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Original Research Article

Saussurea Lappa Enhances Folliculogenesis in the Ovaries of Female Wistar Rats Exposed to Lead

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ABSTRACT

Follicular disorders can lead to infertility, often characterized by elevated levels of inducible Nitric Oxide Synthase (iNOS) and Tumor Necrosis Factor-α (TNF-α), which activate apoptotic pathways and reduce the number of functional ovarian follicles. *Saussurea lappa* is believed to have the capacity to inhibit apoptosis in follicles by reducing the levels of iNOS and TNF-α. This study aimed to evaluate the activity of *Saussurea lappa* in reducing iNOS and TNF-α levels, inhibiting apoptosis, and increasing the number of tertiary follicles in the ovaries. This study used a true experiment post-test control group design. A total of 24 female Wistar rats (*Rattus norvegicus*) were administered lead acetate ($Pb(CH_3COO)_2$) orally at a dose of 1.5 mg/kg for 21 days, divided into one control group (K-1) and three treatment groups with *Saussurea lappa* extract doses: 200 mg/kg (K-2), 400 mg/kg (K-3), and 600 mg/kg (K-4). The results showed that at a dose of 600 mg/kg, there was a significant decrease in iNOS, TNF-α, and apoptosis, as well as a significant increase in the number of primary, secondary, and tertiary follicles ($p < 0.05$). *Saussurea lappa* extract, given at a dose of 600 mg/kg, may mitigate i-NOS, TNF-α, and apoptosis while promoting folliculogenesis in the ovaries of female Wistar rats subjected to lead acetate exposure.

Keywords: *Saussurea Lappa* Extract, Lead-Induced, Folliculogenesis.

Introduction

According to the World Health Organization (WHO) 2023, 8-10% of the world's reproductive age population were estimated to have fertility disorders, and the number will continue to rise. Among these infertility cases, female-related cases contribute to around 40-50 % of the total.[1,2,3] Environmental toxicants are one of the causes of infertility.[4,5] In the reproductive system, lead exposure triggers oxidative stress, which increases reactive oxygen species (ROS) that trigger cell damage and disrupt ovarian function. Increased production of ROS will increase the production of pro-inflammatory cytokines.[6,7] Elevated levels of tumor necrosis factor-α (TNF-α) promote apoptosis in ovarian cells, disrupting follicular development, reducing ovarian reserve, and ultimately contributing to infertility.[2,7,8,9,10]

Various environmental factors influence folliculogenesis, including exposure to lead pollution, which can induce stress on ovarian follicles, disrupt oocyte maturation, and adversely affect follicle development and oocyte quality. Elevated pollutant concentrations, particularly in certain developing nations, will exacerbate these disturbances to the female reproductive system. The utilization of medicinal herbs has been anticipated to mitigate these disruptions.[9] Kust, or costus, denotes the desiccated root of *Saussurea lappa*, a perennial herb indigenous to the Himalayan region, which has several main components including dehydrocostus lactone, costunolide, flavonoids, and sesquiterpene-lactone. The roots have a strong aromatic odor and bitter taste, containing two dominant compounds, namely dehydrocostus lactone (55.39%) and costunolide, [11,12] that inhibit nitric oxide and pro-inflammatory cytokines [13,14] and reduces levels of TNF-α, which plays an important role in follicular development – affecting the number of follicles.[2] The efficacy of *Saussurea lappa* as an anti-inflammatory and antioxidant has been established [11,12,14]; nevertheless, its impact on follicle growth, namely tertiary follicles, remains uncertain.

The essential oil in the roots of *Saussurea lappa* showed a higher sesquiterpenoid content (79.80%) than monoterpenoids (13.25%).[15] The compounds contained in the roots have anti-inflammatory effect i.e. they can inhibit nitric oxide and pro-inflammatory cytokines.[13] This inhibition leads to a reduction in the levels of TNF-α, which has an important role in the ovaries, thereby potentially affecting the number of follicles. Decreased levels of TNF-α will increase levels of P45 aromatase and inhibin-A, further triggering ovulation, resulting in an increased number of follicles.[8]

This study aims to elucidate the effects of *Saussurea lappa* extract on the ovaries of rats subjected to lead acetate solution ($Pb(CH_3COO)_2$), focusing on the levels of TNF-α, iNOS, apoptosis, and its impact on the quantity of primary, secondary, and tertiary follicles. This study also intends to investigate the effects of lead exposure on ovarian function, with a particular focus on its impact on follicular development. The findings are expected to contribute to strategies that mitigate lead-induced reproductive toxicity.

Materials and Methods

Extract preparation

Dry roots of *Saussurea lappa* were sourced from local herb store/market in Malang City, Indonesia (-7.966122, 112.637823) in the month of February 2024. Taxonomic identification was carried out by Dr. Iman Hidayat using the Deoxyribonucleic acid (DNA) sequencing method in the BRIN laboratory (Indonesian National

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Research and Innovation Agency). Then the plant was deposited in a herbarium under the voucher number 09/UN10.8/FAR/II/2024. Afterwards the plant was cleaned with running water to remove dirt. The samples were dried without being exposed to direct sunlight, cut into small pieces, and ground into powder.

Extraction of plant material

Approximately 200 grams of powder were dissolved in 1000 mL of 70% ethanol in a 1000 mL Erlenmeyer flask then sonicated with an ultrasonicator for 30 minutes at 45°C, and this method was repeated 3 times. The resulting samples were filtered using Whatman filter paper and concentrated using a rotary vacuum evaporator at 45°C, followed by maceration for five days at room temperature. The mixture was then filtered until the solvent became nearly colorless, and the filtrate was evaporated to obtain a thick extract.[16]

Experimental procedures

The experimental procedures were approved by the Ethics Committee, Faculty of Medicine, University of Muhammadiyah Malang (No. E.5.a/372/KEPFKUMM/XII/2023). The research method was a true experimental study, with a post-test only control group design. In regards to determining the sample size using the resource equation method, 24 female Wistar rats (*Rattus norvegicus*) were used in this study, aged around 8-12 weeks and weighing 100-200 grams. All rats received oral administration of lead acetate solution ($PbAc/Pb(CH_3COO)_2$) at a dosage of 1.5 mg/kg for a duration of 21 days. This study was divided into four groups: one control group (K-1) and three treatment groups (K-2, K-3, K-4). On the 22nd day, *Saussurea lappa* extract was administered at different doses thus, 200 mg/kg for K-2, 400 mg/kg for K-3, and 600 mg/kg for K-4 for a duration of 21 days. Upon completion of the treatment, the experimental animals underwent ovariectomy, with the specimens preserved in 10% formalin. The expressions of iNOS, TNF-α, and apoptosis were analyzed using immunohistochemical techniques. The quantity of tertiary follicles was enumerated utilizing hematoxylin and eosin (H/E) staining.

