Original Article

Effect of *Thymus Vulgaris* on Hormonal Profile and Immunohistochemistry of Ovarian and Uterine Vascular Endothelial Growth Factor in Lead Acetate-treated Rats

Muna Hassan Youssef*, Sadiq Jaffer Ramadhan, Majida Abdul Khaliq Jafaar Al-Qayim

Departments of Physiology, Biochemistry, and Pharmacology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

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ABSTRACT

Background: *Thymus vulgaris* is a plant rich in essential oils acclaimed for the management of oxidative stress and inflammation in the organs. Meanwhile, the heavy metal lead is widely distributed in nature and continued exposure to lead acetate causes reduced fertility.

Objectives: The present study aimed to investigate the effects of *T. vulgaris* on ovarian and uterine structural and functional characteristics in female rats exposed to lead acetate.

Methods: Three groups of 18 mature Wistar albino female rats (*Rattus norvegicus*), 15 weeks old and weighing between 200 and 210 g, were established and handled for 60 days as follows: Group A (control group) received 0.5 mL of distilled water (DW) daily; group B received 5 mg/kg body weight (BW) of lead acetate via oral gavage; and group C received 5 mg/kg BW of lead acetate via oral gavage followed by 75 mg/kg BW of *T. vulgaris* extract 2 hours later. Blood and tissue samples (uterus and ovary) were collected from euthanized animals.

Results: Lead acetate caused oxidative stress, as indicated by increased malondialdehyde (MDA) levels and decreased superoxide dismutase (SOD) activity. It also caused a decrease in serum estrogen and an increase in progesterone levels. Meanwhile, *T. vulgaris* caused a decrease in progesterone and MDA levels and an increase in estrogen levels and SOD activity. The histological changes of the ovary and uterus in the lead acetate group showed vascular degeneration and necrosis, and the expression of vascular endothelial growth factor (VEGF) revealed an increase in positive cells. All these changes were restored to normal by *T. vulgaris*.

Conclusion: Using alcoholic extracts of *T. vulgaris* acts as an antioxidant, helping to restore ovarian and uterine structure and function to near-normal levels in lead acetate-exposed rats.

Keywords: Immunohistochemistry, Lead, *Thymus vulgaris*, Uterine index, vascular endothelial growth factor (VEGF)

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*** Corresponding Author:**
Muna Hassan Youssef
Address: *Departments of Physiology, Biochemistry, and Pharmacology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.*
Phone: *+964 (781) 3701503*
E-mail: *muna.hasan@covm.uobaghdad.edu.iq*

Introduction

Heavy metal lead (Pb) is widely distributed in nature and persists in biotopes for an extended time in both humans and animals. This hazardous metal enters the body through various organs and accumulates, causing significant tissue damage that can range from cancer to cellular degeneration and disease (Harshitha et al., 2024). Continued exposure to lead and high blood levels of lead have both been linked to reduced fertility (Alabbassi et al., 2008; Osowski et al., 2023). Lead is thought to have an adverse effect on the female reproductive system in many species because it causes oxidative stress and increases reactive oxygen species production (Massányi et al., 2020). During pregnancy, lead can cross the placenta and has been associated with intrauterine mortality, preterm birth, and low gestational weight. Additionally, chronic exposure to Pb in laboratory animals may prevent monkeys from ovulating, menstruating, or developing follicles (Vermande-Van Eck & Meigs, 1960). In lead-exposed mice, the ovaries showed a drop in the number of primordial follicles and a rise in the number of atretic follicles. When present in the uterus, it harms the endometrium, myometrium, and perimetrium in mice, while also reducing the size of the uterine gland and the height of columnar cells (Qureshi & Sharma, 2012).

Vascular permeability factor (VPF), commonly known as vascular endothelial growth factor (VEGF) is a strong mitogen for the vascular endothelium. The action of VEGF involves three members of the tyrosine kinase family of receptors, and it has five isoforms resulting from the alternative splicing of the same gene. Numerous studies suggest that VEGF / VPF may play a role in the physiological control of ovarian angiogenesis. The production and release of this growth factor in the ovary suggest its involvement in cyclic angiogenesis and the regulation of vascular permeability, both of which are essential for ovarian folliculogenesis and healthy reproductive function. Defects in ovarian angiogenesis can lead to anovulation and infertility, pregnancy loss, ovarian hyperstimulation syndrome, and ovarian neoplasms (Rauniyar et al., 2023).

Thymus vulgaris is a plant rich in essential oils and a high content of oxygenated monoterpenes and monoterpene hydrocarbons (Capatina et al., 2020). Particularly, the largest concentrations of thymol, carvacrol, P-cymene, borneol, trans-caryophyllene, and cis-sabinene hydrate can be found (Noroozisharaf & Kaviani, 2018). Additionally, thymus species include phenolic compounds, including such as rosmarinic acid and flavonoid derivatives. *T. vulgaris* is ranked among the plant foods with the highest antioxidant activity, free radical scavenging activity, and proapoptotic effects due to these phytochemicals (Al-Kassie, 2009; Al-Naqqash et al., 2014). Through the removal of excess free radicals, this antioxidant activity either directly or indirectly influences the body's antioxidant system, therefore defending the organism. Therefore, this study was designed to evaluate the effect of *T. vulgaris* extract on lead-induced female reproductive system impairment.

Materials and Methods

T. vulgaris extract preparation

The ethanolic extract of *T. vulgaris* was prepared according to the standard method by soaking 30 g of dried plant leaf powder in 300 mL of 70% ethanol for 72 hours with intermittent shaking at room temperature, followed by filtration through the Whatman-2 filter paper. This step was repeated three times. The solvent was evaporated using a rotary evaporator, resulting in a net extract weight of 11 g of the dried powder, with a clearance ratio of 36% (Hmidani et al., 2019).

