" Kerbala Journal of Veterinary Medical Sciences, Vol. 1, Supplement I, 2025 "
"Proceedings of the 6th Scientific Conference, College of Veterinary Medicine, University of Karbala"

The protective effects of Urtica Dioica ethanolic extract against ovarian damage induced by 4-vinylcyclohexene diepoxide in a rat model of menopause

Aisha Abdullah khalifa [1] , Wafaa Kadhim Jasim [1], Mariam abdul-hussein kadhem [1] , Basheer ali al-janabi [2]

[1]Department of physiology, College of Veterinary Medicine, University of Kerbala, Kerbala, Iraq
[2]Al-furat Al-awsat technical university ,Al-mussaib technical institute
Corresponding author: aisha.a@s.uokerbala.edu.iq
Received: 29/4/2025 **Accepted:** 25/5/2025 **Published:** 3/7/2025

ABSTRACT-This study aimed to investigate the protective effects of ethanolic extract of Urtica dioica on the ovarian physiological state in chemically induced menopausal rats. Menopause was induced in female rats using 4-vinylcyclohexene diepoxide (VCD) at dose160 mg/kg/d via IP for 20 days, after which they were divided into four groups, each group contains 10 rats: a control group, a group treated with Urtica dioica leaves ethanolic extract, a group treated with VCD, and a group receiving both VCD and Urtica dioica extract. The *Urtica dioica* extract was administered at a dosage of 100 mg/kg body weight via gastric gavage for 20 consecutive days throughout a total study period of 52 days. Blood samples were collected at three separate time points to assess reproductive hormone levels, while histological studies of the ovary and hypothalamus were performed at the conclusion of the study. The results showed that the a significant increase (P≤0.05) in serum concentrations of LH, FSH hormone, as well as a Significant decline (p≤ 0.05) in serum concentrations of Testosterone and Estradiol in the VCD group, but when administered animals Urtica Dioica ethanolic extract demonstrated an improvement in the parameters. Histopathological analysis of both the ovary and hypothalamus revealed extensive damage in the VCD group, Whereas when given Urtica Dioica ethanolic extract in fourth group were effective in arresting histopathological alterations . In contrast, The observations suggest that Urtica dioica may possess protective potential in mitigating ovarian malfunction and neuroendocrine abnormalities associated with menopause.
KeyWord: Urticadioica, 4vinylcyclohexenediepoxide, Hormones. menopausal Rats

INTRODUCTION

Menopause, signifying the cessation of the menstrual cycle, elevates a woman's susceptibility to various conditions, including ovarian cancer, diabetes, osteoporosis, cardiovascular disease, and metabolic syndrome. The majority of women undergo perimenopause, characterized by a gradual decline in ovarian function over several years, prior to reaching menopause while retaining any residual ovarian tissue.(1).The ovary serves as the principal site for the generation of female sex steroid hormones, encompassing estrogens and progestins. At birth, the mammalian ovary possesses its whole array of oocyte-containing follicles. Oocytes cannot be produced postnatal, rendering the quantity of primordial follicles a limited reservoir of germ cells for ovulation. Proper follicular development is essential for effective ovulation, requiring the follicle to progress through several developmental stages (2).

"Primordial" denotes the earliest phase of follicular development, characterized by the predominance of atresia, or cellular apoptosis, rather than progression to ovulation. The ovary undergoes continuous follicular atresia from birth until the depletion of follicle reserves. Menopause, or ovarian failure, occurs when the ovary is devoid of primordial follicles (3).

The five to 10 years preceding menopause are referred to as perimenopause, characterized by irregular cycle durations and fluctuating estrogen levels. Prolonged intervals of low estradiol are alternated with periods of elevated estradiol, and after menopause, circulation levels of 17β-estradiol consistently decline to low levels. However, post-menopause, the residual ovarian tissue retains the capacity to secrete androgens (4).

It is essential to formulate and utilize a medication that minimizes problems and maximizes safety. Medicinal herbs are natural substances employed in traditional medicine to address menopausal symptoms. Multiple studies have demonstrated that both pharmacological and herbal medicinal agents, whether administered alone or in conjunction, may influence reproductive function (5)

Urtica dioica (UD), commonly known as Nettle, is a flowering herbaceous perennial belonging to the family Urticaceae , has been recognized as a medicinal plant utilized in traditional medicine for its extensive biological activity, This plant contains a broad range of phytochemicals, such as phenolic compounds, sterols, fatty acids, alkaloids, terpenoids, flavonoids, and lignans, that have been widely reported for their excellent pharmacological activities, including antiviral, antimicrobial, antihelmintic, anticancer, nephroprotective, hepatoprotective, cardioprotective, antiarthritis, antidiabetic, antiendometriosis, antioxidant, anti-inflammatory, and antiaging effects.(6).

