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PROTECTIVE EFFECTS OF COMBINED VITAMIN E AND ZINC SULFATE ON OVARIAN AND UTERUS WEIGHT OF ALBINO RATS (*Rattus norvegicus*) EXPOSED TO LEAD ACETATE

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Abstract

Lead exposure exerts toxic effects on the female reproductive system, particularly on the ovaries and uterus, resulting in hypofunction and organ atrophy. Oxidative stress induced by lead accumulation may be attenuated by antioxidants such as vitamin E and zinc sulfate. This study aimed to evaluate the protective effects of vitamin E, zinc sulfate, and their combination on ovarian and uterus weights in albino rats (Rattus norvegicus) exposed to lead acetate. Twenty-five female rats were randomly divided into five groups (K, T0, T1, T2, and T3) and treated orally for 21 days. On day 22, the rats were euthanized, and the ovaries and uterus were collected and weighed. The average ovarian weights for K, T0, T1, T2, and T3 were 25.80 ± 2.49; 18.62 ± 4.36; 28.40 ± 5.67; 33.78 ± 6.09; and 36.84 ± 5.15 mg, respectively. The average uterus weights for K, T0, T1, T2, and T3 were 292.48 ± 36.81; 249.96 ± 25.97; 315.86 ± 26.56; 325.54 ± 31.53; and 375.00 ± 36.55 mg, respectively. In conclusion, vitamin E and zinc sulfate demonstrated protective effects against lead-induced reproductive toxicity, with the combined treatment showing the greatest improvement in ovarian and uterus weights.

Keywords: *Antioxidant Protection, Lead Acetate, Ovarian and Uterus weight, Oxidative Stress, Reproduction Health*

INTRODUCTION

Industrial development has contributed to improved human living standards while simultaneously increasing environmental contamination, particularly from heavy metals such as lead (Swain, 2024). Lead is a toxic substance that can accumulate in the body and enter metabolic pathways through ingestion of contaminated food or water, as well as through inhalation of airborne dust particles, thereby inducing dysfunction in various organ systems, including the reproductive system (Mumtaz *et al*., 2020; Suksmerri., 2018; Sundari *et al*., 2016). Based on data reported by the Indonesian Food and Drug Authority (BPOM RI), in 2016 there were 4,643 cases of food poisoning outbreaks, with 975 cases caused by lead exposure.

Meanwhile, in 2017 there were 2,401 outbreak cases, with 576 cases related to lead exposure (BPOM RI, 2018). These data show that the risk of lead exposure through the food chain remains very high and potentially dangerous to public health in the long term (Kumar et al., 2020).

One major pathway underlying lead toxicity involves the induction of oxidative stress through increased generation of Reactive Oxygen Species (ROS) (Aitken et al., 2020). Elevated ROS levels create an imbalance between free radical production and the body's antioxidant defense system, resulting in impaired cellular function, including within the reproductive system (Lopes et al., 2016). Oxidative stress triggered by lead exposure has been reported to decrease Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) levels. Reduced concentrations of these hormones inhibit Graafian follicle maturation and lower estrogen synthesis, which may impair normal uterus function (LiC et al., 2018; Saeed et al., 2017).

The body actually has a natural defense system to combat free radicals, known as endogenous antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Ighodaro et al., 2018). However, under toxic exposure conditions such as those caused by heavy metals, this system is often insufficient to counteract excessive oxidative stress (Taiwo et al., 2023). Therefore, exogenous antioxidants such as vitamin E are needed to help neutralize free radicals. vitamin E is a lipid-soluble antioxidant that works by protecting cell membranes from oxidative damage and inhibiting lipid peroxidation (Beredugo et al., 2022). In addition to vitamin E, zinc also possesses antioxidant properties by maintaining cell membrane stability and stimulating the formation of antioxidant proteins that are capable of neutralizing ROS (Prasad., 2014; Khafaji., 2023). Accordingly, this study aims to evaluate the protective effects of combined vitamin E and zinc sulfate administration on ovarian and uterus weights in albino rats (*Rattus norvegicus*) exposed to lead acetate.