Experimental design

Female albino Wister rats (*Rattus norvegicus*), 15 weeks old and weighing between 200 and 210 g, were used in this study. After two weeks of acclimation in the animal house of the College of Veterinary Medicine, University of Baghdad, the experimental protocols were evaluated by an ethics committee to confirm their accordance with the Animal Use Protocol and compliance with the guidelines of the University of Baghdad. Subsequently receiving approval. Eighteen rats were used and distributed into three equal groups, which were handled as follows: Control group (A): 0.5 mL of distilled water (DW) daily; Group B: Lead acetate 5 mg/kg BW daily (Alqayim & Asis, 2013), Group C: Lead acetate 5 mg/kg BW + *T. vulgaris* ethanolic extract at 75 mg/kg BW, administered 2 hours after lead acetate administration daily for 60 days (Abdulrazzaq et al., 2023).

Experimental animals were housed in a well-ventilated room in plastic cages, and they had free access to food and water during the experimental period. Room temperature was kept at 22±2 ˚C with a 12 h light/dark cycle during the period of acclimatization and experiment. Blood samples were taken by the heart puncture method from rats after being sedated with intramuscular injections of ketamine-HCl (90 mg/kg BW) and xylazine (40

mg/kg BW) (Switzerland). The serum was extracted by centrifugation at 3000 rpm for 15 min and stored in a deep freezer for later examination of the following parameters. The experimental animals were housed in a well-ventilated room in plastic cages and had free access to food and water during the experimental period. The room temperature was maintained at 22±2 ˚C with a 12-hour light/dark cycle during both the acclimatization and experimental periods. Blood samples were collected using the heart puncture method from the rats after they were sedated with intramuscular injections of ketamine-HCl (90 mg/kg BW) and xylazine (40 mg/kg BW) (Switzerland). The serum was extracted by centrifugation at 3000 rpm for 15 minutes and stored in a deep freezer for later examination of the following parameters.

Body weight, female reproductive hormones, and antioxidant status measurements

The body weight of animals was measured at the beginning of the experiment and weekly until the end of the experiment. Weight gain was also measured. Serum estrogen and progesterone were measured using the COBAS e411 kit from Hitachi Roche Diagnostics GmbH, Mannheim, Germany (Laufer et al., 1982). The levels of serum malondialdehyde (MDA) were determined by a modified procedure (Guidet & Shah, 1989), and the activity of superoxide dismutase (SOD) was measured by spectrophotometric analysis (Weydert & Cullen, 2010).

Ovarian and uterine histopathology and immunohistochemistry evaluation

The ovarian and uterine paraffin sections were stained with hematoxylin and eosin for histopathological investigation by an expert pathologist. The VEGF levels were evaluated in ovarian and uterine sections using the immunohistochemistry technique, following the protocol of PathnSitu's highly sensitive and specific PolyExcel detection system. In brief, around 4 cm-thick sections of paraffin-embedded ovarian and uterine samples from three rats per group were collected on charged slides and dried overnight at 60 ˚C.

The sections were then deparaffinized, rehydrated, and subjected to heat-induced epitope retrieval (HIER) by boiling the tissue in the PT module for 20 minutes at 95 ˚C in vitro. EDTA buffer, pH 8.4. After this step, the sections were rinsed with 3-5 changes of distilled or deionized water and allowed to chill for 20 minutes at room temperature. Endogenous peroxidase was blocked for 10 minutes at room temperature using a peroxidase solution (ref. MAD-021540Q-125). The primary antibody was applied to the tissue slices and incubated for 30 minutes. The antibodies were diluted 1:50 in a solution containing bovine serum albumin and 0.05% sodium azide (NaN_3). The Master Polymer Plus Detection System-HRP (DAB included; ref. MAD-000237QK) was used for detection, utilizing the kit from Dako Denmark A/S (Pérez-Gutiérrez & Ferrara, 2023).

Statistical analysis

SAS statistical software, version 9.1 was used to perform data analysis. One-way ANOVA was employed to assess significant differences among means. The least significant difference (LSD) post-hoc test was used to compare all possible pairs of means, as all pairwise comparisons were of interest. The significance level was set at $P<0.01$ to maintain a stringent criterion for declaring differences significant.

Results

The present results revealed that lead acetate caused a significant reduction ($P\leq0.01$) in body weight gain in both groups treated with lead acetate alone and in combination with *T. vulgaris* (Table 1). The activity of the antioxidant enzyme SOD, as shown in Table 2, was decreased in the lead acetate group and increased in the T. vulgaris-treated group compared with the control group ($P<0.01$). In contrast, MDA levels showed a significant increase ($P\leq0.01$) in the lead acetate group and a decrease in the *T. vulgaris* group within the Pb-Ac group.

The female reproductive hormones (Table 3) in the serum of the lead group showed a significant increase ($P\leq0.01$) in progesterone and a decrease in estrogen. The group treated with *T. vulgaris* and lead showed significantly ($P\leq0.01$) decreased progesterone and increased estrogen levels. The uterus index (Table 4) revealed a significant decrease ($P\leq0.01$) in the lead group.

Ovarian and uterine histopathology evaluation

Histopathology of the examined tissues (ovaries and uterus) from animals in control group A showed normal architecture of the ovarian tissue, with mature follicles in the cortex and a normal orientation of vasculature in the medulla (Figure 1A). Occasionally, newly formed follicles were observed, characterized by multiple layers of follicular cells and theca placida (secondary follicle). The endometrium appeared normal in sections of the uterus; smooth muscle bundles of the myometrium were also noted, as were endometrial glands lined with a single layer of cuboidal epithelium (Figure 2A).