LC/MS (Liquid Chromatography/ Mass Spectrometry) Method

The extract was examined for the presence of active compounds using the LC/MS method. UHPLC (Vanquish)-MS/MS (TSQ) – Triple Quadrupole Thermo Scientific was employed for the LC/MS analysis.[17]

Immunohistochemical method

Immunohistochemistry (IHC) is a method that utilizes the specific binding of antibodies to antigens for the detection of certain antigens in cells and tissues, analyzed under a light microscope. To conduct an IHC test, one must isolate the antigen (AR), introduce primary antibodies, apply secondary antibodies that attach to the primary antibodies, and incorporate detection reagents that indicate the location of the primary antibodies.[18,19] The ImageJ software was used to measure antibody expression. It examined pictures of immunohistochemistry (IHC)-stained samples to find out how much specific antibody expression there was for TNF-α, iNOS, and apoptosis antibodies that showed up as brown. We conducted the calculations using ImageJ software across five distinct visual fields. This observation used a light microscope (Nikon Eclipse-type Ei) with an Optilab microscope camera.[20,21,22]

Haematoxylin Eosin (H/E) staining method

The ovaries were stained using the Hematoxylin-eosin (HE) technique and observed under a light source, using a microscope with 400x magnification in all fields of view. The number of primary, secondary, and tertiary follicles counted in the study was mostly about the follicles that made up the granulosa and cumulus oophorus membranes, which had layers of internal and external theca. To prepare the ovaries for histology, the paraffin method and hematoxylin-eosin staining were done the following method of Schütz et al.[23]

The tertiary follicle count in the ovaries was conducted via microscopic examination of the preparation. Granulosa cells encase tertiary follicles, which possess a more expansive antrum compared to secondary follicles. The oocyte was encased by the zona pellucida and a layer of granulosa cells. A cumulus oophorus developed when the oocyte relocated to one side of the follicle. The tertiary follicles enumerated in this study were characterized by the presence of granulosa and cumulus oophorus membranes, together with internal and external theca layers.[23,24,25]

Statistical analysis

We performed a statistical analysis to compare the treatment group to the control group. The research data was spread out in a normal and uniform way. The one-way ANOVA test and the Tukey/HSD post hoc test were used, and a *p*-value of less than 0.05 meant that the results were significant. Multiple regression tests were performed to determine the relationship between iNOS and TNF-α with apoptosis. All statistical analyses were carried out using IBM SPSS Statistics (Version 30; IBM Corp., Armonk, NY, USA).

Results and Discussion

Multiple regression test between TNF-α ($p>0.05$) and iNOS ($p<0.05$) with regards to apoptosis showed that TNF-α did not have a significant effect, while iNOS had a significant effect on the level of apoptosis.

LC/MS results

This instrument allowed for the qualitative reading of certain compounds. Total Ion Chromatogram (TIC) data confirmed the presence of costunolide and dehydrocostus lactone (Figures 1). Mass spectrometry was performed using Selective Reaction Monitoring (SRM) mode with the positive ion source as a Heated Electron Spray (H-ESI).

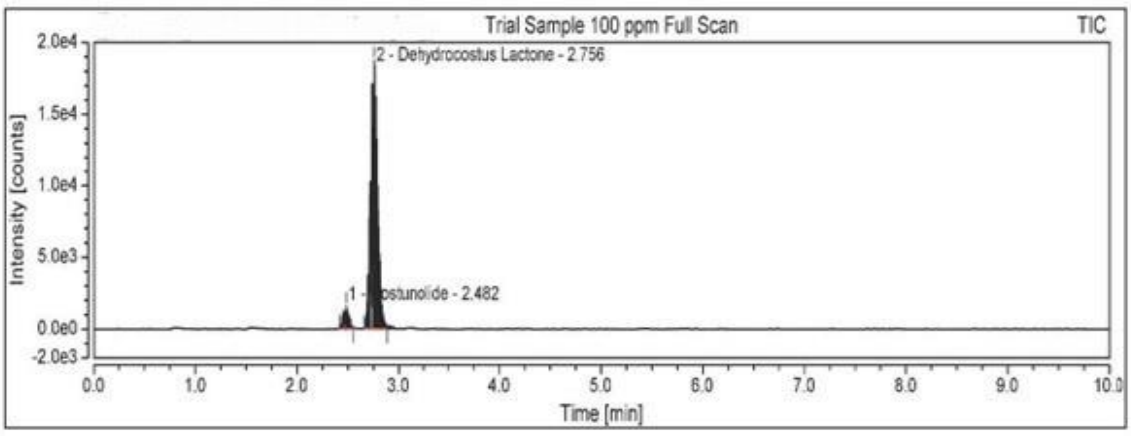

Figure 1: Outcomes of the LC/MS (total ion chromatography) analysis of a 70% ethanol extract of *Saussurea lappa*.

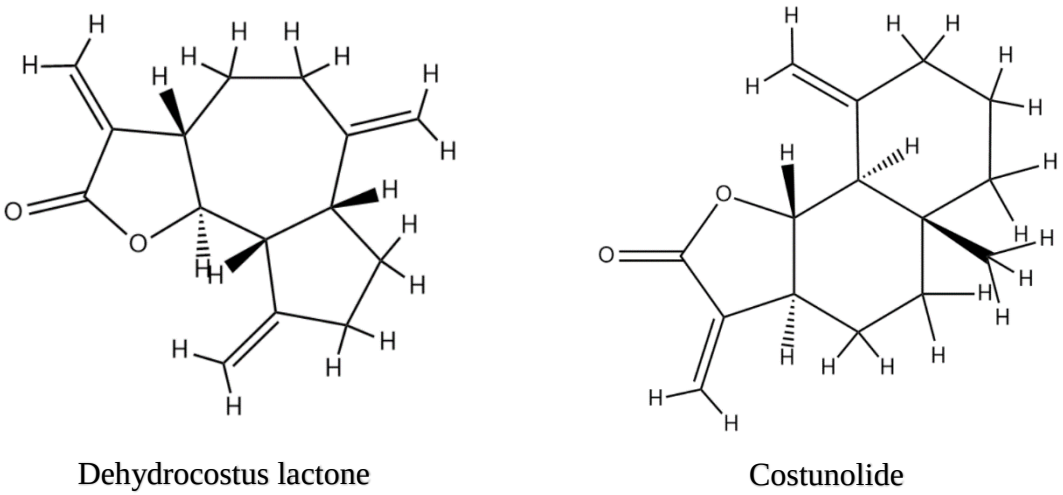

Figure 2: The chemical structure of *dehydrocostus lactone* and *costunolide*

After undergoing protonation and fragmentation, the the compounds present were recognized as dehydrocostus lactone and costunolide (Figure 2).[26]

Immunohistochemical and Hematoxylin Eosin (HE) results

The rat ovaries were processed for immunohistochemical examination to see the expression of TNF-α (Figure 3), iNOS (Figure 4), and apoptosis (Figure 5). The hematoxylin-eosin technique, at 400x magnification, depicted the tertiary follicle (Figure 6). We counted the number of primary, secondary, and tertiary follicles using hematoxylin-eosin (HE) staining (Figure 7). Theca internal cells synthesized the hormone estrogen, which affects follicular growth. Folliculogenesis commences with primary follicles. Primordial follicles progressed to secondary follicles, or preantral follicles, which then matured into tertiary follicles. The tertiary follicles would undergo apoptosis-mediated cell death. Certain tertiary follicles persisted during the follicular phase. Instead, they persisted in developing into preovulatory follicles, referred to as Graafian follicles, which releases an ovum.[27,28,29] Lead (Pb) could affect the female reproductive system through adverse endocrine changes in the structure and function of the ovaries. Pb accumulation triggered oxidative stress in the ovaries through increased reactive oxygen species (ROS) and reactive nitrogen, which caused deoxyribonucleic acid (DNA) damage, thereby increasing the activation of NF-kB (a transcription factor associated with the expression of TNF-α and IL-6) and apoptosis.[30,31,32]