It is indigenous to Europe, substantial regions of temperate Asia, and western North Africa, and is now globally distributed. The plant has a longstanding history of utilization as a source of traditional medicine, food, tea, and textile raw materials in both ancient and contemporary countries (7). The aim of this study was to investigate the antioxidant properties and protective effects of ethanolic extract of Urtica dioica on the physiological state of the ovary in induced-menopausal rats through changes in reproductive hormone levels, oxidative stress status, and histological changes in the ovary and hypothalamus.

MATERIALS AND METHODS

Forty adult female rats at 49 days were divided equally and randomly into four groups. Before treatment, the animals were allowed to acclimate to the laboratory environment for 7 days until the age of 56 days treatment period lasted for 20 days with the following groups: The rats were divided into 4 groups (10 rats in each group) (the control group) was administered only with normal saline, Group Urtica Dioica was administered (100 mg/kg) Urtica Dioica leaves ethanolic extract orally for 20 days (8), The VCD group received 160 mg/kg/day of VCD via intraperitoneal injection (IP) for 20 days (9), The VCD + Urtica dioica group received 160 mg/kg/day of VCD via IP and 100 mg/kg/day of Urtica dioica orally for 20 days. Blood samples were collected through heart puncture technique in three stages, and the serum was stored for hormonal analysis. The ovaries and hypothalamus were removed for histopathological examination. The organs were then preserved in sterile plastic containers in 10% formalin.

STATISTICAL ANALYSIS

The Statistical Packages of Social Sciences- (10). program was used to detect the effects of different groups and time periods on study parameters. The Least Significant Difference (LSD) test (two-way ANOVA) was used to determine significant differences among group means.

RESULT & DISCUSSION

Hormonal analysis revealed statistically significant alterations among the experimental groups. The VCD-treated group showed marked hormonal disturbances compared to the control, indicating that VCD successfully induced ovarian failure and mimicked menopausal status.

1-Luteinizing Hormone (LH)

The VCD group exhibited a significant elevation in LH levels at all measured intervals, peaking on day 35 at 8.00 ± 0.45. Treatment with Urtica dioica significantly reduced LH levels in the VCD + plant group (4.87 ± 0.15 on day 35), approaching the control group levels (3.12 ± 0.06).

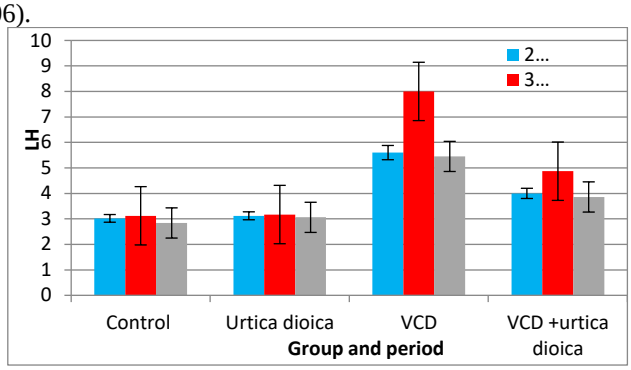

Figure 1. Effect of treatment group and duration on LH levels

2-Follicle Stimulating Hormone (FSH):

FSH levels increased significantly in the VCD group, peaking on day 35 (19.50 ± 0.64). In contrast, the VCD plus plant group showed a decrease in FSH levels (14.05 ± 0.21), indicating a regenerative effect of Urtica dioica. Both the control and plant-only groups maintained stable and normal levels.

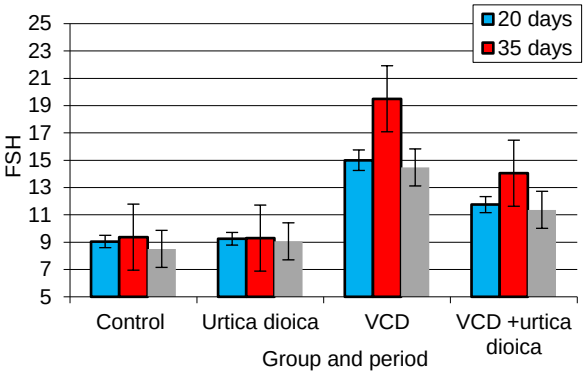

Figure 2. Effect of treatment group and duration on FSH levels

3-Testosterone

A significant decrease in testosterone levels was observed in the VCD group, particularly on day 35 (0.287 ± 0.02). Administration of nettle in the VCD group increased testosterone levels (0.475 ± 0.03), suggesting a partial recovery towards normal levels, although still lower than those in the control group (0.822 ± 0.02).