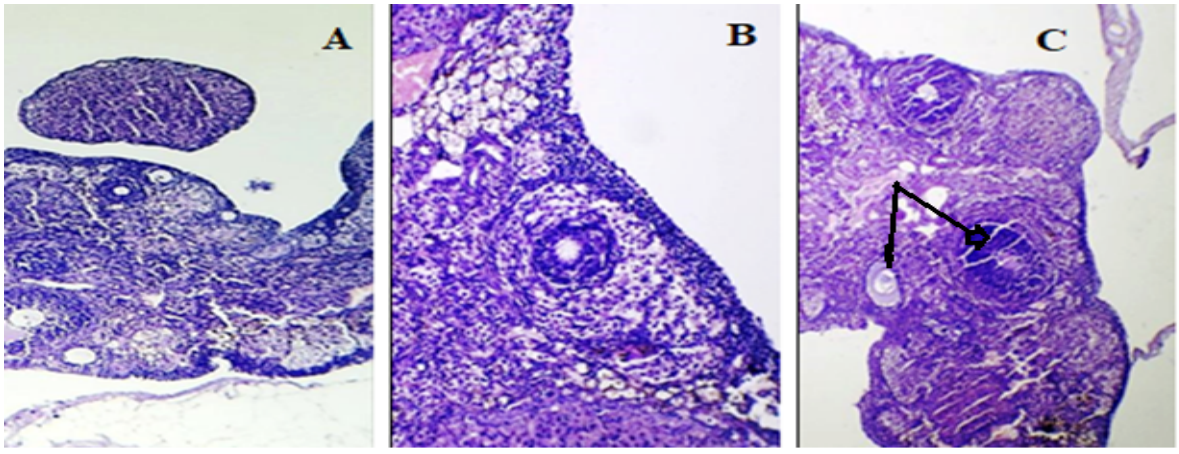

Figure 1. Light microscopic image of the ovary

A) Mature follicles in the tunica albuginea with the normal orientation of vasculature in the medulla (control group), B) Vacuolar degeneration of follicular cells and congestion of blood vessels (Pb group), C) Showing proliferation of follicles (Pb + *T. vulgaris* group) (H&E stain, ×40: A, ×100: B&C).

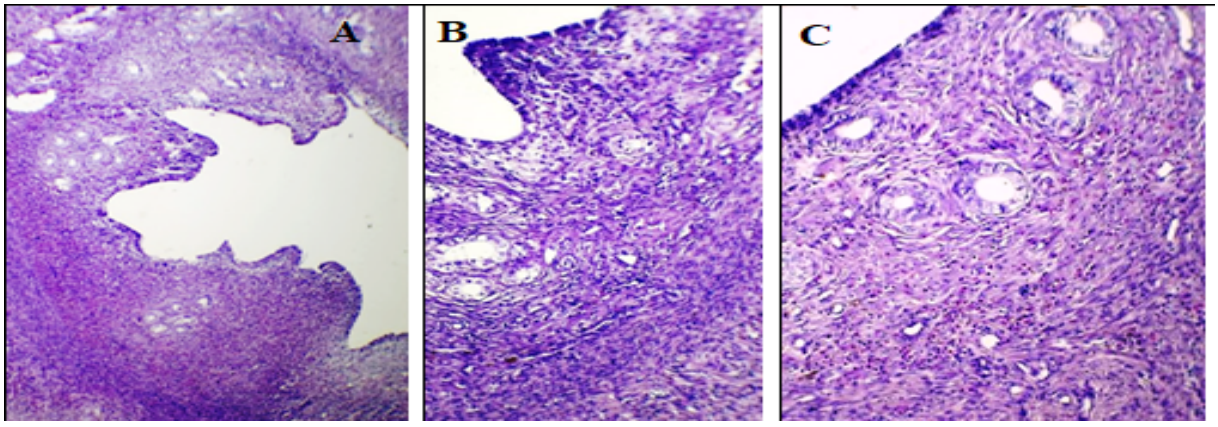

Figure 2. Light microscopic image of the uterus

A) Normal endometrium and smooth muscle bundles of the myometrium(control group), B) Desquamation and vacuolar degeneration of the lining epithelium (Pb group), C) Mild hyperchromatic lining epithelium (Pb + T. vulgaris group) (H&E stain: ×40: A, ×100: B&C).

In group B treated with lead acetate, there was shrinkage and distortion of ovarian tissue due to follicle atresia in the tunica albuginea, and no further proliferation of follicles was observed. Additionally, there was severe vacuolar degeneration of the follicular epithelium, dilation of medullary blood vessels, and fibroplasia of the stroma (Figure 1B). The uterus sections revealed vacuolar degeneration of the lining epithelium in the endometrium, as well as enlargement of the secretory glands in the submucosa due to swelling of the cuboidal lining epithelium (Figure 2B).

In group C, which was exposed to lead acetate and treated with *T. vulgaris* extract, there was a proliferation of secondary follicles (Figure 1C). Most of the uterine tissues appeared to have normal architecture; however, mild changes were occasionally noted, including epithelial degeneration and cystic dilation of endometrial glands (Figure 2C).

Ovarian and uterine VEGF by immunohistochemistry

The immunohistochemistry results related to the detection of angiogenesis marker, VEGF, indicated by brown color resulting from the reaction of the specific antibody with VEGF antigen, and Dap staining indicated positive results. Uterine sections (Figure 3) from the control

