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The modulatory effects of *Urtica Dioica* Leaves extract on gene expression of GnRH-I , GnRH-II and reproductive functions in induced –menopausal Rats

A Thesis

Submitted to the Council of the College of Veterinary Medicine/ University of Kerbala, as a Partial Fulfillment of the Requirements for the Degree of Master in Science of Veterinary Medicine/ Physiology

By

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

ٱلَّذِى جَعَلَ لَكُمُ ٱلْأَرْضَ مَهْدًا وَسَلَكَ لَكُمْ فِيهَا سُبُلًا وَأَنزَلَ
مِنَ ٱلسَّمَآءِ مَآءً فَأَخْرَجْنَا بِهِۦٓ أَزْوَٰجًا مِّن نَّبَاتٍ شَتَّىٰ ﴿٥٣﴾

صدق الله العلي العظيم

(من سورة طه الاية 53)

Supervisor Certificate

We certify this thesis entitled (**The modulatory effects of Urtica Dioica Leaves extract on gene expression of GnRH-I , GnRH-II and reproductive functions in induced –menopausal Rats**) was carried out by (**Aisha Abdullah khalifa**) has been prepared under my supervision at the College of Veterinary Medicine/University of Kerbala as a Partial Fulfillment of the requirements for the degree of Master in the Sciences of Veterinary Medicine in Veterinary/ physiology.

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2025/ /

I

Committee Certification

We, the members of examining committee, certify that we have read this thesis entitled (**The modulatory effects of Urtica Dioica Leaves extract on gene expression of GnRH-I , GnRH-II and reproductive functions in induced –menopausal Rats**) presented by the student (**Aisha Abdullah khalifa**) from the department of (Veterinary Physiology, Biochemistry and pharmacology) and we have examined the student in its contents. We have found that it is adequate for the award of the Degree on Master in science of veterinary medicine/ .physiology

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Certification of Linguistic Evaluator

I certify that this thesis entitled « **The modulatory effects of Urtica Dioica Leaves extract on gene expression of GnRH-I , GnRH-II and reproductive functions in induced –menopausal Female Rats.**» for the student **(Aisha Abdullah khalifa)** was linguistically reviewed by me, and the necessary correction has been made. Thus, it is linguistically ready for examination

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III

Declaration

We hereby declare that this thesis is my original work except for equations and citations, which have been fully acknowledged. We also declare that it has not been previously, and is not concurrently, submitted for any other degree at University of Kerbala or other institutions.

Aisha Abdullah khalifa

DEDICATION

To My College teachers, especially branch physiology, pharmacology and biochemistry of you were keen on my success, you were the watchful eyes and the bright minds. To you I dedicate this letter.

…

To those who instilled in me the values of ambition and patience. To those who offered love and support. To the candle that lights my path and whose prayers accompany me. My mother and father.

…

To the one who believed in me and was with me every step of the way. To the one who seeks my comfort and happiness, the inexhaustible spring that weaves my happiness with the threads of his beloved heart, my husband.

…

To the one who, when I embrace him, makes me feel as if I own the world in an instant. To the one who made me dwell in heaven on earth. To the children of my heart,(Sanad and Noah). I hope you see in this work a message of love and giving, just as I always see you as flowers pulsating with life with every step I take.

…

To the one who smiles in my life. To my support and companion. To the one who has encouraged me: my brothers and sisters.

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To every teacher, professor, and doctor who has taught me a word in my life.

…

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Summery

The current study was conducted at the College of Veterinary Medicine/University of Karbala. and aimed to evaluate the antioxidant properties and protective effects of urtica dioica on the physiological status of the ovaries in menopausal rats. The effect of urtica dioica on the expression pattern of the GnRH gene was evaluated.

The experiment used 40 adult female rats for 52 days and was dosed for 20 days. The animals were divided into four groups: the control group (10 rats) received a daily dose of sterile water orally for 52 days, the urtica dioica group (10 rats) received 100 mg/kg orally for 20 days, the 4-vinylcyclohexene group (10 rats) received 160 mg/kg intraperitoneally for 20 days, and the VCD + nettle group (10 rats) received 160 mg/kg intraperitoneally and 100 mg/kg orally for 20 days.