MATERIALS AND METHODS

Experimental animals consisted of 25 albino rats (Rattus norvegicus), Wistar strain, with body weights ranging from 250 to 300 g. The materials used in this study included lead acetate trihydrate (EMSURE® ACS, Merck, Germany) at a dose of 1.5 mg/kg body weight based on Uchewa et al. (2019), vitamin E ((±)-α-tocopherol synthetic, ≥96% HPLC, Sigma-Aldrich, USA) at a dose of 100 mg/kg body weight according to Soemardini (2016), and zinc sulfate heptahydrate (EMSURE® ACS, Merck, Germany) at a dose of 0.54 mg/kg body weight following Larasati et al. (2019). Aquadest, physiological NaCl, and corn oil were also used during the experiment. Before the treatment phase began, the rats underwent a one-week acclimatization period to adapt to their environment, cages, and diet. Throughout the experiment, the animals were maintained in standard laboratory cages with free access to food and drinking water (ad libitum) under stable environmental conditions according to laboratory animal care guidelines (National Research Council et al., 2010).

Research Procedure

Female albino rats (*Rattus norvegicus*), wistar strain, were randomly assigned into five treatment groups. Animals in the control group were administered 0.5 mL of aquadest and 0.5 mL of corn oil, followed four hours later by an additional 0.5 mL of aquadest. The T0 group received 0.5 mL of aquadest and 0.5 mL of corn oil, and after a four hours interval, lead acetate was administered at a dose of 1.5 mg/kg body weight dissolved in 0.5 mL of aquadest. The T1 group was given 0.5 mL of aquadest and vitamin E at a dose of 100 mg/kg body weight dissolved in 0.5 mL of corn oil, followed four hours later by lead acetate at a dose of 1.5 mg/kg body weight dissolved in 0.5 mL of aquadest. The T2 group received zinc sulfate at a dose of 0.54 mg/kg body weight dissolved in 0.5 ml of aquadest along with 0.5 mL of corn oil, and after four hours, lead acetate was administered at a dose of 1.5 mg/kg body weight in 0.5 mL of aquadest. The T3 group received zinc sulfate at a dose of 0.54 mg/kg body weight dissolved in 0.5 mL of aquadest and vitamin E at a dose of 100 mg/kg body weight dissolved in 0.5 mL of corn oil. Four hours after administration, lead acetate was given at a dose

of 1.5 mg/kg body weight dissolved in 0.5 mL of aquadest. All treatments were administered orally once daily for 21 consecutive days (Uchewa et al., 2019).

Sample Collection

The final body weights of all experimental animals were recorded on day 22 using a digital scale. Data from both initial and final body weights were used to ensure homogeneity and normal distribution. After the final body weights were recorded, the animals were euthanized using the cervical dislocation method according to the AVMA Guidelines for the Euthanasia of Animals (AVMA, 2020). Following euthanasia, the rats were necropsied to collect the ovaries and uterus. The organs were rinsed with physiological saline (NaCl), dried using filter paper, and weighed using an Ohaus® analytical balance with a precision of 0.0001 g. The recorded weights were then converted into milligrams. After weighing, the ovary and uterus samples were placed in sample containers for further analysis.

Data Analysis

Data analysis was performed using Statistical Product and Service Solution (SPSS) version 20 (IBM Corp., Armonk, NY, USA). Ovarian and uterus weight data were evaluated by one-way analysis of variance (ANOVA) for normally distributed data with homogeneous variances. When significant differences were observed, further analysis was performed using Duncan's test (Setiawan et al., 2022).

RESULTS

Data were collected to evaluate the effects of vitamin E and zinc sulfate on ovarian and uterus weights in albino rats (*Rattus norvegicus*) exposed to lead acetate. A total of 25 albino rats were assigned into five experimental groups (K, T0, T1, T2, and T3), with 5 repetitions in each treatment group.

Table 1: Mean percentage and standar deviation of right ovary weight, left ovary weight, and average ovary weight of albino rats (*Rattus norvegicus*) subjected to lead acetate.

Treatment Group	Right Ovary Weight (X ± SD)	Left Ovary Weight (X ± SD)	Average Ovary Weight (X ± SD)
K	$25,16^{b} \pm 2,45$	$26,42^{b} \pm 2,92$	$25,80^{b} \pm 2,49$
T0	$18,48^{a} \pm 3,24$	$18,70^{a} \pm 5,52$	$18,62^{a} \pm 4,36$
T1	$28,04^{bc} \pm 5,52$	$28,68^{b} \pm 6,16$	$28,40^{bc} \pm 5,67$
T2	$33,72^{cd} \pm 6,34$	$33,80^{bc} \pm 6,02$	$33,78^{cd} \pm 6,09$
T3	$36,76^{d} \pm 4,85$	$36,88^{c} \pm 5,61$	$36,84^{d} \pm 5,15$

Note: Different superscripts (a, b, c, d) in the same column indicate a significant difference ($p < 0.05$)

Control group (K) showed significant differences compared with T0, T2, and T3, but not with T1. Group T0 differed significantly from all other groups. T1 was significantly different from T0 and T3, but not from K and T2. Group T2 differed from K and T0, but not from T1 and T3. Meanwhile, T3 showed significant differences from K, T0, and T1, but not from T2.