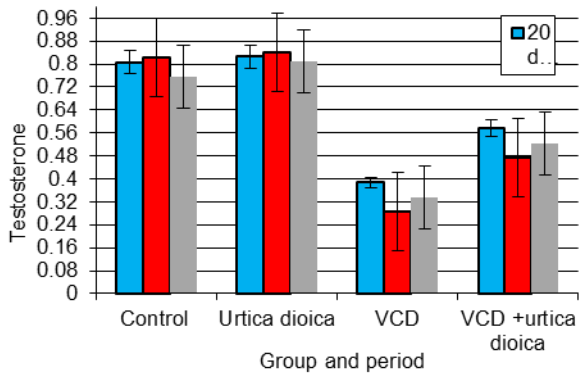

Figure 3. Effect of treatment group and duration on Testosterone levels

4-Estradiol (E2)

Mice treated with VCD showed a significant decrease in estradiol levels, particularly on day 35 (10.50 ± 0.64). However, nettle treatment improved estradiol levels in the VCD group (18.50 ± 0.64), partially restoring them toward the control range (28.25 ± 0.63).

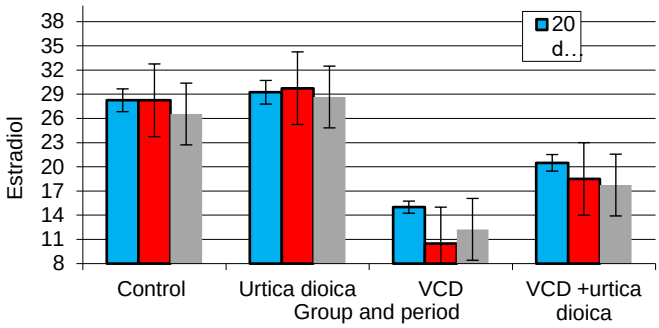

Figure 4. Effect of treatment group and duration on Estradiol levels

5-Anti-Müllerian Hormone (AMH)

AMH levels in the VCD group were markedly reduced at all time intervals, attaining a value of 3.01 ± 0.66 on day 20. Conversely, the VCD + plant group exhibited elevated AMH levels (6.69 ± 0.52 at day 52), signifying a retained ovarian reserve relative to the untreated VCD group. Only the Urtica dioica group exhibited the highest AMH values, indicating its beneficial involvement in preserving follicular health.

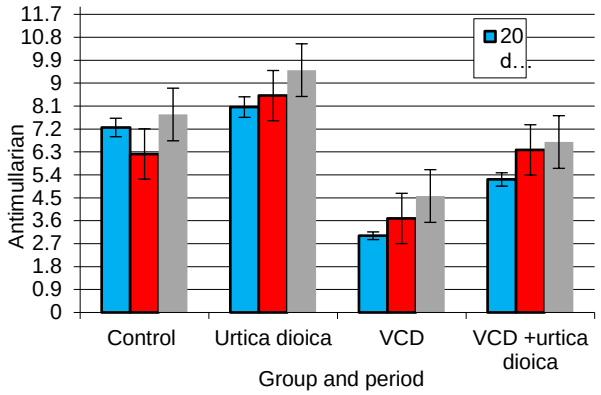

Figure 5: Effect of treatment group and duration on Anti-Müllerian Hormone levels

6-Histological Findings

The control group had active folliculogenesis, characterized by a normal germinal layer and ovarian stroma (Fig. 6).