Blood samples were taken to assess testosterone and follicle-stimulating hormone levels. FSH, luteinizing hormone (LH), estradiol, and anti-Müllerian hormone (AMH) levels were assessed, as were reduced glutathione (GSH) and malondialdehyde (MAD) gene expression in the hypothalamus (GnRH-I and GnRH-II). Histological examinations were performed to assess changes in the ovaries and hypothalamus.

The current study showed a significant increase in VCD group in MDA, FSH, and LH levels,.along with a decrease in GSH, estradiol, testosterone, and AMH. VCD also increased the expression of the GnRH-I and GnRH-II genes, with clear histological damage such as follicular atrophy, stromal fibrosis, and neuronal degeneration. Combined treatment with Urtica Dioica improved these changes by restoring oxidative balance, modifying hormone levels, reducing GnRH gene expression, and improving the histological structure of the ovaries and neuronal tissues.

Conclusion: These findings indicate that, the results indicate the possibility of using *Urtica Dioica* to alleviate the complications of transitional menopause due to its antioxidant properties and its protective effect through its effect on reproductive hormone levels, oxidative stress status, and histological changes of the ovary and hypothalamus

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List of abbreviations

Abbrev	Full Form
AMH	Anti Mulleria hormone
DTNB	dithiobis-(2-nitrobenzoic acid)
E2	Estradiol
FMP	final menstrual period
FSH	follicle-stimulating hormone
GCMS	Gas chromatography Mass Analysis
GnRH-I	Gonadotropin-Releasing Hormone (GnRH1)
GnRH-II	Gonadotropin-Releasing Hormone GnRH2
GnSAF	gonadotropin surge-attenuating factor
GSH	Glutathione
HRT	Hormone replacement treatment
LH	luteinizing hormone
MDA	Malondialdehyde

Chapter One
Introduction

1.Introduction

Perimenopause, or the menopausal transition, refers to the phase preceding the cessation of a woman's reproductive years, characterized by considerable reproductive and hormonal alterations, which markedly elevate the risk of ischemic stroke and Alzheimer's disease. While our comprehension of the exact timeframe and definition of perimenopause is constrained, it is evident that menopause has two distinct periods. The early menopausal transition is characterized by predominantly regular menstrual cycles with little disruptions, while the late menopausal transition is marked by prolonged menopause lasting a minimum of 60 days until the final menstrual period **(McCarthy and Raval, 2020).**

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**(Kamińska *et al* .,2023)**

The ovary is the principal location for the synthesis of female sex steroid hormones, encompassing estrogens and progesterone At birth, the mammalian ovary possesses its whole array of oocyte-containing follicles. Oocytes cannot be produced Postpartum 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 **(Selvakumar *et al* ., 2022)**

"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. **(Inman and Flaws, 2024).**

Urtica dioica (UD), often known as Nettle, has been recognized as a medicinal urticia dioica and utilized in traditional medicine for its extensive biological activity**(Moayeri *etal* .,2018**).

Urtica dioica is a member of the Urticaceae family and is often known as nettle. Urtica dioica is categorized as a significant urticia dioica group in the European Pharmacopoeia. It is recognized for possessing several pharmacological qualities, including antioxidant, anti-inflammatory, anti-ulcer, anti-cancer, antibacterial, and antifungal effects **(Martz and Kankaanpää ,2025).**

The hypothalamus produces gonadotropin-releasing hormone (GnRH), a crucial hormone that regulates the vertebrate reproductive system. It functions by inducing the anterior pituitary gland to release two crucial hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which control the ovaries' and testes' functions, including the generation of gametes and sex hormones. GnRH-I and GnRH-II is the second form of GnRH identified in vertebrates and is highly conserved across species. Unlike GnRH-I, which is the primary regulator of FSH and LH secretion from the pituitary, the exact role of GnRH-II remains less clear. However, evidence suggests that GnRH-II contributes to the modulation of reproductive hormone release, influences sexual behavior, and may play a role in linking metabolic status with reproductive function. **(Mate *etal*.,2024)**

Aims of Study :

The present work was conducted to study the following aims :

1-To evaluate the effects of Urtica dioica extract on reproductive hormones in .VCD-induced menopausal rats

2-To investigate its influence on oxidative stress markers and histological . .changes in the ovary and hypothalamus

3-To determine the modulatory role of Urtica dioica on GnRH-I and GnRH-II gene expression.