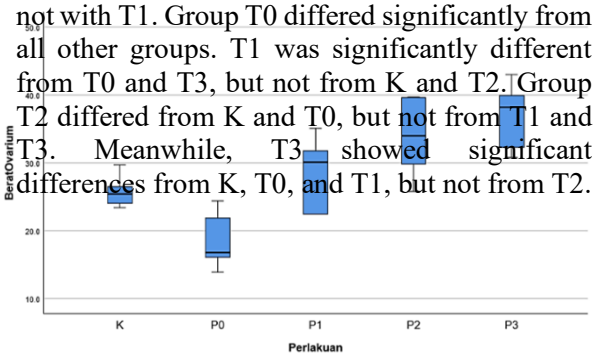

Figure 1. Box plot of ovarian weight in albino rats (*Rattus norvegicus*) following lead acetate exposure and antioxidant treatment with vitamin E and zinc sulfate. Data are presented as mean ± SD (n = 5).

Table 2: Mean percentage and standard deviation of uterus weight of albino rats (*Rattus norvegicus*) exposed to lead acetate.

Treatment Group	Uterus Weight (X ± SD)
K	$292,48^{b} \pm 36,81$
T0	$249,96^{a} \pm 25,97$
T1	$315,86^{b} \pm 26,56$
T2	$325,54^{b} \pm 31,53$
T3	$375,00^{c} \pm 36,55$

Note: Different superscripts (a, b, c, d) in the same column indicate a significant difference ($p < 0.05$).

Evaluation of uterus weight revealed that the control group (K) exhibited significant differences compared with the T0 and T3 groups, while no significant differences were observed compared with the T1 and T2 groups. The T0 group showed significant differences compared with all other experimental groups. Significant differences were also observed between the T1 group and the T0 and T3 groups; however, no significant differences were detected between the T1 group and the K and T2 groups. Similarly, the T2 group differed significantly from the T0 and T3 groups but did not differ from the K and T1 groups. In contrast, the T3 group demonstrated significant differences compared with all other groups.

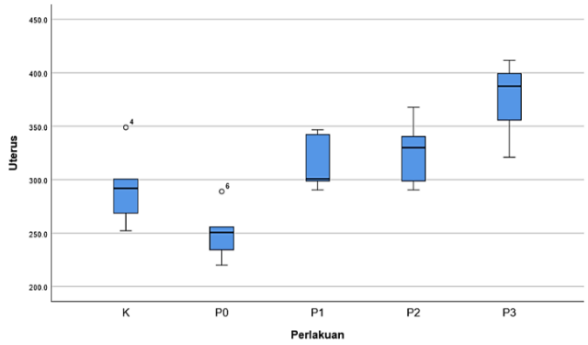

Figure 2. Box plot of uterus weight in albino rats (*Rattus norvegicus*) following lead acetate exposure and antioxidant treatment with vitamin E and zinc sulfate. Data are presented as mean ± SD (n = 5).

The ovaries and uterus organs of the albino rats (*Rattus norvegicus*) can be seen in **Figure 3.** The organs were then weighed using an Ohaus® analytical balance with an accuracy of 0.0001g and converted to milligrams.

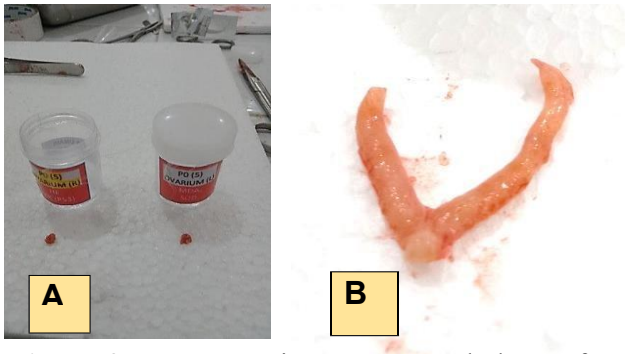

Figure 3. Representative gross morphology of ovaries and uterus collected from albino rats (Rattus norvegicus) following lead acetate exposure and antioxidant treatment with vitamin E and zinc sulfate. (A) Ovaries. (B) Uterus.