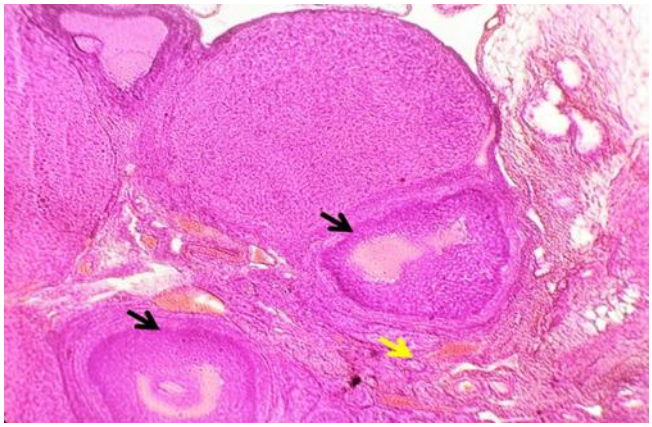

Figure 6: Photography of ovary (control group) show normal and appearance highly activity germinal layer. Stromal ovarian tissue appearance normal state with numerous of follico-genesis (black arrows). H&E stain. 100X.

The Urtica dioica Group had improved follicular growth and little degeneration, showing the favorable benefits of the extract alone (Fig. 7)

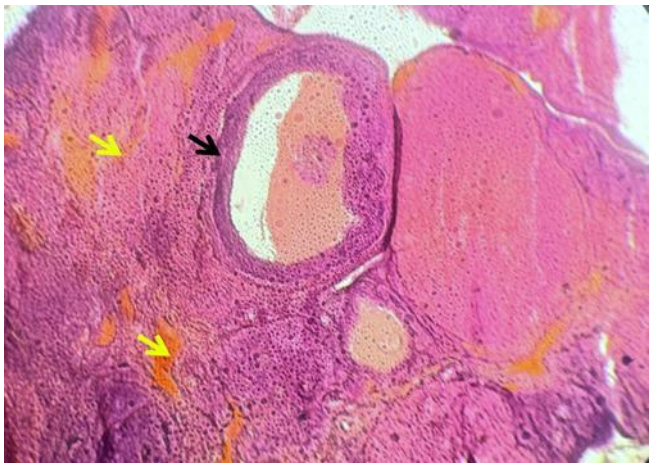

Figure 7. Photography of ovary (plant group) show the rise in the variety of mature follicle (black arrow) and the ovarian cortex should show well-functioning with minimal degeneration (yellow arrows). H&E stain.100X..

VCD Group: Ovaries exhibited atresia, fibrotic stromal alterations, and reduced follicular count. (Fig. 8) demonstrating perimenopausal induction.

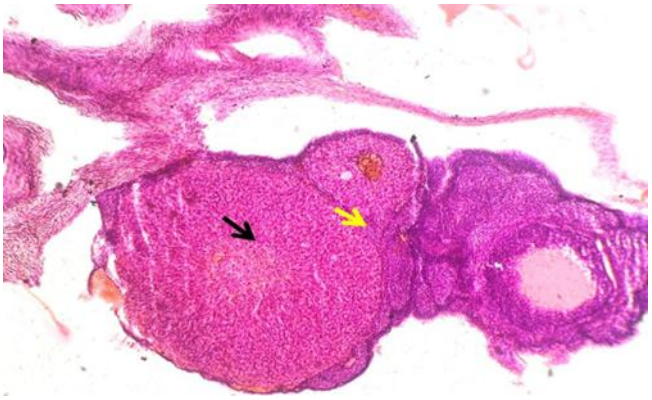

Figure 8. Photography of Ovary (VCD group) show which is seen as a reduction in healthy follicles(black arrow) and an increase in atretic follicles. VCD can lead to changes in the stromal cells, causing fibrosis or thickening of the ovarian stroma (yellow arrow).H&E stain.100X.

VCD + Plant Group: Histological analysis indicated restoration of follicular architecture, a rise in viable follicles, normalization of stroma, and evidence of corpus luteum development (Fig. 9)

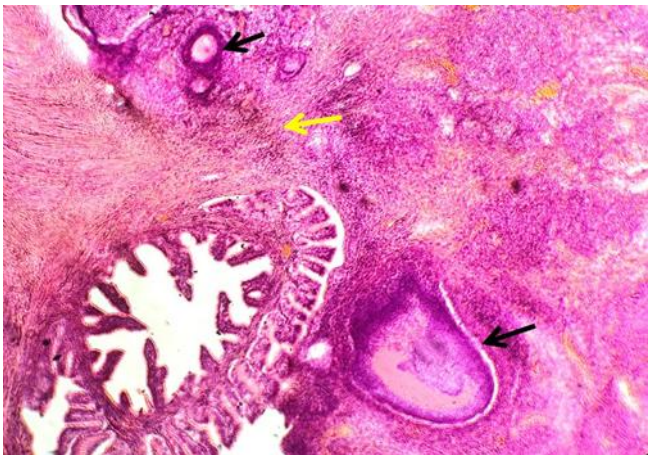

Figure 9. Photography of Ovary (VCD +Plant group) show Increased number of mature follicles compared to the VCD group (black arrow). Normalization of ovarian stroma and architecture and Potential maintenance of corpus luteum and healthy oocyte development. H&E stain.100X.