Objectives:

-The reproductive hormone levels :Testosterone,follicle-stimulating hormone(FSH) and luteinizing hormone (LH) estradiol, Anti Mulleria hormone(AMH)

-Oxidative stress biomarkers: reduced Malondialdehyde(MDA) glutathione(GSH),

- gene Expression of GnRH-I and GnRH-II gene .

Histopathology: -

- Histopathological changes of ovary and hypothalamus .

Chapter Two

Review of Related Literature

2. Review of Related Literature:

2.1.Ovaries

The ovaries are diminutive, paired reproductive glands situated next to the lateral borders of the pelvic cavity. These organs are tasked with the production of ova and the secretion of hormones. Ovulation is the process by which an egg cell is liberated. Ovulation occurs in a cyclical manner and influences the duration of the menstrual period. Subsequent to ovulation, the oocyte traverses the fallopian tube en route to the uterus **(Gibson and Mahdy,2019).**

Fertilization often takes place in the fallopian tube; subsequently, the fertilized egg may imurticia dioica in the uterine lining. During fertilization, the egg cell produces specific chemicals that direct the sperm and facilitate adhesion between the surfaces of the egg and sperm. The ovum can then assimilate the sperm, initiating the fertilization process**(Cook ,2022).**

2.2.Functions of female reproductive systems

The primary function of the reproductive system is to facilitate the generation of progeny. The reproductive system facilitates sexual function and generates hormones that promote sexual growth and sustain pregnancy. The ovaries generate ova**. (Mancini and Pensabene,2019).** The eggs are subsequently transferred to the fallopian tube during ovulation, where fertilization by sperm may take place. The fertilized ovum subsequently migrates to the uterus, where the endometrial lining proliferates in reaction to the endogenous hormones of the menstrual cycle, also referred to as the reproductive cycle. Upon entering the uterus, the fertilized ovum can adhere to the thicker endometrium and proceed to develop**(Al-Suhaimi et al ., 2022).**

If imurticia dioicaation fails, the uterine lining is expelled during menstruation. Furthermore, the female reproductive system generates sex hormones that regulate the menstrual cycle. During menopause, the female

reproductive system progressively ceases the production of hormones essential for menstruation. At this stage, menstrual cycles may become erratic and ultimately cease. Menopause is defined as the absence of a menstrual menstruation for a whole year **(Boyd *et al* .,2018).**

2.3. Menstrual cycle

The menstrual cycle is a periodic physiological process in female primates, including humans, regulated by a complex hormonal interaction involving the hypothalamic-pituitary-gonadal axis. It generally lasts around 28 days and is segmented into four overlapping phases: the menstrual phase, follicular phase, ovulation, and luteal phase**(Patricio and Sergio ,2019)**.

The cycle commences with the desquamation of the endometrial lining resulting from a decline in estrogen and progesterone, succeeded by the follicular phase, wherein increasing concentrations of follicle-stimulating hormone (FSH) promote the maturation of ovarian follicles. A single dominant follicle grows and produces escalating quantities of estrogen, resulting in endometrial growth. An increase in luteinizing hormone (LH) subsequently initiates ovulation, resulting in the release of a mature ovum. **(Krishna and Witchel ,2024).**

Following ovulation, the ruptured follicle converts into the corpus luteum, which secretes progesterone to sustain the endometrium for possible imurticia dioicaation. In the absence of fertilization, the corpus luteum deteriorates, hormone levels decrease, and menstruation recommences. **(Thiyagarajan *et al*., 2024)**

Each cycle transpires in phases determined by events occurring in either the ovary (ovarian cycle) or the uterus (uterine cycle). The ovarian cycle comprises the follicular phase, ovulation, and luteal phase, while the uterine cycle includes

the menstrual, proliferative, and secretory phases. Day 1 of the menstrual cycle marks the commencement of the cycle, which typically endures for approximately five days. Approximately on day 14, the egg is often expelled from the incubator**(Fedorcsak,2024).**