The increase in ROS-induced iNOS levels, along with the increase in TNF-α levels due to inflammation, occurs when exposed to lead acetate. The concentration of iNOS indirectly triggered pro-caspase 9 into caspase-9. The elevated levels of TNF-α then created a combination with TRADD, thereby activating pro-caspase 8 into caspase-8. Caspase-9 and caspase-8 activate pro-caspase-3, resulting in the formation of caspase-3 and initiating apoptosis. Excessive apoptosis is known to impair folliculogenesis, potentially leading to reduced follicle survival. *Saussurea lappa* contained phytochemicals that could stop the production of iNOS and TNF-α, hence preventing cells apoptosis, which promotes folliculogenesis.

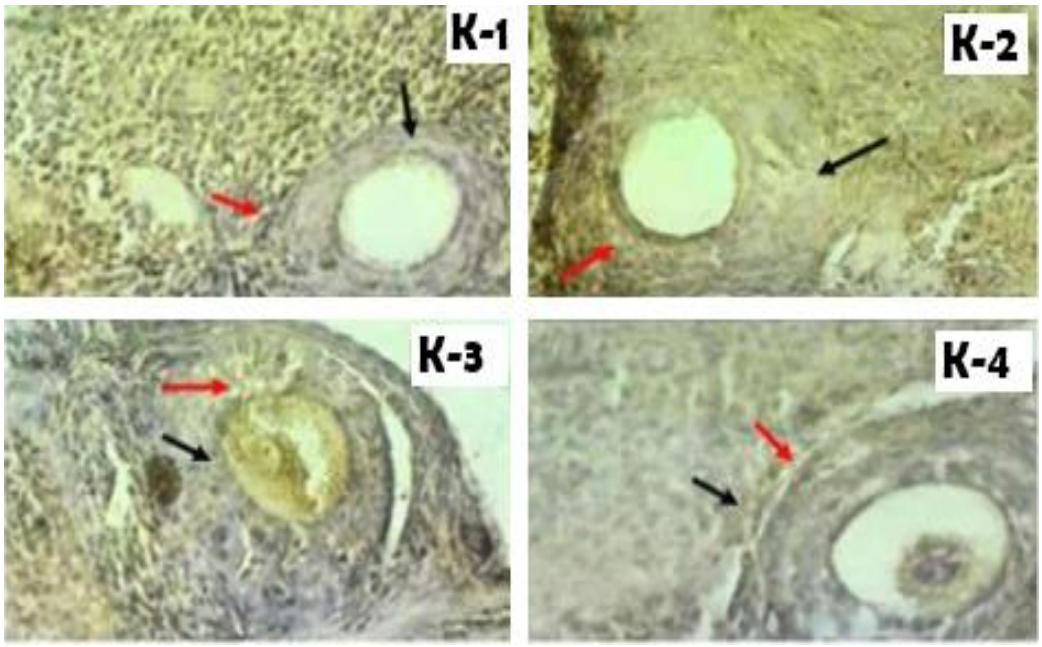

Figure 3: Immunohistochemical expression of TNF-α on Ovarium.
A red arrow indicates positive, and a black arrow indicates negative. K-1 (Control), K-2 (Extract SL 200 mg/kg), K-3 (Extract SL 400 mg/kg), K-4 (Extract SL 600 mg/kg), SL (*Saussurea lappa* 70% ethanolic extract)

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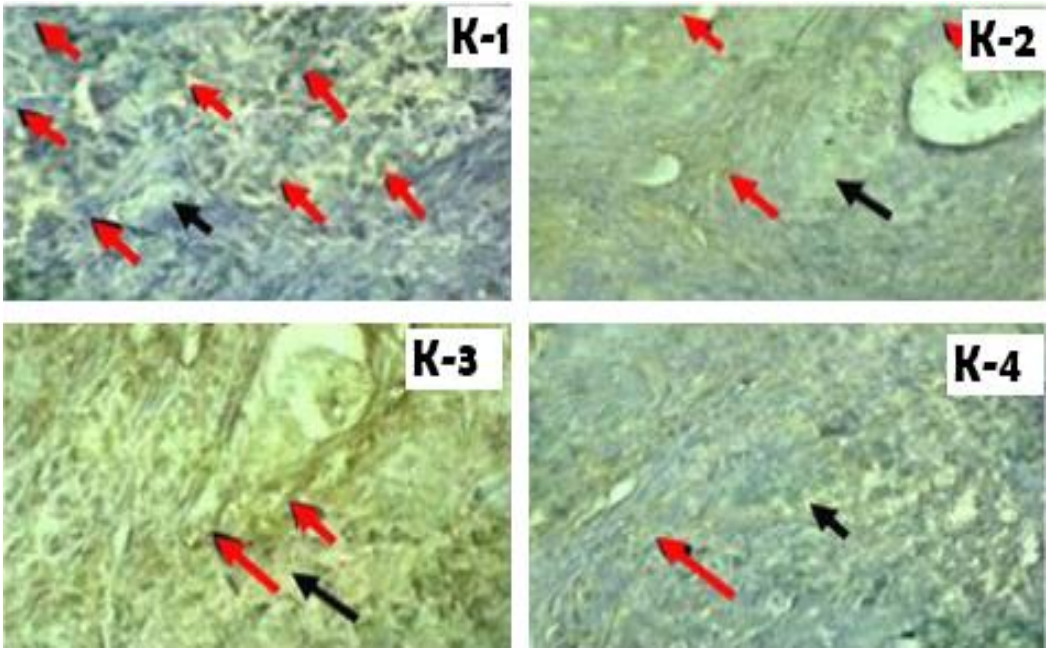

Figure 4: Immunohistochemical expression of iNOS on Ovarium.
A red arrow indicates positive, and a black arrow indicates negative. K-1 (Control), K-2 (Extract SL 200 mg/kg), K-3 (Extract SL 400 mg/kg), K-4 (Extract SL 600 mg/kg), SL (*Saussurea lappa* 70% ethanolic extract)