The hypothalamic tissue had a normal cellular architecture with clearly delineated neurons and an even distribution of glial cells, signifying a non-inflammatory and healthy neuroenvironment.

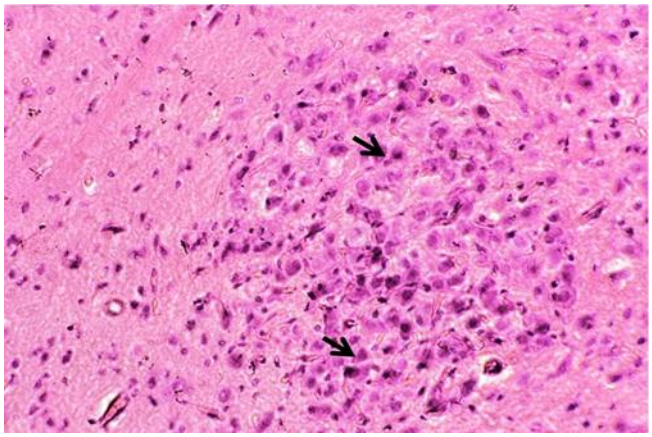

Figure 10. Photography of Hypothalamus (control group) show the hypothalamus would indicate a normal cellular arrangement. The neurons will be well-defined, and the glial cells (such astrocytes and microglia) would be in their typical proportions (black arrows).H&E stain.400X.

The hypothalamic tissue exhibited diminished glial activation and reduced reactivity, indicating a decrease in neuroinflammatory processes relative to the control group

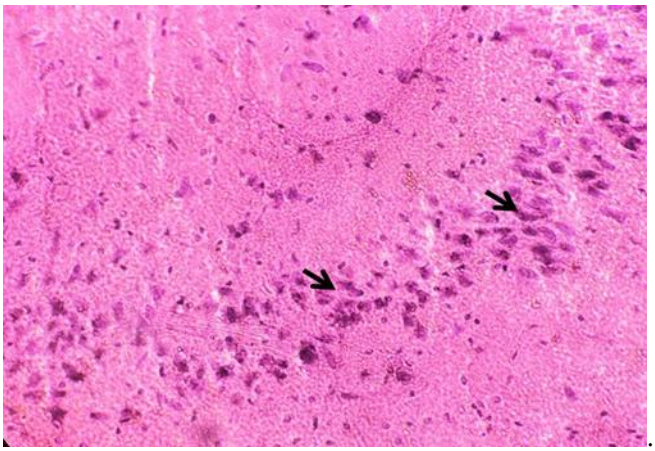

Figure 11. Photography of hypothalamus (plant group) show Possible reduction in glial activation, indicating decreased neuroinflammation and Presence of less reactive or atrophic glial cells compared to control. H&E stain. 400X.

The hypothalamus in the VCD group exhibited neuronal degeneration, a reduced count of intact neurons, and the presence of cellular debris. A significant rise in reactive glial cells was observed, suggesting neuroinflammation and potential neurotoxicity.

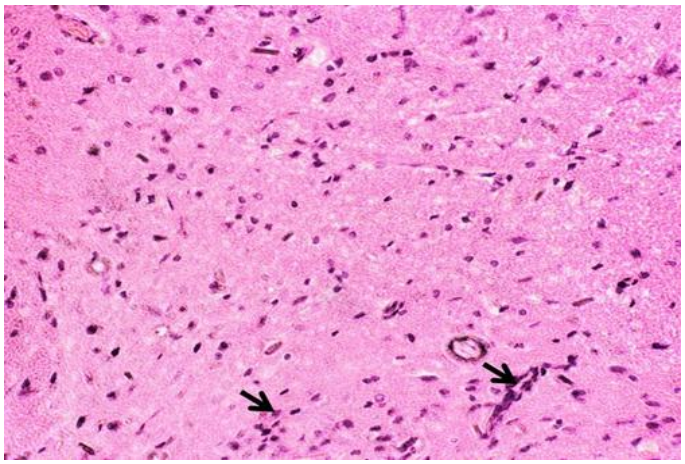

Figure 12. Photography of hypothalamus (VCD group) show Neuronal degeneration or shrinkage, with a decrease in the number of intact neurons with Presence of cellular debris due to apoptosis or necrosis (increased numbers of reactive glial cells (black arrow. H&E stain.400X.

