity due to cadmium/mercury [22,23]. The bioactive constituents of ginger include gingerols, shogaols, and paradols which have antioxidant effects through their scavenging of free radicals, enhancement of enzymatic antioxidant defences, and modulation of inflammation [24,25,26]. Emerging scientific evidence suggests that ginger has the ability to mitigate lead-induced oxidative stress; and it can also preserve cellular integrity and function [27].

Despite extensive research on lead toxicity, limited studies have explored the potential of ginger in counteracting lead-induced ovarian dysfunction. Therefore, this study evaluated the protective effects of ginger against lead-induced ovarian toxicity in female Wistar rats.

2. Methodology

2.1 Materials and Reagents

Ginger roots, distilled water, and normal saline were utilised in this study. Fresh ginger roots were sourced from a local market in Osogbo, Osun State, Nigeria. The ginger was thoroughly washed, cut into smaller pieces, and subsequently dried using an oven at 39°C. Once completely dried, it was ground into fine powder using a Rico mixer grinder.

2.2 Experimental Animals

Healthy female Wistar rats used for this study were obtained from an animal facility located in the Igbo Sai area of Ogbomoso, Oyo State, Nigeria. Upon acquisition, the animals were transported to the Animal Research Facility at the University of Ilesa, Osun State, Nigeria, where they were allowed a fourteen-day acclimatisation period before the commencement of the study. The rats were housed in plastic cages with dimensions of 25 × 15 × 14 inches, under a controlled temperature of 22.5 °C with a margin of ±2.5 °C. A 12-hour light-dark cycle was maintained, with light exposure beginning at 7:00 a.m. Throughout the experiment, the rats were provided unrestricted access to food and water. All handling and procedures were performed following the ethical guidelines of the Faculty of Basic Medical Sciences, University of Ilesa, and adhered to the European Council Directive (EU2010/63) on the care and use of laboratory animals.

2.3 Diet

The rats were fed a commercially formulated diet in mashed form to meet their dietary requirements. The standard pellet diet contained essential macronutrients, including carbohydrates, proteins, fats, and calcium. Ginger powder was incorporated into the mashed diet at two supplementation levels (1% and 5%) by thoroughly mixing the powdered ginger with the feed, to ensure homogeneity and consistent distribution of the supplement. The experimental diets were supplied *ad libitum* for a period of 28 days.

2.4 Experimental Methods

A total of twenty-four (24) female Wistar rats, weighing between 150–170 g each, were randomly assigned to four experimental groups, with each group consisting of six (6) animals:

Group A: Healthy control group, received standard diet and vehicle (distilled water).

Group B: Lead control group, received standard diet and lead carbonate at 30 mg/kg orally via gavage.

Group C: Lead-treated group, administered lead carbonate at 30 mg/kg and diet supplemented with dried powdered ginger at 1%.

Group D: Lead-treated group, administered lead carbonate at 30 mg/kg and diet supplemented with dried powdered ginger at 5%.

The period of experimentation was 28 consecutive days. On the 29th day, the animals were euthanised by cervical dislocation, following the ethical protocols outlined by the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals (2020) [28]. Blood samples were

collected via intracardiac puncture and placed in heparinised tubes. The samples were centrifuged and subsequently analysed for biochemical markers such as MDA (Malondialdehyde), TAC (Total Antioxidant Capacity), TNF- α , and IL-6. Additionally, ovary tissues were harvested and preserved in 10% neutral buffered formalin for histological examination.

2.5 Biochemical Assays

2.5.1 Determination of Tumor Necrosis Factor- α and Interleukin-6 Levels

Concentrations of tumor necrosis factor alpha (TNF- α), and interleukin-6 (IL-6) were quantified in samples using enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer's protocols [36].

2.5.2 Assessment of Lipid Peroxidation

Lipid peroxidation was measured by determining malondialdehyde (MDA) levels in plasma using the thiobarbituric acid reactive substances (TBARS) assay, according to established protocols. In this method, MDA in the samples reacts with thiobarbituric acid to form a coloured complex, which is then quantified by measuring absorbance at 532 nm with a spectrophotometer [37].