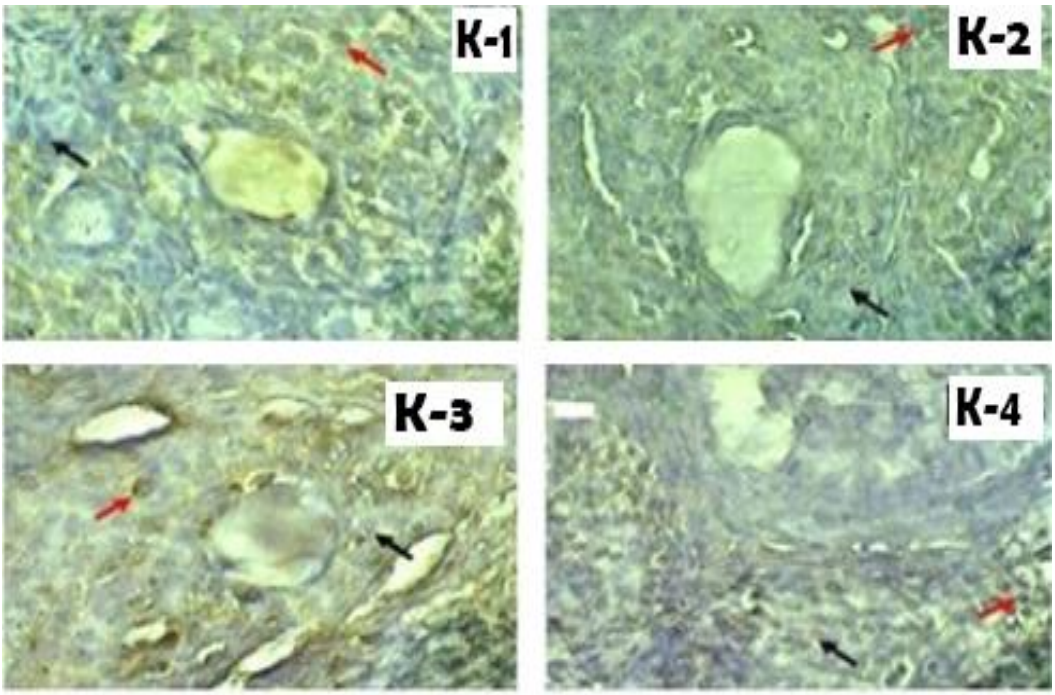

Figure 5: Immunohistochemical expression of apoptosis on Ovarium.
A red arrow indicates positive, and a black arrow indicates negative. K-1 (Control), K-2 (Extract SL 200 mg/kg), K-3 (Extract SL 400 mg/kg), K-4 (Extract SL 600 mg/kg), SL (*Saussurea lappa* 70% ethanolic extract)

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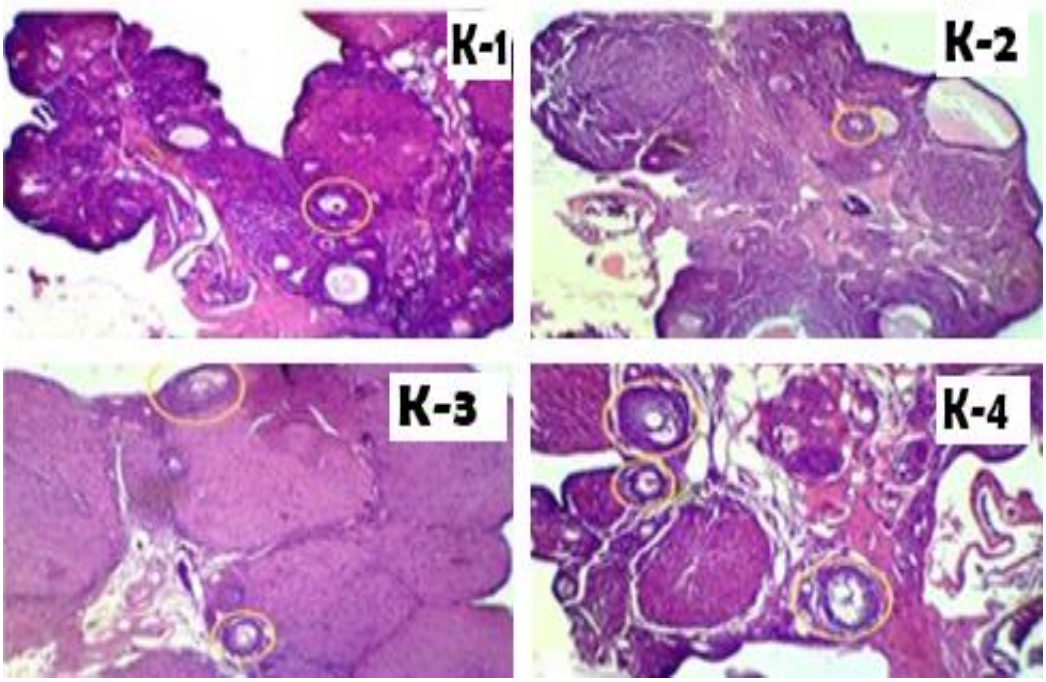

Figure 6: Microscopic image of rat ovarian tertiary follicles with Haematoxylin Eosin (HE) staining at 400x magnification.
Yellow circle indicates antral follicles. K-1 (Control), K-2 (Extract SL 200 mg/kg), K-3 (Extract SL 400 mg/kg), K-4 (Extract SL 600 mg/kg), SL (*Saussurea lappa* 70% ethanolic extract)

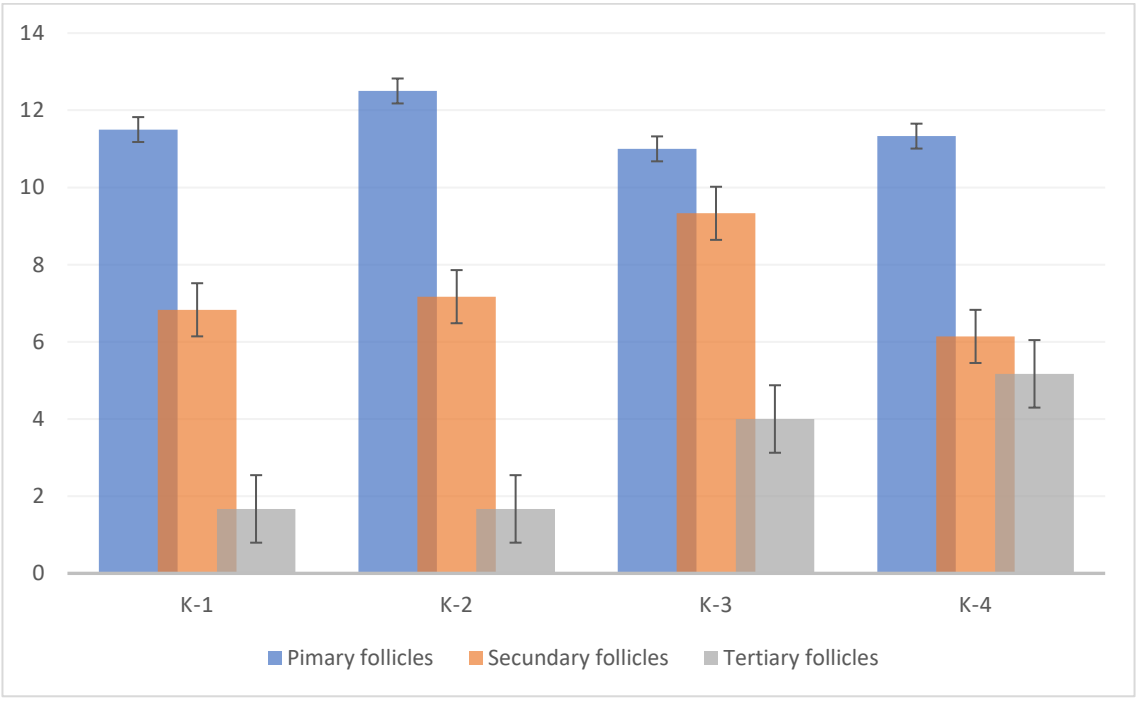

Figure 7: Number of primary, secondary and tertiary follicles
*p<0.05. Mean±SD. K-1 (control group), treatment groups at doses K-2 (200 mg/kg), K-3 (400 mg/kg), K-4 (600 mg/kg). The number of primary, secondary, and tertiary follicles in each group. Primary follicles: K-1 11.5±5.13; K-2 12.5±2.59; K-3 11±0.63; K-4 11.33±0.82. Secondary follicles: K-1 6.83±5.12; K-2 7.17±3.92; K-3 9.33±3.01; K-4 6.14±2.79. Tertiary follicles K-1 1.67±1.51; K-2 1.67±1.03; K-3 4.00±2.00; K-4 5.17±1.72.
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The detrimental effect of lead on ovarian structure resulted in the atresia of tertiary follicles, which was indicative of a significant ovarian injury.[18,30,33,34]