2.3.1. Cycles and phases of menstrual cycle

The menstrual cycle is a complex series of hormonally regulated physiological events that routinely occur in the female reproductive system. Its primary function is to prepare the body for potential fertilization and pregnancy. The cycle generally lasts approximately 28 days, although it can vary from 21 to 35 days in healthy women. It is regulated by the hypothalamic-pituitary-ovarian axis through the coordinated release of gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen, and progesterone **(Ussher *et al* .,2020).**

The menstrual cycle consists of the ovarian and uterine cycles. The ovarian cycle determines the changes that occur within the ovarian follicles, while the uterine cycle determines the changes in the endometrium .**show in Figure (2-1)** The ovarian cycle consists of the follicular phase, ovulation, and luteal phase, while the uterine cycle includes the menstrual cycle, proliferative phase, and secretory phase. Each stage is characterized by specific hormonal patterns and physiological changes that regulate egg maturation and release, thickening of the uterine lining, and, in the absence of fertilization, shedding of this lining through the menstrual cycle **(Tortora and Derrickson, 2018); (Asher et al., 2020).**

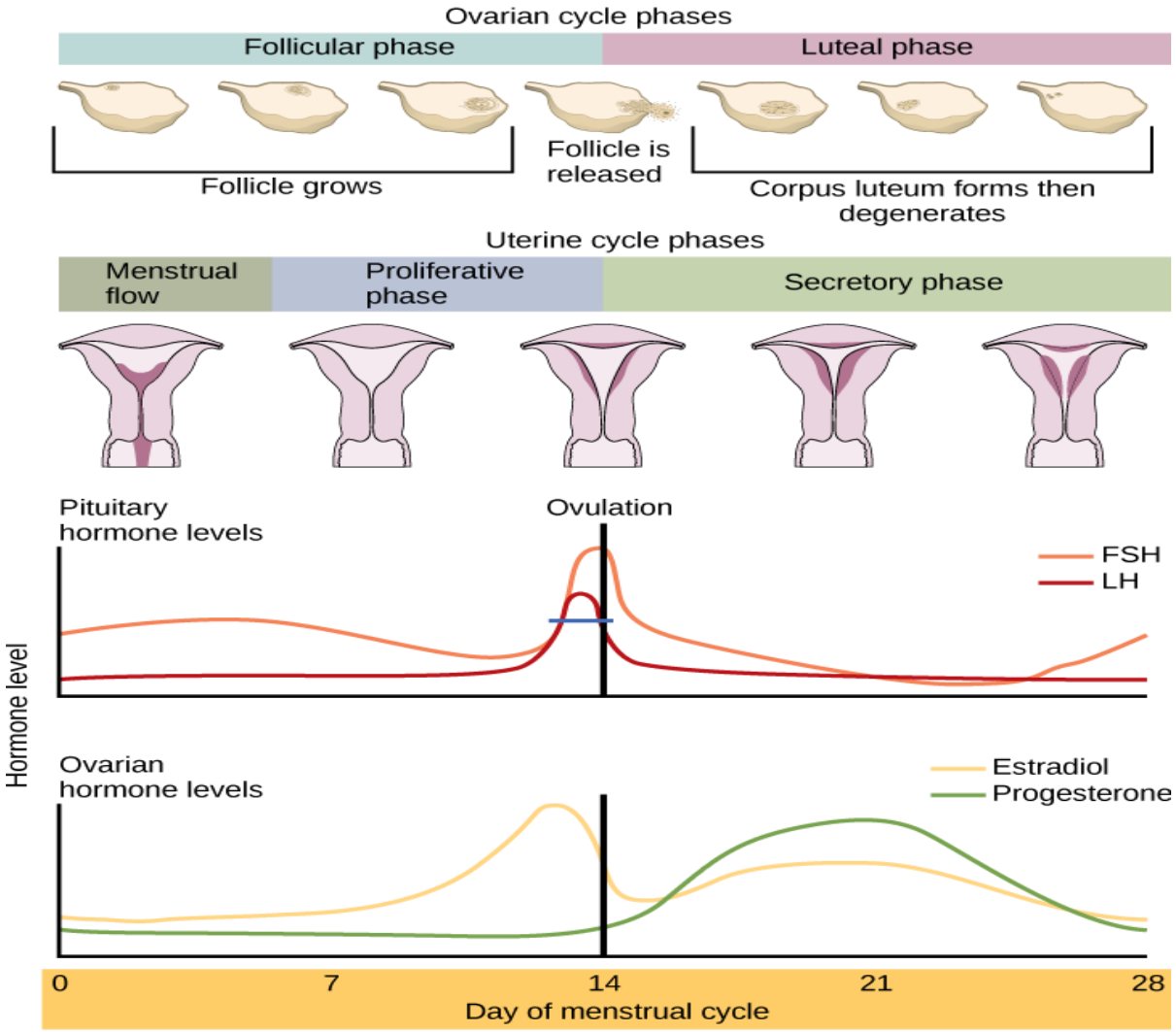


Figure (2-1) Cycles and phases of menstrual cycle (Patricio and Sergio, 2019)

2.3.2 .Ovarian cycle

The ovarian cycle is a crucial physiological process in sexually mature female governing the maturation and release of oocytes and the hormonal conditions necessary for possible fertilisation and pregnancy. **(Thiyagarajan *et al* .,2024).**

The process is segmented into three primary phases: the follicular phase, ovulation, and the luteal phase. This cycle is predominantly regulated by the interactions of the hypothalamus, pituitary gland, and ovaries, via the actions of gonadotropins (LH and FSH) and ovarian steroid hormones (oestrogen and progesterone). During the cycle, several follicles initiate development, although

typically only one attains dominance and achieves full maturation. **(Davies,2021).**

The ovarian cycle facilitates the regular discharge of an ovum and prepares the endometrial lining of the uterus for imurticia dioicaation. In the absence of fertilisation, hormone levels decline, prompting the cycle to recommence. This dynamic mechanism is essential for female fertility and reproductive well-being. **(Makhanova *et al* .,2025).**

2.3.2.1. Follicular phase

Follicular phase:the follicular phase commences on the initial day of menstruation and continues until ovulation occurs. The process is marked by the development and maturation of ovarian follicles stimulated by increasing levels of FSH**(Jinno,2025).**