DISCUSSION

Ovary Weight

Findings of the present study indicate that treatment in the T0 group, involving exposure to lead acetate at a dose of 1.5 mg/kg body weight for 21 days, resulted in a reduction in ovarian weight in albino rats (*Rattus norvegicus*). This observation is consistent with the study reported by Uchewa et al. (2019), which demonstrated that lead administration at the same dose and duration induced reproductive toxicity and interfered with normal reproductive organ development. Reproductive dysfunction associated with lead exposure is primarily related to the induction of Reactive Oxygen Species (ROS). Accumulation of lead in the body enhances ROS production, leading to oxidative stress (Li et al., 2018). Oxidative stress can cause damage to lipid membranes and DNA. Lipid membrane injury promotes lipid peroxidation, thereby altering membrane composition, structure, and integrity, which ultimately results in cellular, tissue, and reproductive organ damage (Simanjuntak et al., 2020). Oxidative stress may also disrupt hypothalamic function, leading to reduced secretion of Follicle Stimulation Hormone (FSH) from the anterior pituitary gland. Since FSH plays a critical role in ovarian follicle development, suppression of this hormone can inhibit folliculogenesis, reduce follicle numbers, and consequently decrease ovarian weight (Nurkarimah et al., 2017).

Treatment in the T1 group, which received vitamin E combined with lead acetate exposure, resulted in a higher ovarian weight compared with the T0 group. Vitamin E contributes to the maintenance of ovarian follicular development by preventing lipid peroxidation and protecting cellular membranes from oxidative damage. Previous studies reported that vitamin E can attenuate degenerative changes in ovarian function and support normal ovarian physiology (Beredugo, 2022). The antioxidant mechanism of vitamin E involves inhibition of lipid peroxidation, thereby preserving cell membrane

integrity and supporting normal folliculogenesis (Ningtyas et al., 2019). This finding is consistent with Soemardini (2016), who reported that oral administration of vitamin E at 100 mg/kg body weight effectively mitigates oxidative stress induced by lead exposure. Vitamin E is recognized as a major lipid-soluble radical scavenger that interrupts oxidative chain reactions by neutralizing lipid peroxyl radicals. This mechanism plays a critical role in reducing lead-induced oxidative stress characterized by excessive reactive oxygen species (ROS) production and lipid peroxidation in reproductive tissues (Niki, 2014).

Results obtained from the T2 group, which received zinc sulfate in combination with lead acetate exposure, demonstrated an increase in ovarian weight. The mean ovarian weight in this group was higher than that observed in the T1 and T0 groups. These findings are consistent with the report by Soussi et al (2018), indicating that zinc sulfate functions as an antioxidant by stabilizing cell membranes and protecting tissues from oxidative stress-induced demage. Zinc also plays a critical role in the regulation of ovarian function and ovulation in female reproduction (Garner et al., 2021). Supplementation with zinc may increase systemic zinc levels, maintain the stability of Follicle Stimulating Hormone (FSH), and enhance lymphocyte activity, which subsequently contributes to increased Superoxide Dismutase (SOD) activity. Elevated SOD activity plays an important role in strengthening the body's antioxidant defense system against oxidative stress (Faghfouri et al., 2021).

This study also showed that group T3, which received a combination of zinc sulfate and vitamin E, exhibited a significantly higher increase in ovarian weight in albino rats (Rattus norvegicus) exposed to lead acetate compared with the T1 and T2 groups, which received vitamin E and zinc sulfate respectively. The increase in ovarian weight was attributed to antioxidant administration, which may protect reproductive tissues from oxidative stress induced by lead acetate exposure. This effect is likely due to the synergistic interaction between zinc sulfate and vitamin E. Vitamin E acts as a chain-breaking antioxidant by neutralizing free radicals induced by lead acetate and limiting oxidative damage, whereas zinc plays an essential role in maintaining cellular structure and membrane integrity (Mesalam et al., 2023).

Uterus Weight

Results obtained from the T0 group, which was exposed to lead acetate at a dose of 1.5 mg/kg body weight for 21 days without antioxidant supplementation such as zinc sulfate or vitamin E, demonstrated a reduction in uterus weight when compared with the control group (K). Exposure to lead acetate alone resulted in a significant decrease in uterus weight. Continuous exposure to lead leads to its accumulation in the body, resulting in toxicity that may adversely affect the reproductive system. Elevated levels of accumulated lead are toxic and may cause dysfunction in various organs (Sari et al., 2018).