In this study, the lead acetate was administered orally and on entering the digestive tractwas absorbed into the bloodstream and spread throughout the body, including the ovaries. Lead acetate accumulation, if it reached a threshold, would be toxic by binding to sulfhydryl groups, which resulted in a decrease in antioxidants, including glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD), which could increase Reactive Oxygen Species (ROS), leading to oxidative stress resulting in DNA damage. [31,32]

Inducible nitric oxide synthase (iNOS) overexpression in ovarian tissue had been associated with oxidative stress-mediated damage to follicular cells, impairing oocyte maturation and folliculogenesis. Excess nitric oxide and ROS produced by iNOS could disrupt the hormonal and microenvironmental balance of the follicle, contributing to follicular atresia and reduced fertility.[33,34]

TNF-α play a role in pro-inflammatory responses, regulated cell renewal and differentiation, and was expressed in human oocytes and granulosa cells, and played a role in follicle development, ovarian hormone synthesis, ovulation, and granulosa cell necroptosis.[7,34,35]

TNF-α had a two-phase effect. At low doses, it binds to tumor necrosis factor receptor (TNFR2), which helps cells grow. At high doses, it could bind to both tumor necrosis factor receptor (TNFR1) and TNFR2 at the same time, which kills cells. This gives endocrine and ovarian follicle development a two-phase effect.[7,18]

DNA damage changes the intrinsic pathway of apoptosis by starting a P53 response. This response could interact with the mitochondrial membrane to start pro-apoptosis (BAX). As a result, granulosa cell mitochondria released cytochrome c, bound to APAF-1, and then activated caspase 9. The extrinsic pathway was caused by an increase in pro-inflammatory cytokines (TNF-α) due to oxidative stress.[33,34] TNF-α, which bound to adapter proteins (Fas-Associated Death Domain protein (FADD) and TNFR1-Associated Death Domain protein (TRADD)), would activate caspase 8. Both caspase 9 and caspase 8 activated caspase 3 (executor caspase), resulting in apoptosis. Lead exposure might also increase apoptosis indirectly by impairing pituitary-hypothalamic function, resulting in disrupted hormonal regulation of the ovaries. Gonadotropin-releasing hormone (GnRH) consequently releases less Follicle-Stimulating Hormone (FSH). A decrease in FSH could inhibit follicular growth. Imbalanced follicle development triggers apoptosis and increases the risk of infertility.[28]

Many studies were conducted to address the consequences of ovarian stress, such as the administration of vitamins C and E. Furthermore, garlic was administered to determine whether it could improve folliculogenesis. The results, particularly when examining tertiary follicles, were still insufficient for stimulating folliculogenesis. [36,37]

The plant *Saussurea lappa* contains dehydrocostus lactone and costunolide that are excellent in reducing inflammation and protecting cells from damage. Dehydrocostus lactone could inhibit the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α,[11] and costunolide could inhibit the activation of NF-kB, TNF-α, NO, iNOS, and COX-2.[38,39,40]

Dehydrocostus lactone possessed the ability to suppress the production of inducible nitric oxide synthase (iNOS) and act as a pharmacological inhibitor of NF-κB activation. [37,38,39] Costunolide, with its anti-inflammatory properties, was able to inhibit apoptosis in Lipopolysaccharide (LPS)-induced BV2 microglia cells by reducing several neuroinflammatory mediators, such as iNOS and TNF-α, through inhibition of NF-κB and Mitogen-Activated Protein Kinase (MAPK) activation through blockade of IκBα phosphorylation.[38,39] Costunolide could activate SOD as an antioxidant agent, and was able to prevent NF-kB activation, thereby reducing nitric oxide and TNF-α levels, which are pro-inflammatory.[38] e Costunolide can inhibit the activation of NF-kB, TNF-α, NO, iNOS, and COX-2 in intestinal mucositis mouse models.[39]

Based on figure 8, apoptosis showed in K-1: 45.96±4.08, K-2: 55.64±9.42, K-3: 43.26± 4.93 and K-4: 37.54±4.08*. iNOS levels showed K-1: 50.00±3.65, K-2: 55.48±4.78, K-3: 47.21±7.29 and K-4: 43.59±4.64*. TNF-α levels showed K-1: 56.49±11.21, K-2: 51.38±5.28, K-3: 50.94±8.80 and K-4: 46.50±2.05*.

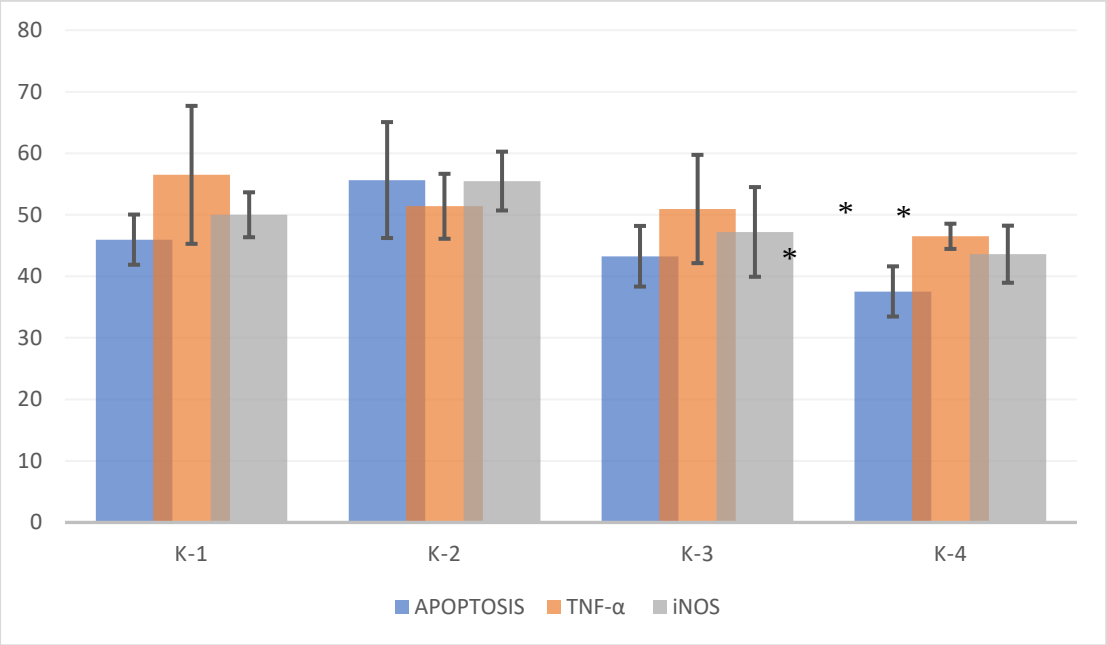

Figure 8: Results of the analysis of apoptosis, iNOS and TNF-α levels in the ovaries.