2.5.3 Measurement of Total Plasma Antioxidant Capacity

Total antioxidant capacity (TAC) in plasma was evaluated using a commercial antioxidant assay kit in 96-well plates, following the manufacturer's instructions [38]. Briefly, metmyoglobin and chromogen were added to both plasma and standard samples, and the reaction was initiated by adding hydrogen peroxide working solution. The plate was incubated at room temperature for 5 minutes, after which absorbance was read at either 750 nm using a microplate reader [38].

2.6 Histological Examination and Photomicrography

Fixed ovary samples were processed, sectioned, and stained for histological evaluation. Microscopic examination was performed, and images of tissue sections were captured for further analysis.

2.7 Statistical Analysis

Statistical evaluation of the data was conducted using Graph Pad Prism 9 software for Windows. The results were expressed as mean $\pm$ standard error of the mean (S.E.M.). The threshold for statistical significance was set at $p < 0.05$.

3. Results

3.1 Effects of Ginger (*Zingiber Officinale*) Root on Biochemical parameters

Table 1, Figures 1 and 2 show the effects of powdered ginger supplement on oxidative stress and inflammatory markers in lead-exposed female rats. Lead administration (Group B)

caused a significant increase ($p < 0.05$) in malondialdehyde (MDA), Tumor necrosis factor (TNF)- α , and interleukin (IL)-6 levels, alongside a significant decrease in Total antioxidant capacity (TAC), compared to the control group (Group A). These changes indicate increased oxidative damage and inflammation due to lead toxicity.

Supplementation with ginger powder at both 1% (Group C) and 5% (Group D) significantly ameliorated these effects. Specifically, MDA levels were reduced and TAC levels increased in ginger-treated groups relative to the lead-only group, as shown in Table 1 and Figure 1. Similarly, ginger supplementation significantly lowered TNF- α and IL-6 concentrations induced by lead administration, as presented in Table 1 and Figure 2. The 5% ginger group exhibited greater improvements across all biochemical markers compared to the 1% group.

Table 1: Effects of *Zingiber officinale* on Biochemical Markers

Groups	MDA ($\mu\text{M/L}$)	TAC (mmol/l)	TNF- α (pg/mL)	IL-6 (pg/mL)
Control	1.05 $\pm$ 0.054	2.34 $\pm$ 0.17	99.55 $\pm$ 1.394	15.50 $\pm$ 0.89
Lead	2.13 $\pm$ 0.112 ^{ac}	1.05 $\pm$ 0.05 ^{ac}	143.5 $\pm$ 4.573 ^{ac}	29.32 $\pm$ 0.81 ^{ac}
Lead+G1	1.90 $\pm$ 0.004 ^{abc}	1.62 $\pm$ 0.02 ^{ab}	142.3 $\pm$ 5.340 ^a	25.48 $\pm$ 1.11 ^{abc}
Lead+ G5	1.48 $\pm$ 0.045 ^a	1.63 $\pm$ 0.15 ^a	114.3 $\pm$ 4.675	19.28 $\pm$ 0.53 ^a

Data presented as mean $\pm$ SEM; $p < 0.05$ Groups: A=control, B=lead, C=lead+1% ginger, D=lead+5% ginger). a, b, and c, represents significant change when compared to Control, Lead only, and Lead + 5% Groups respectively. G1: Ginger 1%, G5: Ginger 5%

3.2 Effects of Ginger (*Zingiber Officinale*) Root on Histomorphology of the ovaries

Plate 1 shows representative haematoxylin and eosin-stained sections of the slides of rat ovaries. Examination of photomicrographs of rats in the Control group (Plate 1A) revealed normal ovarian morphology with intact oocytes, well-structured follicles including preovulatory follicle and secondary follicle dispersed within a healthy ovarian stroma. Animals in the Lead-control group (Plate 1 B) showed evidence of toxicity with presence of numerous atretic follicles including damaged granulosa cells and abnormal stroma. In the groups treated with 1 % Ginger (Plate 1C) there was evidence of partial protection with reduced atresia and improved ovarian stroma. While in the animals treated with 5% ginger (Plate 1D) substantial protection with preserved follicles, healthy granulosa cells, and near-normal stroma was observed.