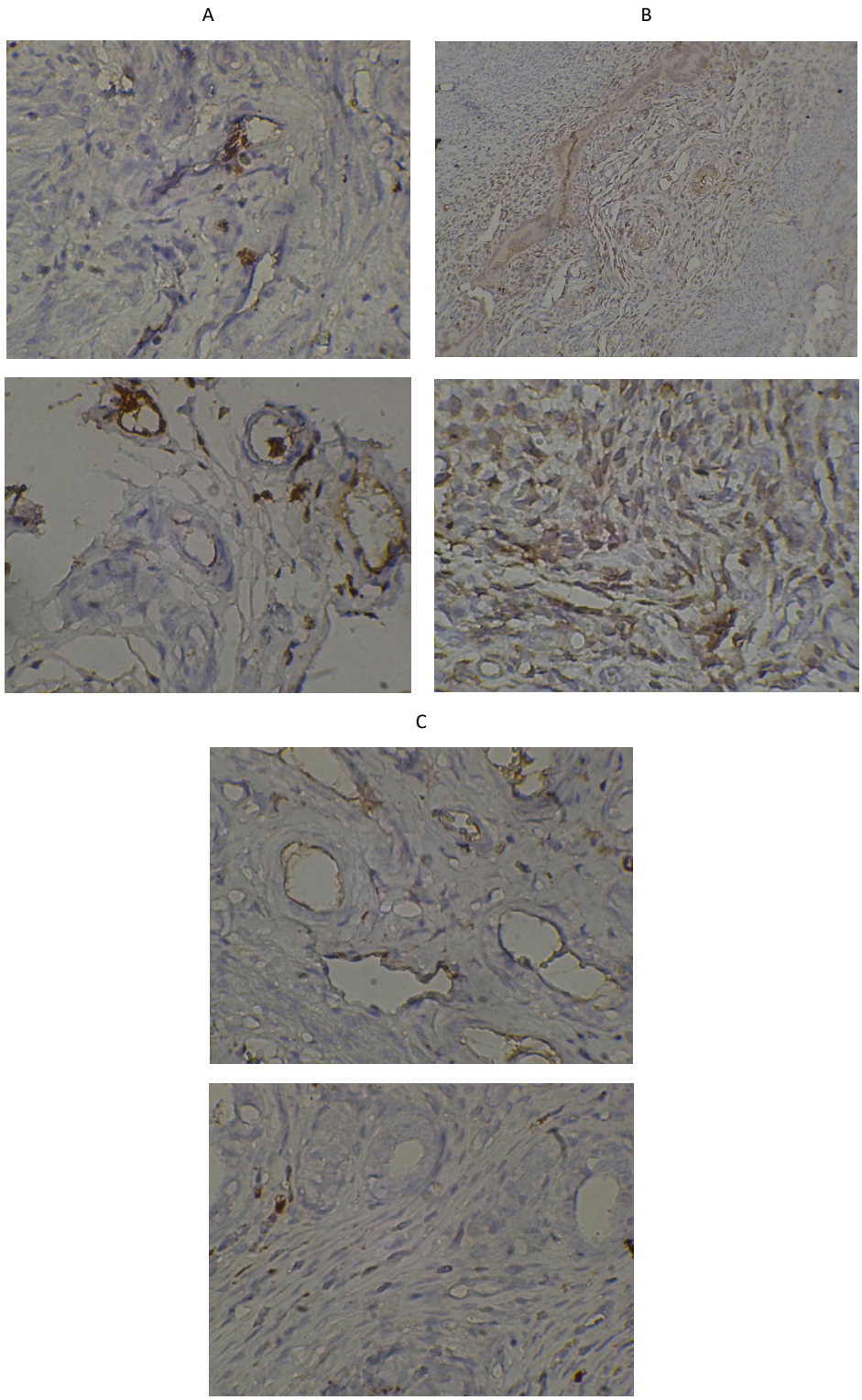

Figure 3. Light microscopic immunoreactivity for VEGF in the uterus

A) Normal minimum presence of immune-positive cells for VEGF-(control group); B) High levels of immune-positive VEGF cells, mostly scattered in the stromal gland (Pb group), C) Marked immune positivity for VEGF in uterine stromal blood vessels with a rare appearance in stromal layers (Pb+ *T. vulgaris* group)

Note: DAB reaction at ×10 line 1 and ×40 line 2.

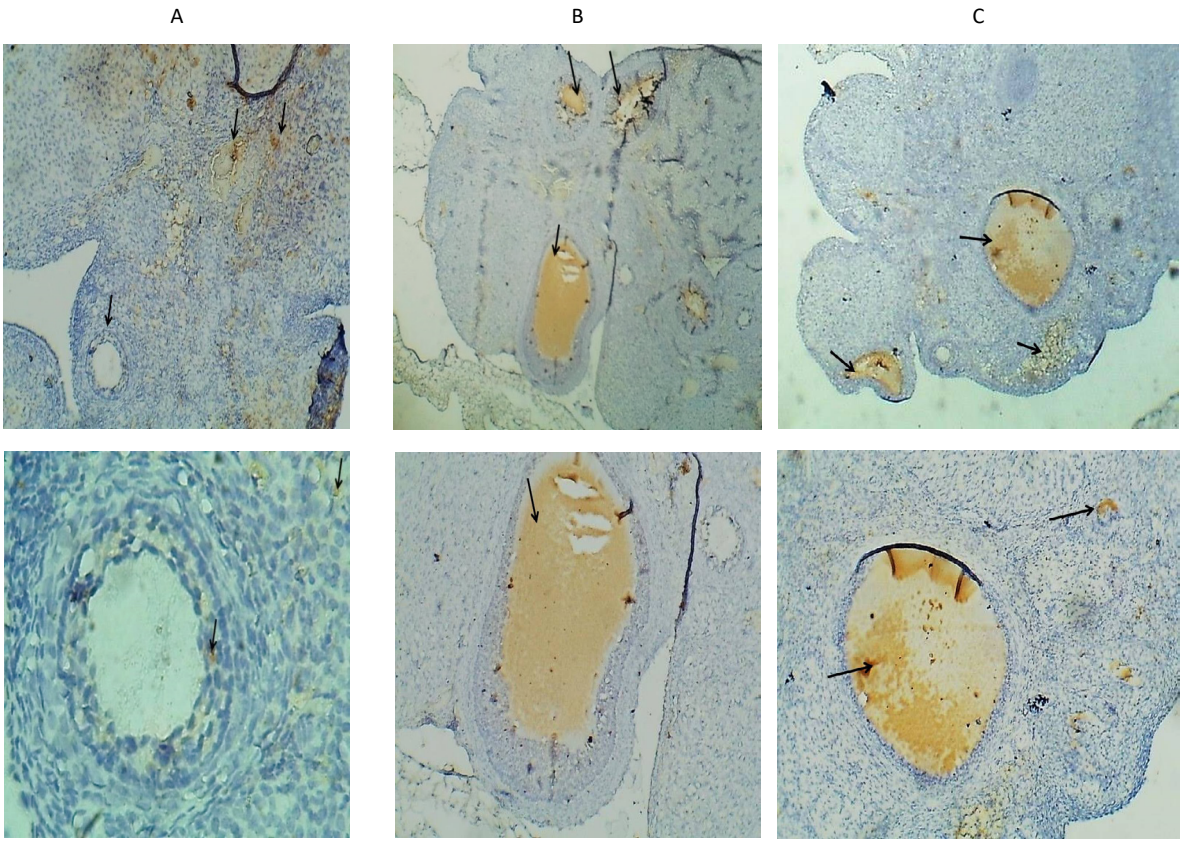

Figure 4. Light microscopic image revealing the immunoreactivity for vascular endothelial growth factor in the ovary