Group T1, which was administered vitamin E at a dose of 100 mg/kg body weight in conjunction with lead acetate exposure, exhibited a higher uterus weight than group T0. This effect is attributed to the antioxidant activity of vitamin E, which protects cell membranes from oxidative damage induced by free radicals, as reported by Raharjo et al. (2018). Furthermore, Mumtaz (2020) stated that vitamin E neutralizes peroxyl radicals and inhibits lipid peroxidation, thereby maintaining oxidant–antioxidant balance. When there is an excess of ROS from physiological metabolism, the -OH group of vitamin E donates a hydrogen radical to lipid radicals or lipid hydroperoxide radicals (Ainun et al., 2021). Vitamin E acts as a lipid-soluble chain-breaking antioxidant that terminates lipid peroxidation reactions by neutralizing lipid peroxyl radicals, thereby preventing oxidative damage and supporting cellular integrity in reproductive organs (Traber & Atkinson, 2007). In addition, zinc contributes to antioxidant defense by enhancing endogenous antioxidant enzymes such as superoxide dismutase (SOD), thereby strengthening cellular protection against reactive oxygen species (ROS)-induced damage (Prasad, 2014).

Group T2, which received zinc sulfate at a dose of 0.54 mg/kg body weight along with lead acetate exposure, exhibited an increase in uterus weight compared with groups T1 and T0. According to Wardani (2018), zinc is an essential mineral with an effective antioxidant effect in

body tissues. Zinc can induce the formation of proteins that neutralize ROS and acts as a cofactor for important enzymes contributing to the function of the antioxidant defense system. Moreover, zinc protects cells from oxidative damage. Adequate zinc concentration plays an important role in preventing oxidative stress by reducing the oxidation of lipids, proteins, and DNA (Khadivi et al., 2020).

The findings in the T3 group, which received a combination of antioxidant consisting of zinc sulfate and vitamin E, demonstrated a greater increase in uterus weight in albino rats (*Rattus norvegicus*) exposed to lead acetate when compared with the T1 group (vitamin E) and the T2 group (zinc sulfate) under the same lead exposure. This outcome aligns with the biological role of antioxidants in reducing oxidative stress through stabilization of free radicals by donating electrons, thereby interrupting free-radical chain reactions (Chaudhary et al., 2023). Zinc sulfate functions as a primary antioxidant by enhancing the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD), which protects cells against oxidative damage, whereas vitamin E acts as a secondary antioxidant that scavenges and neutralizes free radicals (Marreiro et al., 2017). Another factor that can influence ovarian and uterus weights in albino rats (Rattus norvegicus) is the reproductive cycle. However, a limitation of this study is the absence of reproductive cycle examination, where the reproductive cycle consists of the proestrus, estrus, metestrus, and diestrus phases. The proestrus phase involves the growth of Graafian follicles under the influence of FSH and lasts for 12 hours. The estrus phase is marked by the peak increase of estrogen hormone in the blood; high estrogen levels cause FSH secretion to decrease, while LH secretion increases to stimulate oocyte maturation. This phase lasts for 10–20 hours. The metestrus phase is characterized by the formation of the corpus luteum, which produces progesterone and lasts for approximately 21 hours. Diestrus is the final and longest phase of the reproductive cycle. During this phase, follicular development resumes and prepares the reproductive system to re-enter the proestrus phase (Ajayi & Akhigbe, 2020). In addition, the present study evaluated only gross ovarian and uterus weights and did not include histopathological examination of reproductive tissues.

CONCLUSION

The results of the present study indicate that lead acetate exposure adversely affects reproductive organs, as evidenced by a decrease in ovarian and uterus weights in albino rats (*Rattus norvegicus*). Administration of the antioxidant vitamin E and zinc sulfate provided a protective effect against oxidative stress induced damage in these organs. Notably, the combined use of both antioxidants was more effective in restoring organ weight than either treatment alone. These results suggest that the combination of vitamin E and zinc sulfate holds potential as a therapeutic strategy to mitigate reproductive dysfunction caused by heavy metal exposure.

ETHICAL APPROVAL

The experimental protocol was approved by the Animal Care and Use Committee of Universitas Airlangga (Approval No. 884/HRECC.FODM/XII/2022).

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