Discussion

The increasing environmental prevalence of heavy metals like lead poses significant risks to female reproductive health, with the ovary being particularly vulnerable due to its high metabolic activity and sensitivity to oxidative stress [29, 30]. Lead

exposure disrupts ovarian function through multiple mechanisms, including lipid peroxidation, inflammatory cytokine upregulation, and follicular atresia; via oxidative damage to granulosa cells [31]. These pathological changes correlate with altered steroidogenesis, hormonal imbalances, and reduced fertility [32].

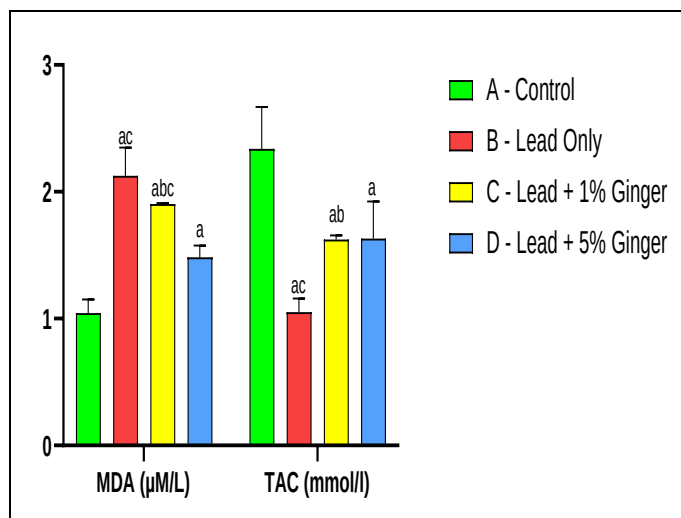


Fig 1: Effects of ginger on the levels of malondialdehyde (MDA), and total antioxidant capacity (TAC) in lead-administered female rats

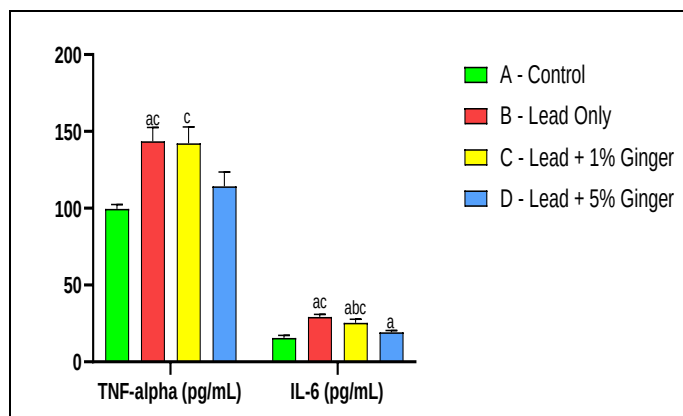


Fig 2: Effects of ginger on the levels of Tumor Necrosis Factor (TNF- α), and interleukin 6 (IL-6) in lead-administered female rats

The results from this study show a significant increase in malondialdehyde (MDA), a marker of lipid peroxidation, in the lead-exposed group (Group B), compared to the healthy control group (Group A). This elevation in MDA indicates substantial oxidative stress induced by lead exposure. Conversely, the total antioxidant capacity (TAC) was significantly reduced in Group B, reflecting a depletion of antioxidant defences. Ginger supplementation, particularly at 5% (Group D), effectively mitigated lead-induced oxidative stress. Malondialdehyde level in Group D was significantly lower than in Group B, and TAC level was significantly higher, indicating an augmentation of antioxidant capacity. The 1% ginger diet (Group C) also showed a trend towards improvement, but the effects were less pronounced compared to the 5% diet, as represented in Table 1 and Figure 1. These findings suggest that ginger, rich in

bioactive compounds such as gingerols and shogaols [16], effectively scavenges free radicals and enhances antioxidant defences, thereby protecting against lead-induced oxidative damage [9]