A) Normal positive reaction in the primary follicle and stroma (control group), B) Positive reaction in different stages of follicles (Pb group), C) Moderate positive reaction in both stroma and follicles (Pb+ *T. vulgaris* group)

Note: DAB reaction at ×10 line 1 and ×40 line 2.

group (Figure 3A) showed the normal presence of immune-positive cells that contained VEGF. Rats exposed to Pb-Ac for an extended period, classified as the chronic exposure group, showed that the uterine glands exhibited high levels of immune-positive cells, which were mostly scattered in the stromal gland (Figure 3B). The uterine sections of rats administered *T. vulgaris* along with Pb-Ac (Figure 3C) showed marked immune positivity for VEGF in uterine stromal blood vessels, with a rare appearance in stromal layers.

Ovaries from different animals analyzed for VEGF immunohistochemistry revealed the expression of this marker as a brown coloration (Figure 4). The control group (Figure 4A) showed a weak appearance of positive cells, with the exception of blood vessels. Meanwhile, the Pb-Ac rats (Figure 4B) demonstrated a marked positive reaction for VEGF in the oviduct and ovarian follicles at different stages, with fewer positive cells in the stroma. In contrast, the number of markedly positive cells was reduced in the ovarian follicles and moderate in the stroma of the group that received *T. vulgaris* extract (Figure 4C).

Discussion

The present experiment represents a simple and realistic model for lead exposure since one of the main ways that both humans and animals are exposed to lead is through oral exposure. Following lead exposure, it is distributed to various internal organs, including the brain. Changes in body weight changes or decreased weight may be linked to lead's effect on eating behavior via the central nervous system or growth hormone release (Assi et al., 2016). Oxidative stress results from exposure to lead acetate, stemming from an imbalance between the production of ROS and the activity of the body's antioxidant system. Lead acetate has the potential to induce oxidative stress, leading to the generation of high levels of free radicals and a reduction in antioxidant responses. The elevated level of MDA is produced as the final product during the process

Table 1. Body weight changes (g) in the lead acetate and *T. vulgaris* groups

Groups	Mean±SE		
	Initial Body Weight (g)	Final Body Weight (g)	Body Weight Gain (g)
A	257.17±3.86	288.86±4.02^{a}	31.69±1.05^{a}
B	251.38±1.42	271.51±2.07^{b}	20.38±1.42^{b}
C	258.38±6.46	274.85±7.05^{ab}	16.46±3.51^{b}
LSD value	13.767 (NS)	14.58*	6.845**
P	0.502	0.05	0.0007

NS: Not significant.

*(P≤0.05), **(P≤0.01).

Note: Different letters mean significant differences between means, small letters between groups. N=6/each groups. A: Control group rats were given DW; B: (Pb) group received 5 mg/kg/B.W/daily of lead acetate, C: Received Pb 5 mg/kg BW + *T. vulgaris* 75 mg/kg BW for 60 day.

of lipid peroxidation (LPO) in cells exposed to lead acetate (Ghazi & Al-Qaiym, 2023).

On the other hand, lead acetate reduces the antioxidant activity of SOD, which is derived from the effectiveness of the sulfhydryl (SH) group's affinity for scavenging ROS. Additionally, lead is excreted from the body via its conjugation to the SH group (Ramadhan & Khudair, 2019; Diab et al., 2024). The high affinity of lead cations for thiol-containing molecules is now considered the main mechanism, by which these metals disrupt the functions of these molecules and SOD could be one of those affected in the present trial. The primary antioxidant in the mitochondrial matrix is mitochondrial SOD, which is responsible for the formation of superoxide in the mitochondria during metabolic processes (Palma et al., 2020).

The female reproductive system, including the ovary, oviduct, and uterus in the present study, was targeted by lead. This targeting manifested in several ways, the most significant of which is cellular damage caused by ROS, leading to dysfunction in steroid hormone production. In the ovaries, the lack and inactivity of granulosa cells under the influence of lead-induced oxidative stress results in low estrogen levels. On the contrary, excessive quantities of progesterone indicate the pathogenesis of granulosa cells. Granulosa cell tumors have been shown to secrete high levels of estrogen and progesterone (Dumitrescu et al., 2015; Ahmed & Mohammed, 2022).

Table 2. Serum MDA and SOD in the lead acetate and *T. vulgaris* groups

Groups	Mean±SE	
	MDA (umol/L)	SOD (U/mg)
A	9.79±0.2^{c}	8.10±0.32^{b}
B	15.39±0.28^{a}	4.53±0.33^{c}
C	12.68±0.18^{b}	9.54±0.25^{a}
LSD value	0.676**	0.919**
P	0.0001	0.0001

**P≤0.01.

Note: Different letters indicate significant differences between means, with lowercase letters denoting differences between groups. N=6 per group. A: The control group received DW, B: The Pb group received 5 mg/kg/BW daily of lead acetate, C: The Pb+ *T. vulgaris* group received 5 mg/kg BW of Pb and *T. vulgaris* at 75 mg/kg BW for 60 days.

Table 3. Serum progesterone and estrogen levels in the lead acetate and *T. vulgaris* groups

Groups	Mean±SE	
	Progesterone (nmol/L)	Estrogen-E2 (pmol/L)
A	46.13±4.37^{c}	65.53±8.81^{b}
B	164.89±13.71^{a}	26.98±2.38^{c}
C	118.13±17.24^{b}	141.82±11.66^{a}
LSD value	39.09**	25.776**
P	0.0001	0.0001

**P≤0.01.

Note: Different letters indicate significant differences between means, with lowercase letters denoting differences between groups. N=6 per group. A: The control group received DW, B: The Pb group received 5 mg/kg/BW daily of lead acetate, C: The Pb+ *T. vulgaris* group received 5 mg/kg BW of Pb and *T. vulgaris* at 75 mg/kg BW for 60 days.