Histological analysis showed improved neuronal morphology with partial repair of previously damaged neurons. There was a noticeable reduction in glial activation compared to the VCD group, suggesting reduced inflammation and neural recovery.

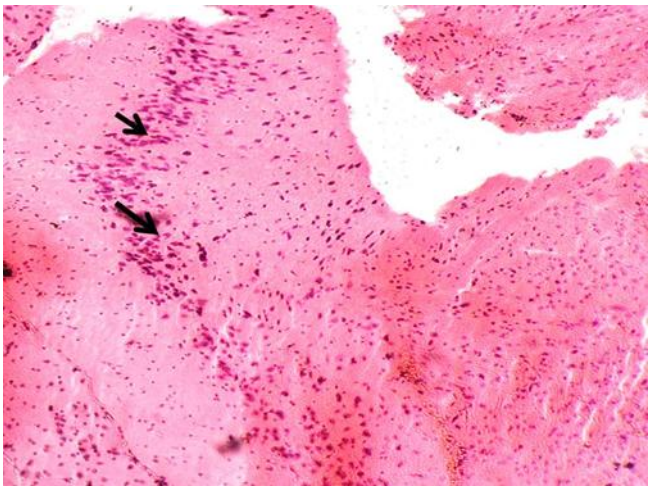

Figure 13. Photography of Ovary (VCD +Plant group) show Improved neuronal morphology, with some repair of damaged neurons Possible restoration of some normal neuronal functions and cellular architecture with Reduced glial activation compared to the VCD group/ (Black arrow).H&E stain.400X.

DISCUSSION

The documented hormonal variations substantiate that VCD proficiently creates a perimenopausal condition by affecting ovarian follicles, as indicated by elevated gonadotropins (FSH, LH) The result described above corresponds to the research undertaken by (11) and (12) and diminished levels of estradiol, testosterone, and AMH, The result described above corresponds to the research undertaken by (13)and (14) These alterations replicate normal menopause in humans, hence substantiating the model. This result is consistent by (15). VCD (4-vinylcyclohexene diepoxide) selectively eliminates primordial and primary ovarian follicles, resulting in a substantial decrease in estrogen synthesis. The feedback pathway to the hypothalamus and pituitary gland is impaired. The decline in estrogen levels prompts an elevation in gonadotropins—specifically FSH and LH—resulting from the absence of negative feedback. This hormonal imbalance resembles the hormonal profile of menopause or premature ovarian failure, marked by diminished estrogen and elevated gonadotropins(25).

The extract of Urtica dioica exhibited a modulatory effect on hormonal levels. Its treatment resulted in the normalization of LH and FSH, as well as the restoration of estradiol and AMH levels, The result described above corresponds to the research undertaken by (16)and (17) indicating its phytoestrogenic and antioxidant properties. The extract may improve ovarian responsiveness, diminish oxidative stress in follicular cells, and maybe regulate the hypothalamic-pituitary-gonadal axis.

Elevated AMH levels in Urtica-treated groups signify a retained or enhanced follicular reserve, demonstrating a crucial preventive mechanism against ovarian aging. The

result described above corresponds to the research undertaken by (18)

These findings corroborate previous research indicating that Urtica dioica may enhance reproductive hormones and mitigate chemically induced ovarian damage These data indicate a standard ovarian histology of healthy, reproductively viable animals. The existence of many follicular phases indicates an appropriate hormonal equilibrium and follicular maturation,The result described above corresponds to the research undertaken by (19).

These observations suggest a potential stimulatory or protective influence of the plant extract on ovarian function. Herbal compounds have demonstrated the ability to affect folliculogenesis via antioxidant and hormonal regulation. The result described above corresponds to the research undertaken (20)

Histological alterations are indicative of chemically caused ovarian damage. VCD (4-vinylcyclohexene diepoxide) is recognized for its affinity for primordial and primary follicles, resulting in premature ovarian failure. The result described above corresponds to the research undertaken (21)

The histological integrity underpins appropriate neuroendocrine activity, which is crucial for reproductive and hormonal regulation ,The result described above corresponds to the research undertaken by (22)

A plant extract with a restorative or protective function against damage to glial activity may mitigate brain health and enhance neuroendocrine signaling. The above finding is consistent with previous research (23).