In this phase, multiple antral follicles are recruited; however, often, only one dominant follicle persists in growth, ultimately developing into the Graafian follicle. **(Doornweerd *et al*.,2025).**

This phase is characterised by heightened oestradiol production from the growing follicles, which provides negative feedback to the brain and pituitary, hence modulating FSH secretion. Oestradiol is crucial in priming the uterine lining for possible imurticia dioicaation. The duration of the follicular phase can range among individuals and significantly contributes to variations in cycle length **(Abumoh'd, 2024).**

2.3.2.2. Ovulation

Ovulation signifies the midpoint of the ovarian cycle and is initiated by a rapid and substantial spike in LH, which is prompted by prolonged elevated levels of oestradiol The surge prompts the mature Graafian follicle to burst,

releasing a secondary oocyte into the fallopian tube, rendering it available for fertilisation **(Pearson *etal* .,2025)**

Ovulation often transpires on day 14 of a 28-day cycle, though this may fluctuate. The LH surge also triggers the luteinisation of the remaining follicular cells, preparing for the subsequent phase of the cycle. The precise timing of ovulation is crucial for fertility and is a focus for both natural conception and assisted reproductive techniques**(Iussig *et al* .,2019)**

2.3.2.3. Luteal phase

The luteal phase occurs post-ovulation and is marked by the conversion of the ruptured follicle into the corpus luteum, which produces significant quantities of progesterone and some oestrogen. Progesterone is essential for stabilising and sustaining the uterine lining, so fostering a conducive environment for embryo imurticia dioicaation **(Przygrodzka *et al* .,2025)**

In the absence of fertilisation and imurticia dioicaation, the corpus luteum deteriorates into the corpus albicans, resulting in a decline in progesterone and oestrogen levels. This hormonal cessation induces menstruation and initiates a new cycle. The luteal phase is typically more uniform in duration than the follicular phase, normally lasting approximately 14 days. **(Kapper *et al* .,2024).**

2.4.Effects of hormone on ovulation

Ovulation often transpires 14 days prior to the commencement of menstruation; hence, in a standard 28-day cycle, ovulation occurs on day 14. During the follicular phase, estradiol levels increase, and near the conclusion of this phase, 17-β estradiol transitions from exerting negative feedback to positive feedback at the anterior pituitary**(Thiyagarajan *et al* .,2024)**.