Lead acetate tends to impair all aspects of reproductive organs, including the ovaries and uterus. Lead exposure causes edema and necrosis of the ovarian follicles, dysfunction of folliculogenesis, and significant impacts on uterine tissue due to chronic Pb exposure, leading to conditions such as endometriosis and endometrial cancer (Qu et al., 2021; Raheem et al., 2023). The explanation for this is that lead acetate can damage the granulosa cells of antral follicles by increased ROS, which produces oxidative stress that destroys DNA and then stimulates apoptosis in granulosa cells. Lead adversely affects the endometrium, myometrium, and perimetrium of the uterus, reducing the size of the uterine glands and the height of columnar cells, as demonstrated in mice. The uterine glands are damaged by lead, mediated by ROS production, resulting in altering uterine gland functions, including hormone secretion (Albishtue et al., 2019; Al-Helaly & Mahmood, 2021). All these histopathological changes in ovarian follicles and uterine glands impair their ability to produce hormones, such as estrogen and progesterone, respectively.

VEGF plays an important role in angiogenesis and is critically involved in the maturation of newly formed blood vessels in ischemic tissues. Based on the present results, the immunohistochemical study denoted that the intensity and distribution of the positive immunoreactivity for the VEGF in the ovary and different layers of the uterus were altered by lead. The specific mechanisms, through which Pb induces dysregulation of VEGF in the reproductive system are not well established. As an angiogenic factor, VEGF is vital for the development and maintenance of the damaged uterine and ovarian tissues

Table 4. Uterus and ovary parameters in the lead acetate and *T. vulgaris* groups

Groups	Mean±SE				
	Final Body Weight (g)	Uterus Weight (g)	Uterus Index (Uterus Weight/ Body Weight) (g)	Ovary Weight (g)	Ovary Index (Ovary Weight/ Body Weight) (g)
A	288.86±4.02^{a}	0.393±0.04^{a}	0.00136±0.00012^{a}	0.197±0.02	0.00067±0.00005
B	271.51±2.07^{b}	0.2016±0.024^{b}	0.00074±0.00008^{b}	0.212±0.005	0.00078±0.00002
C	274.85±7.05^{ab}	0.34±0.039^{a}	0.00124±0.0001^{a}	0.225±0.016	0.00082±0.00006
LSD value	14.58*	0.102**	0.0004**	0.0431 NS	0.0001 NS
P	0.050	0.0031	0.0054	0.396	0.131

*P≤0.05, **P≤0.01.

Note: Different letters indicate significant differences between means, with lowercase letters denoting differences between groups.N=6 per group. A: The control group received DW, B: The Pb group received 5 mg/kg/BW daily of lead acetate, C: The Pb+ *T. vulgaris* group received 5 mg/kg BW of Pb and *T. vulgaris* at 75 mg/kg BW for 60 days.

induced by lead. It has been suggested that increased VEGF concentrations arise from the hypoxic environment within the follicles of older women (Salmasi et al., 2021). The present ovarian dysfunction and alterations in estrogen and progesterone secretion can enhance the expression of VEGF in the uterine stroma and promote angiogenesis (Nikolić et al., 2014).

The capacity of plant extracts to serve as free radical scavengers or the ability of phenolic compounds to bind metal ions contributes to their antioxidant effects. Thymol and carvacrol are the main substances with significant antioxidant activity against oxidative stress caused by lead that have been documented. The restorative activity of SOD and the low MDA levels in *T. vulgaris*-treated rats against lead demonstrate its antioxidant activity (Luaibi et al., 2017; Escobar et al., 2020). Thyme exhibits a strong antioxidant activity, which is attributable to the presence of thymol as a key ingredient in its extract, according to numerous studies on the plant's therapeutic properties (Hammoudi Halat et al., 2022).

The positive effects of thyme observed in the current study are not the first to demonstrate its antioxidant capacity against lead and align with previous findings (Kahalerras et al., 2022; Ghazi & Al-Qaiym, 2023). However, the effectiveness of thyme in protecting the ovaries and uterus from the effects of lead has not been extensively addressed. Our results are consistent with another study (Ali et al., 2024) regarding the protective role of thyme oil supplementation in improving reproductive performance. The present results are also in agreement with other studies on the use of natural substances to enhance reproductive performance in animals exposed to lead acetate, such as *Nigella sativa* (Ouies et al., 2021), *Pelargonium graveolens* (Madouche et al., 2024) and *Moringa oleifera* (Idoko et al., 2024).

Conclusion

The present results conclude the protective role of *T. vulgaris* extract due to its antioxidant activity. Accordingly, the crude extract of *T. vulgaris* may be considered a viable option for managing lead acetate toxicity. The female reproductive system may be directly targeted by lead, which serves as a precursor for cellular and molecular damage as well as alterations in steroid hormone secretion. Prolonged lead exposure and its accompanying changes in reproductive organs, histopathological changes, and vascularization may be carcinogenic. Thus, it is recommended that further studies be conducted to investigate the effects of lead acetate on the male reproductive system and offspring health.

Ethical Considerations

Compliance with ethical guidelines

All procedures used in this study were reviewed and approved by the Scientific Committee of the Department of Physiology, Biochemistry, and Pharmacology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq, as well as the Ethics Committee of the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq, in compliance with the ethical principles of animal welfare (Code: 1355/P.G).