The results suggest that the plant extract may have a restorative or protective function against VCD-induced ovarian damage, possibly via anti-apoptotic and hormone-modulating pathways. The above finding is consistent with research conducted (24).

Neuroinflammation and glial activation may arise as a consequence of systemic toxicity or the direct impact of ovarian failure on the hypothalamic-pituitary axis. This may disrupt reproductive neuroendocrine regulation The result described above corresponds to the research undertaken (13)

These findings further underscore the neuroprotective potential of the plant extract, possibly through the regulation of neuroinflammatory pathways and promotion of neuronal regeneration.

CONCLUSION

This study suggest that nettle extract could help reduce the negative consequences of ovarian hypoplasia-induced ovarian failure. The extract has great potential as a natural protective agent to enhance reproductive function during perimenopause by increasing AMH levels and protecting ovarian tissue more study with bigger samples and comprehensive mechanistic investigations are needed to explain the underlying biological mechanisms

REFERENCES

1) Kamińska, M. S., Schneider-Matyka, D., Rachubińska, K., Panczyk, M., Grochans, E., & Cybulska, A. M. (2023). Menopause Predisposes Women to Increased Risk of Cardiovascular Disease. Journal of clinical medicine, 12(22), 7058. https://doi.org/10.3390/jcm12227058
2) Selvakumar, P. P., Rafuse, M. S., Johnson, R., & Tan, W. (2022). Applying Principles of Regenerative Medicine to Vascular Stent Development. Frontiers in bioengineering and biotechnology, 10, 826807. https://doi.org/10.3389/fbioe.2022.826807
3) Inman, Z. C., & Flaws, J. A. (2024). Impact of Real-life Environmental Exposures on Reproduction: Endocrine-disrupting chemicals, reproductive aging, and menopause. Reproduction (Cambridge, England), 168(5), e240113. https://doi.org/10.1530/REP-24-0113
4) McCarthy, M., & Raval, A. P. (2020). The peri-menopause in a woman's life: a systemic inflammatory phase that enables later neurodegenerative disease. Journal of
5) neuroinflammation, 17(1), 317. https://doi.org/10.1186/s12974-020-01998-9
6) Abasian Z, Rostamzadeh A, Mohammadi M, Hosseini M, Rafieian - Kopaei M. A review on role of medicinal plants in polycystic ovarian syndrome: pathophysiology, neuroendocrine signaling, therapeutic status and future prospects. Middle East Fertil Soc J. 2018;23(4):255– 262.
7) Devkota, H. P., Paudel, K. R., Khanal, S., Baral, A., Panth, N., Adhikari-Devkota, A., Jha, N. K., Das, N., Singh, S. K., Chellappan, D. K., Dua, K., & Hansbro, P. M. (2022). Stinging Nettle (Urtica dioica L.): Nutritional Composition, Bioactive Compounds, and Food Functional Properties. Molecules (Basel, Switzerland), 27(16), 5219. https://doi.org/10.3390/molecules27165219
8) Zade, A. B., Bais, S. K., & Gaikwad, K. P. (2023). A review: medicinal herbs capable for anticoagulant properties. International Journal of Pharmacy and Herbal Technology, 1(3), 264-27
9) JOI, O. (2022). Ethanolic root extract of Urtica dioica exhibits pro-fertility and antioxidant activities in female albino rats. Hormones, 4, 5
10) Mashimo, A., Oshida, R., Oka, Y., Kawabata, S., Takasu, C., Nihei, K., ... & Murata, K. (2025). Hormonal fluctuations in rodent models using 4-vinylcyclohexene diepoxide: A systematic review and meta-analysis. Hormones and Behavior, 168, 105680
11) SPSS (2019). Statistical Packages of Social Sciences-SPSS/ IBM Statistics 26 step by step. 16th Edition.
12) https://doi.org/10.4324/9780429056765
13) Sharokhyan Rezaee, M., Farzinpour, A., Farshad, A., & Hatfaludi, T. (2022). The regulative effect of Urtica dioica on sex hormones imbalance: elevated follicle-stimulating hormone/luteinizing hormone ratio≥ 4.5 is associated with low performance in aged breeder quails. Italian Journal of Animal Science, 21(1), 142-152

14) Carolino, R. O., Barros, P. T., Kalil, B., & Anselmo-Franci, J. (2019). Endocrine profile of the VCD-induced perimenopausal model rat. PLoS One, 14(12), e0226874.