* p <0.05. Mean±SD. K-1 (Control group), treatment groups at doses K-2 (200 mg/kg), K-3 (400 mg/kg), K-4 (600 mg/kg). Apoptosis showed in K-1: 45.96±4.08, K-2: 55.64±9.42, K-3: 43.26± 4.93 and K-4: 37.54±4.08*. iNOS levels showed K-1: 50.00±3.65, K-2: 55.48±4.78, K-3: 47.21±7.29 and K-4: 43.59±4.64*. TNF-α levels showed K-1: 56.49±11.21, K-2: 51.38±5.28, K-3: 50.94±8.80 and K-4: 46.50±2.05*.

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The mean values of iNOS, TNF-α, and apoptosis at a dose of 600 mg/kg (K-4) showed significantly lower levels when compared to the control group (K-1) ($p < 0.05$). The reductions observed in the 400 mg/kg group (K-3) were moderate but not statistically significant when compared with the control, whereas the 200 mg/kg group (K-2) exhibited the least effect. These findings indicated that *Saussurea lappa* extract at a higher dose more effectively suppressed oxidative and inflammatory mediators associated with follicular apoptosis. In line with a previous study on mice with rheumatoid arthritis, the 400 mg/kg and 600 mg/kg doses were reported to be more effective at reducing inflammation than the 200 mg/kg dose. Activation of NADPH oxidase and the expression of iNOS were directly linked to the generation of highly reactive ROS.[41]

There were significantly more tertiary follicles in the *Saussurea lappa* extract treatment group (K-4, 600 mg/kg) compared to the control group (K-1) (Figure 9). The quantity of tertiary follicles in each group was as follows: K-1 1.67 ± 1.51, K-2 1.67 ± 1.03, K-3 4.00 ± 2.00, and K-4 5.17 ± 1.72*. This increase in follicular development was consistent with the lower apoptosis level observed in the K-4 group compared to the control (K-1). Apoptosis levels were as follows: K-1 45.96 ± 4.08, K-2 55.64 ± 9.42, K-3 43.26 ± 4.93, and K-4 37.54 ± 4.08*. In the Kruskal–Wallis test ($p < 0.05$), *Saussurea lappa* extract demonstrated a significant effect on the number of tertiary follicles. *Saussurea lappa* contains phytochemicals such as dehydrocostus lactone and costunolide that helps reduce inflammation and free radicals.[36,42]

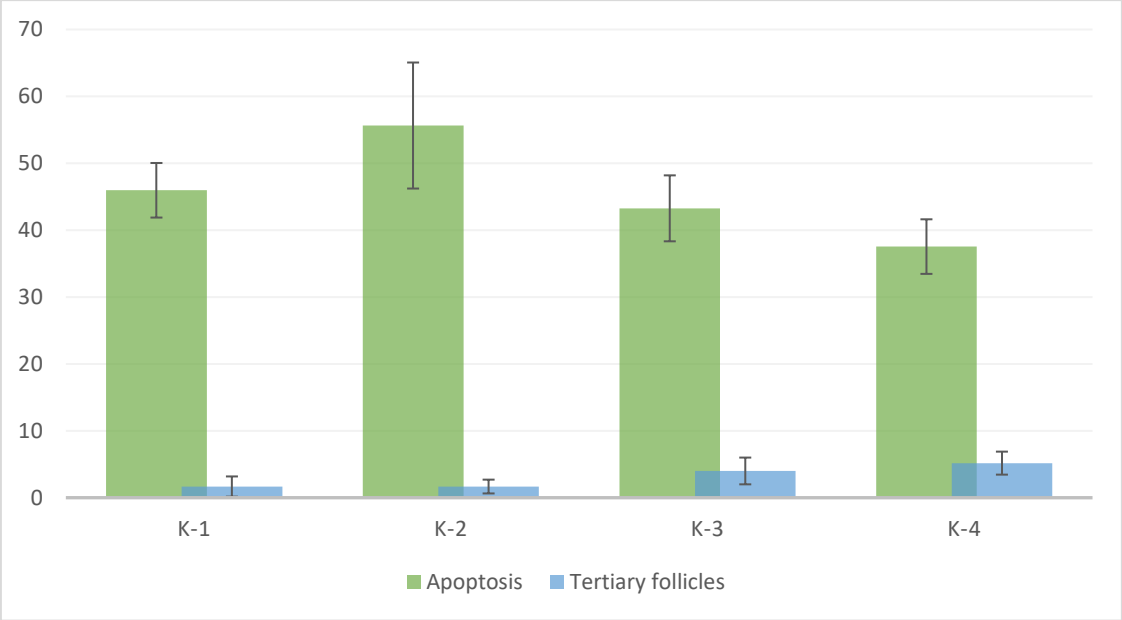

Figure 9: Results of the analysis of apoptosis levels and the number of tertiary follicles.
* p <0.05. Mean±SD. K-1 (control group), treatment groups at doses K-2 (200 mg/kg), K-3 (400 mg/kg), K-4 (600 mg/kg). The quantity of tertiary follicles in each group was as follows: K-1 1.67±1.51; K-2 1.67±1.03; K-3 4.00±2.00; K-4 5.17±1.72*. Apoptosis levels were as follows: K-1: 45.96±4.08, K-2: 55.64±9.42, K-3: 43.26± 4.93 and K-4: 37.54±4.08*.

This helps to prevent the production of iNOS and pro-inflammatory cytokines in the ovaries. Lower levels of iNOS and TNF-α could lead to lower levels of apoptosis. This would promote follicular development and increases the number of tertiary follicles, indicating a vital phase in ovarian folliculogenesis and serving as a marker of reproductive potential. The tertiary follicle served as the primary signal for assessing the progression of folliculogenesis.[33,43,44,45] Meanwhile, the number of primary and secondary follicles also increased. The maximum quantity of primary follicles occurred at a dosage of 200 mg/kg, whereas the secondary follicles peaked at a dosage of 400 mg/kg. Consequently, it might be inferred that the administration of 70% ethanol extract of *Saussurea lappa* could enhance the process of folliculogenesis.