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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References

Abdulrazzaq, O. E., Al-Demirchi, J. Y., & Al-Anssari, H. B. (2023). Effect of Thymus vulgaris on liver tissue in dexamethasone treated female adult rat. *Texas Journal of Agriculture and Biological Sciences, 12*, 78-82. [Link]

Ahmed, R. M., & Mohammed, A. K. (2022). Role of sodium butyrate supplement on reducing hepatotoxicity induced by lead acetate in rats. *The Iraqi Journal of Veterinary Medicine, 46*(2), 29-35. [DOI:10.30539/ijvm.v46i2.1408]

Ali, M. E., Zainhom, M. Y., Abdel Ghfar, S. S., Awad, A. A., Farouk, M. H., & Abdelrahman, M., et al. (2024). Dietary supplementation with thyme oil improves the reproductive characteristics of Barki adult and prepubertal ewes. *BMC Veterinary Research, 20*(1), 536. [DOI:10.1186/s12917-024-04376-2] [PMID]

Alabbassi, M. G., Hussain, S. A., & Ali, S. H. (2008). Therapeutic effects of melatonin in lead-induced toxicity in rats. *Iraqi Journal of Pharmaceutical Sciences, 17*(2), 47-54. [DOI:10.31351/vol17iss2pp47-54]

Albishtue, A. A., Yimer, N., Zakaria, M. Z. A., Haron, A. W., Babji, A. S., & Abubakar, A. A., et al. (2019). The role of edible bird's nest and mechanism of averting lead acetate toxicity effect on rat uterus. *Veterinary World, 12*(7), 1013–1021. [DOI:10.14202/vetworld.2019.1013-1021] [PMID]

Al-Helaly, L. A., & Mahmood, E. S. (2021). Biochemical and histological study of aminoacylase-1 purified from amniotic fluid in rats with oxidative stress induced by lead acetate. *Baghdad Science Journal, 18*(3), 0583-0583. [DOI:10.21123/bsj.2021.18.3.0583]

AL-KASSIE, G. A. (2009). The Effect of Adding Thyme vulgaris and Cinnamomun zeylanicum on production performance and some blood traits in broiler chicken: GALIB AM AL-KASSIE; YASSER J. JAMEL. *The Iraqi Journal of Veterinary Medicine, 33*(2), 84-90. [DOI:10.30539/iraqijvm.v33i2.695]

Al-Naqqash, Z., Jawad, A. L., & Raaof, A. (2014). Evaluation of the activity of crude alkaloids extracts of Zingiber officinale Roscoe, Thymus vulgaris L. and Acacia arabica L. as coagulant agent in lab mice. *Iraqi Journal of Science, 55*(1), 50-60. [Link]

Alqayim, M. A., & Asis, S. A. (2013). Protective role of Vitamin E and/or methionine against lead-induce changes on hematological parameters in rabbits. *Iraqi Journal of Medical Sciences, 11*(2), 187- 194. [Link]

Assi, M. A., Hezmee, M. N., Haron, A. W., Sabri, M. Y., & Rajion, M. A. (2016). The detrimental effects of lead on human and animal health. *Veterinary World, 9*(6), 660–671. [DOI:10.14202/vetworld.2016.660-671] [PMID]

Capatina, L., Todirascu-Ciornea, E., Napoli, E. M., Ruberto, G., Hritcu, L., & Dumitru, G. (2020). Thymus vulgaris Essential Oil Protects Zebrafish against Cognitive Dysfunction by Regulating Cholinergic and Antioxidants Systems. *Antioxidants (Basel, Switzerland), 9*(11), 1083. [DOI:10.3390/antiox9111083] [PMID]

Diab, A. E., Al-Mathal, E., Zahra, M., Dowidar, M., El-Sherbeny, S., & Abu El-Magd, M. (2024). Ameliorative effect of costus speciosus extracts on toxic effects of lead acetate on liver and kidney of male rats. *Egyptian Journal of Veterinary Sciences, 55*(4), 895-901. [DOI:10.21608/ejvs.2023.244473.1652]

Dumitrescu, E., Chiurciu, V., Muselin, F., Popescu, R., Brezovan, D., & Cristina, R. T. (2015). Effects of long-term exposure of female rats to low levels of lead: Ovary and uterus histological architecture changes. *Turkish Journal of Biology, 39*(2), 284-289. [DOI:10.3906/biy-1407-6]

Escobar, A., Perez, M., Romanelli, G., & Blustein, G. (2020). Thymol bioactivity: A review focusing on practical applications. *Arabian Journal of Chemistry, 13*(12), 9243-9269. [DOI:10.1016/j.arabjc.2020.11.009]

Ghazi, M. A., & Al-Qaiym, M. A. (2023). Assessment of Thymus vulgaris protective role on liver and spleen against lead acetate in rats. *Applied Biochemistry and Microbiology, 59*(S1), 235-241. [DOI:10.5281/zenodo.7639165]

Guidet, B., & Shah, S. V. (1989). Enhanced in vivo H2O2 generation by rat kidney in glycerol-induced renal failure.*The American Journal of Physiology, 257*(3 Pt 2), F440–F445. [PMID]

Hammoudi Halat, D., Krayem, M., Khaled, S., & Younes, S. (2022). A focused insight into thyme: Biological, chemical, and therapeutic properties of an indigenous mediterranean herb. *Nutrients, 14*(10), 2104. [DOI:10.3390/nu14102104] [PMID]

Harshitha, P., Bose, K., & Dsouza, H. S. (2024). Influence of lead-induced toxicity on the inflammatory cytokines. *Toxicology, 503,* 153771-153782. [DOI:10.1016/j.tox.2024.153771] [PMID]

Hmidani, A., Bouhlali, E. D. T., Khouya, T., Ramchoun, M., Filali-Zegzouti, Y., & Alem, C., et al. (2019). Antioxidant, anti-inflammatory and anticoagulant activities of three Thymus species grown in southeastern Morocco. *Future Journal of Pharmaceutical Sciences, 5,* 1-6. [Link]

Idoko, G., Onoja, P., Beega, P., Kiekwe, V., Aende, T., Mlumun, M., ... & Saalu, L. (2024). Modulatory Effects of Aqueous Moringa Olifera Extract in Lead Mediated Endometriosis in Wistar Rats. Journal of Innovations in Medical Research, 3(2), 58-74. doi:10.56397/JIMR/2024.06.09. [DOI:10.56397/JIMR/2024.06.09]