15) Pestana-Oliveira, N., Kalil, B., Leite, C. M., Carolino, R. O. G., Debarba, L. K., Elias, L. L. K., ... & Anselmo-Franci, J. A. (2018). Effects of estrogen therapy on the serotonergic system in an animal model of perimenopause induced by 4-vinylcyclohexen diepoxide (VCD). ENeuro, 5(1).

16) Yu, S., Zhang, L., Wang, Y., Yan, J., Wang, Q., Bian, H., & Huang, L. (2023). Mood, hormone levels, metabolic and sleep across the menopausal transition in VCD-induced ICR mice. Physiology & Behavior, 265, 114178.

17) Kumari, R., Ponte, M. E., Franczak, E., Prom, J. C., O'Neil, M. F., Sardiu, M. E., ... & Thyfault, J. P. (2024). VCD-induced menopause mouse model reveals reprogramming of hepatic metabolism. Molecular Metabolism, 82, 101908

18) Kargozar, R., Salari, R., Jarahi, L., Yousefi, M., Pourhoseini, S. A., Sahebkar-Khorasani, M., & Azizi, H. (2019). Urtica dioica in comparison with placebo and acupuncture: a new possibility for menopausal hot flashes: a randomized clinical trial. Complementary therapies in medicine, 44, 166-173..

19) Grigorova, S., Gjorgovska, N., Petkov, E., & Levkov, V. (2022). Evaluation of the Effects of Urtica Dioica L. Supplementation on Egg Quality and Blood Parameters in Laying Hens. Veterinarija ir Zootechnika, 80(1).

20) Orsi, N. M., Baskind, N. E., & Cummings, M. (2024). Anatomy, development, histology and normal function of the ovary. In Pathology of the Ovary, Fallopian Tube and Peritoneum (pp. 1-33). Cham: Springer International Publishing..

21) Armocida, D., Palmieri, M., Tartara, F., Garbossa, D., Cofano, F., Ascenzi, B. M., ... & Frati, A. (2025). The Diencephalon: Hypothalamus. In From Anatomy to Function of the Central Nervous System (pp. 305-330). Academic Press.

22) Niu, Y., Miao, Y., Wan, W., Wang, Q., Ma, Y., Pan, M., ... & Wei, Q. (2025). The 4-vinylcyclohexene dioxide induced mouse ovarian premature failure is caused by down regulation of IGF1R and triggering excessive autophagy. Stress Biology, 5(1), 21.

23) Li, Y., Peng, Y., Nie, G., Cheng, F., Zhou, Y., Liu, J., & Yang, H. (2025). Huyang Yangkun Formula Enhances Ovarian Function and Delays Reproductive Aging by Influencing Hypothalamic GnRH/LH Pulse Release Through GAT-1/GABA/GABAAR2. Drug Design, Development and Therapy, 2677-2691.

24) Lee, H. J., Park, M. J., Joo, B. S., Joo, J. K., Kim, Y. H., Yang, S. W., ... & Kim, K. H. (2021). Effects of coenzyme Q10 on ovarian surface epithelium-derived ovarian stem cells and ovarian function in a 4-vinylcyclohexene diepoxide-induced murine model of ovarian failure. Reproductive Biology and Endocrinology, 19(1), 59

25) Du, S., Wang, L., Wang, Y., Jin, Y., Wang, A., Lv, C., & Abedi-Firouzjah, R. (2023). The modulator role of Urtica dioica on deleterious effects of retinoic acid high doses on histological parameters and fertilization of rats. Heliyon, 9(6).

26) Abdalkareem Jasim, S., Abdul-Jabbar Ali, S., Q Fadhil, O., Kh Rakhmatova, M., H Kzar, H., Margiana, R., E Al-Gazally, M., Turki Jalil, A., Hameed Mahmood, Z., Aravindhan, S., Fakri Mustafa, Y., & Q Sultan, M. (2023). Investigating the Effects of Hydro-alcoholic Urtica Dioica Extract and Retinoic Acid on Follicular Development: An Animal Study. Medical journal of the Islamic Republic of Iran, 37, 1. https://doi.org/10.47176/mjiri.37.1

27) Mayer, L. P., Pearsall, N. A., Christian, P. J., Devine, P. J., Payne, C. M., McCuskey, M. K., ... & Hoyer, P. B. (2002). Long-term effects of ovarian follicular depletion in rats by 4-vinylcyclohexene diepoxide. Reproductive Toxicology, 16(6), 775-781.