The shift from negative to positive feedback remains incompletely comprehended and possibly entails multiple mechanisms; yet, it generally transpires at reaching a key threshold of estradiol. Increased estradiol levels

activate gonadotrophic cells in the pituitary to generate additional GnRH receptors, hence augmenting the sensitivity of these cells to GnRH. Estradiol may inhibit the degradation of GnRH in pituitary cells and reduce the threshold of GnRH required to initiate LH release. A nonsteroidal ovarian product, often known as gonadotropin surge-attenuating factor (GnSAF), is thought to mitigate the sensitizing effects of estradiol throughout the majority of the follicular period. **(Handa *et al* .,2025)**

As estradiol concentrations rise during the follicular phase, GnSAF is inhibited, facilitating the sensitizing effects of estradiol towards the conclusion of the period, culminating in ovulation. These pathways lead to a rapid increase in LH production, with LH levels escalating tenfold during the LH surge, alongside minor elevations in FSH concentrations. **(Urbanski *et al* .,2025).**

The hormonal environment prompts the mature follicle to emit plasminogen activator and other cytokines, resulting in follicular rupture and oocyte release. Ovulation often transpires at 36 to 44 hours following the initiation of the LH surge. The cervical modifications initiated in the follicular phase persist, leading to augmented, aqueous cervical mucus to aid sperm ingress. At the conclusion of ovulation, concentrations of 17-β estradiol diminish **(Asl *et al*.,2023).**

2.5.Estrus cycle

The estrous cycle comprises a series of periodic physiological alterations induced by reproductive hormones in female mammals. Estrous cycles commence post sexual maturity in females and are interspersed with non-estrous phases, referred to as "resting" or gestational phases. Estrous cycles generally last till death. The duration and frequency of these cycles varies significantly among species. Certain animals may have crimson vaginal discharge, frequently mistaken for menstruation. The estrous cycles of numerous mammals utilized in industrial agriculture, including cattle and sheep, can be intentionally regulated

with hormonal pharmaceuticals to maximize productivity **(Lovick and Zangrossi,2021).**

The estrous cycle is a repetitive sequence of hormonal fluctuations that regulates fertility in numerous mammalian species. The recurrent cyclical advancement through the stages of the estrous cycle is governed by hormonal secretion from the hypothalamus-pituitary-gonadal (HPG) axis. Gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus function as a primary conduit for reproductive regulation in both sexes**(Itriyeva, 2022).**

In females, the pulsatile secretion of GnRH prompts the anterior pituitary gland to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which govern the development of ovarian follicles and the synthesis of ovarian steroids, estradiol, and progesterone. Ovarian steroids interact with kisspeptin neurons, which regulate gonadotropin neurons, thereby modifying their activity and managing their release **(Thiyagarajan *et al* .,2024).**

2.5.1.Phases of estrus cycle

The estrous cycle comprises four stages: metestrus, diestrus, proestrus, and estrus, lasting approximately four to five days in female rats and mice. Follicle-stimulating hormone (FSH) and limited amounts of luteinizing hormone (LH) are synthesized in response to infrequent gonadotropin-releasing hormone (GnRH) pulses during metestrus and diestrus. FSH facilitates the development of ovarian follicles, resulting in increased estradiol production by these follicles. Increased estradiol during proestrus induces a surge in LH secretion by enhancing the frequency of GnRH pulses. The GnRH pulse frequency increases during the latter portion of the light phase of proestrus, whereas the LH surge occurs in the hours preceding the transition to the dark phase**(Kumar *et al.*,2021)**.

Approximately ten to twelve hours following the LH surge, the ovary expels the egg from the dominant follicle, a phenomenon termed ovulation,

marked by a significant decrease in estradiol levels. The corpus luteum, formed from the remnants of the first ovarian follicle, synthesizes progesterone and prepares the uterus for potential imurticia dioicaation following fertilization. Post-ovulation and the commencement of estrus, fecundity reaches its zenith. If fertilization and pregnancy do not occur during estrus, the corpus luteum degenerates, progesterone levels decline, and the cycle recommences. The variation in progesterone secretion during the estrous cycle constitutes a primary distinction between mice and rats. In mice, progesterone levels elevate earlier during diestrus, but in rats, he increase occurs subsequent to the LH surge**(Abd-Elkareem *et al* .,2024)**.