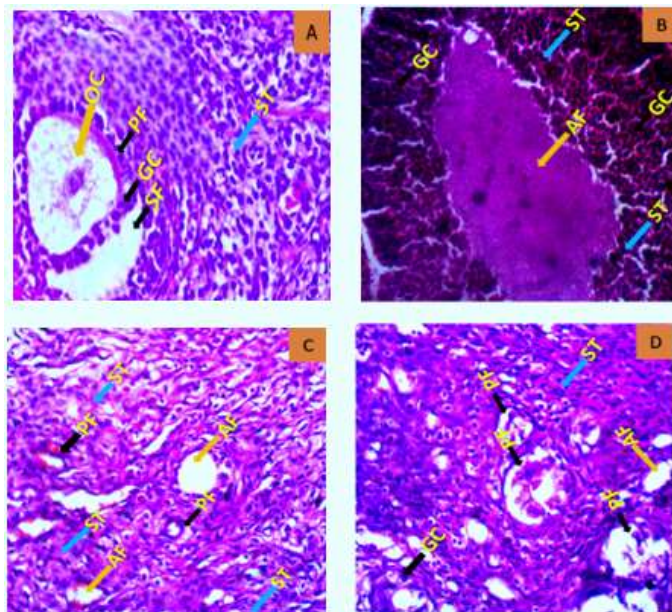


Plate 1: Histological Analysis of Ovarian Tissue in Wistar Rats Exposed to Lead and Treated with Ginger. (A) Control group, showing normal ovarian morphology with intact oocytes, well-structured follicles (PF: preovulatory follicle, SF: secondary follicle), and healthy stroma (ST). (B) Lead-exposed group, demonstrated significant ovarian toxicity with increased atretic follicles (AF), damaged granulosa cells (GC), and abnormal stroma. (C) The lead-exposed group was treated with a 1% ginger diet, showing partial protection with reduced atresia and improved stroma. (D) The lead-exposed group treated with a 5% ginger diet exhibited substantial protection with preserved follicles, healthy granulosa cells, and near-normal stroma. Images were captured at [Magnification 400x].

Lead exposure significantly elevated the levels of pro-inflammatory cytokines, tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6) in Group B, compared to Group A. Tumor necrosis factor- α increased in the lead-exposed group compared to the healthy control; similarly, IL-6 also increased. These results indicate that lead triggers a profound inflammatory response in the ovaries. However, ginger supplementation, particularly at 5%, effectively attenuated lead-induced inflammation as represented in Table 1 and Figure 2, where TNF- α and IL-6 levels in Group D were significantly lower compared to Group B. The 1% ginger diet was also associated with a reduction in inflammatory markers, though the reduction was not as pronounced as that observed with the 5% diet. The ability of ginger to modulate inflammatory responses may be attributed to its inhibitory effects on NF- κ B signaling, a key regulator of pro-inflammatory gene expression [33].

Microscopic examination of ovarian tissues revealed alterations in animals administered lead, with improvement in groups fed with ginger supplemented feed. While the Control Group (Plate 1A), ovarian morphology was normal, with intact oocytes

surrounded by well-organised granulosa cells and healthy stromal tissue; the lead-exposed group (Plate 1B) displayed significant ovarian damage, characterised by an increased number of atretic follicles, disorganised granulosa cells, and stromal alterations. Ginger supplementation conferred a protective effect, with the 1% ginger diet (Group C) showing moderate preservation of follicular structure and reduced atresia. However, the 5% ginger diet (Group D) demonstrated substantial restoration, with follicular integrity closely resembling that of the control group. The reduced atresia and improved granulosa cell organisation suggest that ginger mitigates apoptosis, enhances follicular survival, and prevents mitochondrial dysfunction [34, 35]. These histological improvements align with the biochemical findings, further supporting the protective effects of ginger against lead-induced ovarian damage.

The study revealed a clear protective effect of ginger, with the 5% ginger diet showing more pronounced benefits compared to the 1% diet. This suggests that a high concentration of ginger's bioactive compounds may be necessary to counteract the adverse effects of lead.

Conclusion

The present study provides evidence in support of lead-induced oxidative stress, inflammation, and structural alterations in ovarian tissue. While ginger supplementation appeared to have some protective effects, the findings are limited by the scope and depth of the research. Therefore, the ability of ginger to mitigate lead-induced ovarian toxicity should be further investigated. Further studies are necessary to elucidate the underlying molecular mechanisms, including its potential impact on apoptotic pathways and steroidogenic gene expression, before definitive therapeutic recommendations can be made.

Author Contributions

HLA conceived the idea for the project and played a key role in editing the manuscript. **FKP** was responsible for data analysis. All authors, including **OA**, **AKE**, **OAA**, and **LMB**, carried out the laboratory work and contributed to writing the manuscript. All authors approved the final version of the manuscript.

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Competing interests

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