Conclusion

Treatment with 70% ethanol extract of *Saussurea lappa* at a dose of 600 mg/kg significantly lowered expression of iNOS, TNF-α, and apoptosis, accompanied by a significantly higher number of tertiary follicles compared to the control group. Reducing iNOS and TNF-α can minimize apoptosis, improving folliculogenesis. The use of a specific animal model and the short duration of lead exposure and *Saussurea lappa* treatment may limit the generalizability of these findings. Moreover, the absence of long-term follow-up restricts assessment of the sustained effects on ovarian function and fertility. Future studies should employ multiple animal models and longer durations of lead exposure and *Saussurea lappa* treatment to confirm the reproducibility and sustained effects observed in this study. Long-term follow-up is also needed to assess impacts on ovarian function, reproductive outcomes, and overall fertility. In addition, further mechanistic investigations and evaluation of individual bioactive compounds may clarify the pathways through which *Saussurea lappa* exerts its protective effects.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Feng J, Wu Q, Liang Y, Liang Y, Bin Q. Epidemiological characteristics of infertility, 1990–2021, and 15-year forecasts: an analysis based on the global burden of disease study 2021. Reprod Health. 2025; 22(1):26. https://doi.org/10.1186/s12978-025-01966-7
2. Osowski A, Fedoniuk L, Bilyk Y, Fedchyshyn O, Sas M, Kramar S, Lomakina Y, Fik V, Chorniy S, Wojtkiewicz J. Lead exposure assessment and its impact on the structural organization and morphological peculiarities of rat ovaries. Toxics. 2023; 11(9):769. https://doi.org/10.3390/toxics11090769
3. Walker MH, Tobler KJ. Female infertility. In: StatPearls [Internet]. 2022[cited 2022 Dec 19]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK556033/
4. Canipari R, De Santis L, Cecconi S. Female fertility and environmental pollution. Int. J Environ Res Public Health. 2020; 17(23):8802. https://doi.org/10.3390/ijerph17238802
5. Priya K, Setty M, Babu UV, Pai KS. Implications of environmental toxicants on ovarian follicles: how it can adversely affect female fertility? Environ Sci Pollut Res Int. 2021; 28(48):67925–67939. https://doi.org/10.1007/s11356-021-16489-4
6. Syarifah AS, Sudjarwo SA, Hendarto H, I'tishom R. *Nanocurcumin* potential effect of SOD enzyme and caspase-3 expression in lead-acetate induced rat's ovarian granulosa cells. Indian J Forensic Med Toxicol. 2021; 15(2). https://doi.org/10.37506/ijfmt.v15i2.14650
7. Collin MS, Venkatraman SK, Vijayakumar N, Kanimozhi V, Arbaaz SM, Stacey RS, Anusha J, Choudhary R, Lvov V, Tovar GI, Senatov F. Bioaccumulation of lead (Pb) and its effects on human: a review. J Hazard Mater Adv. 2022; 7:100094. https://doi.org/10.1016/j.hazadv.2022.100094
8. Liang H, Xu D, Tong X, Ling B, Wang W, Feng D, Liang J. Biphasic effect of TNF-α on the survival and endocrine function of human ovarian granulosa cells. Clin Exp Obstet Gynecol. 2022; 49(5):119. https://doi.org/10.31083/j.ceog4905119
9. Fiorentino G, Cimadomo D, Innocenti F, Soscia D, Vaiarelli A, Ubaldi FM, Gennarelli G, Garagna S, Rienzi L, Zuccotti M. Biomechanical forces and signals operating in the ovary during folliculogenesis and their dysregulation: implications for fertility. Hum Reprod Update. 2023; 29(1):1–23. https://doi.org/10.1093/humupd/dmac031
10. Ojo OA, Nwafor-Ezeh PI, Rotimi DE, Iyobhebhe M, Ogunlakin AD, Ojo AB. Apoptosis, inflammation, and oxidative stress in infertility: a mini review. Toxicol Rep. 2023; 10:448–462. https://doi.org/10.1016/j.toxrep.2023.04.006
11. Ali A, Alqaseer K, Fatlawi D, Shehab S, Falah M, Hassan M, Shnain W, Radhi O. *Saussurea lappa*: an important medicinal plant for treatment different diseases: a review. Kufa J Nurs Sci. 2021; 11(1):1–8. https://doi.org/10.36321/kjns.vi20211.427
12. Lammari N, Demautis T, Louaer O, Meniai AH, Casabianca H, Bensouici C, Devouassoux G, Fessi H, Bentaher A, Elaissari A. Nanocapsules containing *Saussurea lappa* essential oil: formulation, characterization, antidiabetic, anti-cholinesterase and anti-inflammatory potentials. Int. J Pharm. 2021; 593:120138. https://doi.org/10.1016/j.ijpharm.2020.120138
13. Alvarenga ES, Macías FA, Ferreira SS, Galindo JC, Molinillo JM. Exploring sesquiterpene lactones from *Saussurea lappa*: isolation, structural modifications, and herbicide bioassay evaluation. Plants (Basel). 2025; 14(7):1111. https://doi.org/10.3390/plants14071111
14. Kumari R, Negi M, Thakur P, Mahajan H, Raina K, Sharma R, Singh R, Anand V, Ming LC, Goh KW, Calina D. *Saussurea costus* (Falc.) Lipsch.: a comprehensive review of its pharmacology, phytochemicals, ethnobotanical uses, and therapeutic potential. Naunyn Schmiedebergs Arch Pharmacol. 2024; 397(3):1505–1524. https://doi.org/10.1007/s00210-023-02694-0
15. Raharjo CO, Akbar CS, Rahmalia MQ. Anticancer activity of *Aucklandia costus* Falc. terpenes: targeting MDM2 protein inhibition for therapeutic advancements. J Pharm Pharmacogn Res. 2024; 12(4):748–758. https://doi.org/10.56499/jppres23.1910_12.4.748
16. Abubakar AR, Haque M. (2020). Preparation of medicinal plants: basic extraction and fractionation procedures for experimental purposes. J Pharm Bioallied Sci. 12(1):1–10. https://doi.org/10.4103/jpbs.jpbs_175_19
17. Khalid AM, Mahmod AI, Rashid HM, Talib W, Hasen E, Al-Najjar M, Afifi F. LC–MS analysis and biological potential of the peel extracts of *Citrus aurantium* grown in Jordan. Trop J Nat Prod Res. 2020; 8(10). https://doi.org/10.26538/tjnpr/v8i10.19
18. Naselli F, Cardinale PS, Volpes S, Martino C, Cruciata I, Valenti R, Luparello C, Caradonna F, Chiarelli R. An alternative approach of TUNEL assay to specifically characterize DNA fragmentation in cell model systems. Histochem Cell Biol. 2024; 162(5):429–442. https://doi.org/10.1007/s00418-024-02306-9
19. Al-Jammas S, Taqa GA, Al-Saraj A. Impact of teriparatide acetate on caspase-9 gene expression in relation to histological and TUNEL assay of apoptosis in rat kidneys and livers. J Appl Vet Sci. 2025; 10(1):101–108. https://dx.doi.org/10.21608/javs.2024.337828.1467
20. Janke LJ, Ward JM, Vogel P. Classification, scoring, and quantification of cell death in tissue sections. Vet Pathol. 2019; 56(1):33–38. https://doi.org/10.1177/0300985818800026
21. Sarma UC, Winship AL, Hutt KJ. Comparison of methods for quantifying primordial follicles in the mouse ovary. J Ovarian Res. 2020; 13(1):121. https://doi.org/10.1186/s13048-020-00724-6
22. Moldovan C, Onaciu A, Toma V, Munteanu RA, Gulei D, Moldovan AI, Stiufiuc GF, Feder RI, Cenariu D. Current trends in luminescence-based assessment of apoptosis. RSC Adv. 2023; 13(45):31641–31658. https://doi.org/10.1039/D3RA05809C
23. Schütz LF, Batalha IM. Granulosa cells: central regulators of female fertility. Endocrines (Basel). 2024; 5(4):547–565. https://doi.org/10.3390/endocrines5040040
24. Morotti F, Miguez-Gonzalez S, Cerezetti MB, Seneda MM. Evaluation of three classification methods of antral follicle count and fertility to the timed artificial insemination in cattle. Anim Reprod. 2022; 19:e20210121. https://doi.org/10.1590/1984-3143-AR2021-0121
25. Liang S, Chen C, Li R, Jiang G, Du L. Costunolide and dehydrocostus lactone alleviate ulcerative colitis via regulating TLR4, NF-κB and PI3K expression. Sci. Rep. 2024; 14(1):29777. https://doi.org/10.1038/s41598-024-77021-5
26. Gershon E, Dekel N. Newly identified regulators of ovarian folliculogenesis and ovulation. Int. J Mol Sci. 2020; 21(12):4565. https://doi.org/10.3390/ijms21124565
27. Orozco-Galindo BV, Sánchez-Ramírez B, González-Trevizo CL, Castro-Valenzuela B, Varela-Rodríguez L, Burrola-Barraza ME. Folliculogenesis: a cellular crosstalk mechanism. Curr Issues Mol Biol. 2025; 47(2):113. https://doi.org/10.3390/cimb47020113
28. Karimah F, Sa'adi A, Widjiati W. The ethanolic extract of *Ocimum basilicum* effect on expression of follicle stimulating hormone receptors (FSHr) and folliculogenesis in female mice model endometriosis. J Med Chem Sci. 2023; 6(12):2896–2905. https://doi.org/10.26655/JMCHEMSCI.2023.12.4
29. Adelodun TS, Okonoboh MJ, Ajibade TP, Abijo AZ, Olatunji SY, Olorunnado ES, Ogunsanya ST, Adewole OS. Ameliorative potentials of *Linum usitatissimum* (flax seed) crude extract against lead acetate-induced neuronal damage on the hippocampal histoarchitecture using Wistar rat model. Trop J Nat Prod Res. 2025; 9(1). https://doi.org/10.26538/tjnpr/v9i1.45
30. Rudnicka E, Suchta K, Grymowicz M, Calik-Ksepka A, Smolarczyk K, Duszewska AM, Smolarczyk R, Meczekalski B. Chronic low-grade inflammation in pathogenesis of PCOS. Int. J Mol. Sci. 2021; 22(7):3789. https://doi.org/10.3390/ijms22073789