The duration of the stages of the estrous cycle differs both within and among species, even though they are typically represented as uniform. The estrous cycles of mice exhibit the following approximate phase durations: menstruation (6–21 hours), estrus (48–72 hours), proestrus (12–14 hours), and estrus (12–48 hours). The durations of the rats' estrous cycle are approximately: proestrus <24 hours, estrus 48–72 hours, menstruation 2–24 hours, and estrus 12–48 hours**(Majeed *et al* .,2025)**

2.5.1.1.proestrus

Proestrus is the initial phase of the reproductive cycle, during which the body commences preparations for mating. The initial development of ovarian follicles is characterized by the influence of follicle-stimulating hormone (FSH). As the follicles mature, they begin secreting estrogen, which has various impacts on the body. The endometrium of the uterus proliferates in response to estrogen, facilitating potential embryo imurticia dioicaation. Behavioral indicators during proestrus encompass a little attraction to males without complete approval of mating. In certain species, such as canines, a significant vaginal discharge is

often observed, often sanguineous, resulting from heightened vascularity of the reproductive tract. **(da Silva *et al* .,2025)**

2.5.1.2.estrus

During the estrus phase, estrogen levels reach their zenith, resulting in notable behavioral and physiological alterations. This is the phase of sexual receptivity, during which the female is prepared to copulate. A spike in luteinizing hormone (LH) initiates ovulation, releasing a mature egg from the dominant ovarian follicle. During this phase, females display more pronounced indicators of sexual receptivity, including standing for mounting in cattle and horses, as well as heightened vocalization and restlessness in certain species such as dogs. The cervix softens, and the vaginal discharge becomes more transparent and fluid, hence enhancing sperm passage during copulation **(Pylova *et al* .,2025).**

2.5.1.3.diestrus

Following ovulation, the cycle transitions into metestrus, commonly referred to as the luteal phase. In this phase, the ruptured follicle converts into the corpus luteum, which commences the secretion of progesterone. Progesterone is essential for sustaining the thicker endometrial lining and establishing an appropriate milieu for possible embryo imurticia dioicaation. In the absence of fertilization, the corpus luteum progressively deteriorates, resulting in a decrease in progesterone levels. Metestrus is generally a phase of non-receptivity, during which females exhibit a lack of sexual interest in mating. The behavioral indicators of estrus are lacking, and the uterine milieu transitions from receptivity to one conducive to early pregnancy, provided fertilization has occurred..**(Batistela *et al*.,2025)**

2.5.1.4.Anestrus

The concluding phase of the estrous cycle is anestrus, characterized by reproductive inactivity. In anestrus, the animal's ovaries are effectively inactive, and no ovarian follicles are developing. Hormonal levels, encompassing estrogen, progesterone, and gonadotropins, are diminished. **(Salemme *et al*.,2024)** This phase is characterized by the absence of sexual behavior, and the animal will reject mating. Anestrus may be seasonal, as observed in species such as sheep or cats, or it can be affected by physiological states like pregnancy or breastfeeding, which inhibit reproductive activity. The length of anestrus differs among species and may be affected by factors including nutrition, stress, and environmental conditions. **(Szucs *et al*.,2024)**

2.6. Differences from the menstrual cycle

The menstrual cycle and the estrous cycle are two distinct reproductive cycles seen in animals, differing markedly in hormonal regulation, physiological processes, and reproductive behavior. The menstrual cycle is distinctive to humans and certain primates, but the estrous cycle is prevalent throughout most non-primate mammals, including domesticated species such as dogs, cows, and horses. A key distinction between the two cycles is the occurrence or non-occurrence of menstrual blood. The menstrual cycle involves the removal of the uterine lining (endometrium) if fertilization does not occur, leading to menstrual bleeding **(Ajayi and Akhigbe,2020).**