Kahalerras, L., Otmani, I., & Abdennour, C. (2022). The Allium triquetrum L. Leaves Mitigated Hepatotoxicity and Nephrotoxicity Induced by Lead Acetate in Wistar Rats. *Biological trace Element Research, 200*(11), 4733–4743. [DOI:10.1007/s12011-021-03052-y] [PMID]

Laufer, N., Navot, D., & Schenker, J. G. (1982). The pattern of luteal phase plasma progesterone and estradiol in fertile cycles. *American Journal of Obstetrics and Gynecology, 143*(7), 808–813. [DOI:10.1016/0002-9378(82)90014-x] [PMID]

Luaibi, N. M., Jasim, M. G., & Mousa, A. H. (2017). A study medicinal and nutritional effects of thyme on hematological changes in male albino rats. *Iraqi Journal of Science, 58*(3A), 1177-1188. [Link]

Madouche, M., Boulila, N., Lakabi, L., Akdader, S., & Medjdoub-Bensaad, F. (2024). Rose geranium essential oil restorative potential on lead acetate induced ovary toxicity using a mouse model. *Polish Journal of Natural Sciences, 39*(3), 187-197. [Link]

Massányi, P., Massányi, M., Madeddu, R., Stawarz, R., & Lukáč, N. (2020). Effects of cadmium, lead, and mercury on the structure and function of reproductive organs. *Toxics, 8*(4), 94. [DOI:10.3390/toxics8040094] [PMID]

Nikolić, M., Glamočlija, J., Ferreira, I. C., Calhelha, R. C., Fernandes, Â., & Marković, T., et al. (2014). Chemical composition, antimicrobial, antioxidant and antitumor activity of Thymus serpyllum L., Thymus algeriensis Boiss. and Reut and Thymus vulgaris L. essential oils. *Industrial Crops and Products, 52,* 183-190. [DOI:10.1016/j.indcrop.2013.10.006]

Noroozisharaf, A., & Kaviani, M. (2018). Effect of soil application of humic acid on nutrients uptake, essential oil and chemical compositions of garden thyme (Thymus vulgaris L.) under greenhouse conditions. *Physiology and Molecular Biology Of Plants: An International Journal of functional Plant Biology, 24*(3), 423–431. [DOI:10.1007/s12298-018-0510-y] [PMID]

Ouies, S. M., Roshdy, S., & Baset, A. (2021). Effect of lead acetate on the ovarian growth in the pre-pubertal and pubertal periods in the offspring of albino rats and the possible protective role of nigella sativa oil. *Egyptian Journal of Histology, 44*(3), 814-827. [DOI:10.21608/ejh.2020.44558.1366]

Osowski, A., Fedoniuk, L., Bilyk, Y., Fedchyshyn, O., Sas, M., & Kramar, S., et al. (2023). Lead exposure assessment and its impact on the structural organization and morphological peculiarities of rat ovaries. *Toxics, 11*(9), 769. [DOI:10.3390/toxics11090769] [PMID]

Palma, F. R., He, C., Danes, J. M., Paviani, V., Coelho, D. R., Gantner, B. N., & Bonini, M. G. (2020). Mitochondrial Superoxide Dismutase: What the established, the intriguing, and the novel reveal about a key cellular redox switch. *Antioxidants & Redox Signaling, 32*(10), 701–714. [DOI:10.1089/ars.2019.7962] [PMID]

Pérez-Gutiérrez, L., & Ferrara, N. (2023). Biology and therapeutic targeting of vascular endothelial growth factor A. *Nature Reviews. Molecular Cell Biology, 24*(11), 816–834. [DOI:10.1038/s41580-023-00631-w] [PMID]

Qu, J., Niu, H., Wang, J., Wang, Q., & Li, Y. (2021). Potential mechanism of lead poisoning to the growth and development of ovarian follicle. *Toxicology, 457,* 152810. [DOI:10.1016/j.tox.2021.152810] [PMID]

Qureshi, N., & Sharma, R. (2012). Lead toxicity and infertility in female swiss mice: A review. *Journal of Chemical, Biological and Physical Sciences (JCBPS), 2*(4), 1849- 1861. [Link]

Raheem, S. S., Falah Hasan, H., Hashim Abid Ali, A., & Mansour Jasim, A. (2023). Effectiveness of Histopathological Changes of Induced Thin Layer Endometrium by Pentoxifylline and Pentoxifylline-Loaded Poly Lactic-co-Glycolic Acid on Female Rats. *Archives of Razi Institute, 78*(6), 1762–1770. [DOI:10.32592/ARI.2023.78.6.1762] [PMID]

Ramadhan, S. J., & Khudair, K. K. (2019). Effect of betaine on hepatic and renal functions in acrylamide treated rats. *The Iraqi Journal of Veterinary Medicine, 43*(1), 138 - 147. [DOI:10.30539/iraqijvm.v43i1.484]

Rauniyar, K., Bokharaie, H., & Jeltsch, M. (2023). Expansion and collapse of VEGF diversity in major clades of the animal kingdom. *Angiogenesis, 26*(3), 437-461. [DOI:10.1007/s10456-023-09874-9] [PMID]

Salmasi, S., Sharifi, M., & Rashidi, B. (2021). Ovarian stimulation and exogenous progesterone affect the endometrial miR-16-5p, VEGF protein expression, and angiogenesis. *Microvascular Research, 133,* 104074. [DOI:10.1016/j.mvr.2020.104074] [PMID]

SAS/STAT Software. (2018). Statistical analysis system, user's guide. Retrieved from: [Link]

Vermande-Van Eck, G. J., & MEIGS, J. W. (1960). Changes in the ovary of the rhesus monkey after chronic lead intoxication. *Fertility and Sterility, 11,* 223–234 [DOI:10.1016/s0015-0282(16)33730-x] [PMID]

Weydert, C. J., & Cullen, J. J. (2010). Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. *Nature Protocols, 5*(1), 51-66. [DOI:10.1038/nprot.2009.197] [PMID]