31. Minhas R, Bansal Y, Bansal G. Inducible nitric oxide synthase inhibitors: a comprehensive update. Med Res Rev. 2020; 40(3):823–855. https://doi.org/10.1002/med.21636
32. Akhtar F, Bokhari SRA. Apoptosis. In: StatPearls [Internet]. 2024 [cited 2024, Apr 30]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK499821/
33. Kim JH, Moon SH, Kim MY. Modulation of A375 human melanoma cell proliferation and apoptosis by nitric oxide. J Appl. Nat Sci. 2022; 14(2):320. https://doi.org/10.31018/jans.v14i2.3365
34. Elnour AA, Abdurahman NH. Current and potential future biological uses of *Saussurea costus* (Falc.) Lipsch: a comprehensive review. Heliyon. 2024; 10(18):e37790. https://doi.org/10.1016/j.heliyon.2024.e37790
35. Rauf A, Khalil AA, Awadallah S, Khan SA, Abu-Izneid T, Kamran M, Hemeg HA, Mubarak MS, Khalid A, Wilairatana P. Reactive oxygen species in biological systems: pathways, associated diseases, and potential inhibitors — a review. Food Sci. Nutr. 2024; 12(2):675–693. https://doi.org/10.1002/fsn3.3784
36. Jafari F, Khalilzadeh S, Nejatbakhsh F. Therapeutic effects of garlic (*Allium sativum*) on female reproductive system: a systematic review. Heliyon. 2023; 9(12):e22555. https://doi.org/10.1016/j.heliyon.2023.e22555
37. Sapmaz T, Kale E, Pence ME, Sevgin K, Tekayev M, Topkaraoglu S, Basol G, Yilmaz MB, Sapmaz E, Irkorucu O. Treatment strategies with vitamin E and C in autologous intraperitoneal ovarian transplantation and its impact on ovarian surface epithelium and follicle reserve. Biochem Biophys Res Commun. 2023; 677:81–87. https://doi.org/10.1016/j.bbrc.2023.08.002
38. Wang L, Yang M, Zhang C, Huang F. The protective effects of dehydrocostus lactone against TNF-α-induced degeneration of extracellular matrix (ECM) in SW1353 cells. Aging (Albany NY). 2020; 12(17):17137. https://doi.org/10.18632/AGING.103657
39. Silva JR, Lima FE, Souza AL, Silva AW. Interleukin-1β and TNF-α systems in ovarian follicles and their roles during follicular development, oocyte maturation and ovulation. Zygote. 2020; 28(4):270–277. https://doi.org/10.1017/S0967199420000222
40. Sukmawati IM, Muchtaridi M, Fristiohady A, Ikram NK. Anti-inflammatory activity of quts al-hindi extract (*Saussurea lappa*) using erythrocyte membrane stability and prediction of its selectivity for COX-2 and iNOS enzymes. J Hunan Univ. Nat Sci. 2023; 50(10).
41. Tag HM, Khaled HE, Ismail HA, El-Shenawy NS. Evaluation of anti-inflammatory potential of the ethanolic extract of the *Saussurea lappa* root (costus) on adjuvant-induced monoarthritis in rats. J Basic Clin. Physiol. Pharmacol. 2016; 27(1):71–78. https://doi.org/10.1515/jbcpp-2015-0044
42. Kim DY, Choi BY. Costunolide — a bioactive sesquiterpene lactone with diverse therapeutic potential. Int. J Mol. Sci. 2019; 20(12):2926. https://doi.org/10.3390/ijms20122926
43. Lee SH, Cho YC, Lim JS. Costunolide, a sesquiterpene lactone, suppresses skin cancer via induction of apoptosis and blockage of cell proliferation. Int. J Mol. Sci. 2021; 22(4):2075. https://doi.org/10.3390/ijms22042075
44. Jabori EE, Khaleel LW, Ismail HK, Flaih AN. Modulatory effect of *Saussurea costus* ethanolic extract on kidney male rats exposed to oxidative stress. Iraqi J Vet Sci. 2023; 37:81–88. https://doi.org/10.33899/ijvs.2022.1373070.2668
45. Nadda RK, Ali A, Goyal RC, Khosla PK, Goyal R. *Aucklandia costus* (syn. *Saussurea costus*): ethnopharmacology of an endangered medicinal plant of the Himalayan region. J Ethnopharmacol. 2020; 263:113199. https://doi.org/10.1016/j.jep.2020.113199