This procedure, which initiates the cycle, is exclusive to humans and certain primates. Conversely, the estrous cycle is devoid of menstrual blood. If fertilization does not take place, the endometrial lining is reabsorbed, and the cycle proceeds without tissue shedding. A significant distinction pertains to the sexual receptivity of females. During the menstrual cycle, females may partake in sexual activity at any point; however, sexual receptivity is generally not

associated with a particular phase. Conversely, during the estrous cycle, females exhibit sexual receptivity solely during a designated phase termed estrus or "heat." During estrus, elevated estrogen levels induce behavioral modifications, including heightened activity, vocalization, and receptivity to mating**.(Shukla *etal* .,2025)**

This phase is generally short-lived and transpires just once per cycle. The cycle durations vary between the two. The menstrual cycle generally endures approximately 28 days in humans, however variations may occur. The estrous cycle differs markedly among species, with durations ranging from a few days to many weeks, contingent upon the animal. The estrous cycle in canines endures approximately 6 months, whereas in bovines, it often spans 21 days. Moreover, the hormonal regulation of each cycle is distinct. During the menstrual cycle, estrogen and progesterone levels fluctuate, with estrogen reaching its zenith prior to ovulation and progesterone increasing post-ovulation to sustain the**(Zafar, 2025).**

During the estrous cycle, estrogen levels reach their zenith before to ovulation; however, progesterone assumes a more prominent role in the luteal phase (metestrus), when the corpus luteum synthesizes progesterone to facilitate pregnancy in the event of fertilization. The menstrual cycle and the estrous cycle both fundamentally prepare the female reproductive system for conception and pregnancy; yet, they differ in physiological processes, hormonal regulation, and sexual behavior patterns. Comprehending these distinctions is essential for researchers engaged with diverse species, as it impacts reproductive management, breeding methodologies, and the identification of fertility complications. **(Griffith *et al* .,2025)**

2.7. Menopause

Menopause is a natural biological process that signifies the end of a woman's reproductive capacity, happening when the ovaries cease egg production and the levels of reproductive hormones, especially estrogen and progesterone, markedly decrease. This transition generally takes place between the ages of 45 and 55, although the precise time may differ. Menopause is established when a woman has had 12 consecutive months without menstruation, indicating the cessation of the menstrual cycle **(Wang *et al* ., 2025).**

The transition to menopause consists of three phases: perimenopause, menopause, and postmenopause. Perimenopause, commencing several years prior to menopause, may induce irregular menstruation, hot flashes, nocturnal perspiration, and mood fluctuations in women. These symptoms arise from the variable amounts of estrogen and progesterone, which influence multiple body systems. As ovarian egg production diminishes, ovulation becomes erratic, and menstrual periods may vary in duration. **(Bertone-Johnson *et al* .,2018). (Crandall,2019).**

The duration and severity of perimenopause differ significantly among women. Menopause is characterized by the total cessation of menstruation, indicating the conclusion of a woman's capacity for natural conception. The hormonal fluctuations during this era may result in diverse physical and emotional ailments. Alongside the commonly recognized hot flashes and night sweats, women may encounter vaginal dryness, diminished libido, and alterations in sleep habits **(Uddenberg *et al* .,2024)**

The decline in estrogen levels adversely impacts bone density, hence elevating the risk of osteoporosis. Postmenopause is the phase that occurs after a woman's final menstruation. During this phase, menopausal symptoms may persist, but they often diminish over time. The long-term reduction in estrogen

levels elevates the chances of osteoporosis, cardiovascular illnesses, and other health complications. **(Hamoda and Sharma,2024)**

Hormone replacement treatment (HRT) is occasionally employed to mitigate these symptoms, while it has possible hazards and is often assessed on an individual basis. Although menopause is a natural aspect of aging, it can entail considerable health consequences. The reduction in estrogen elevates the risk of cardiovascular illnesses and osteoporosis, prompting postmenopausal women to prioritize a healthy lifestyle, including weight-bearing workouts, a balanced diet, and possibly medication to enhance bone health **(Davis and Baber,2022).**

2.7.1.menopausal signs and symptoms

The menopausal transition often last for an average duration of four years. Menopause symptoms often commence modestly and increase in prevalence during the transition, as hypoestrogenism and prolonged amenorrhea become more prevalent. One of the initial signs of the menopausal transition may be irregular bleeding patterns, often associated with anovulation, encompassing fluctuations in the frequency and duration of menstruation **(Hamoda and Sharma,2024)**

The initial one to two years after the final menstrual period (FMP) is when signs and symptoms are most prevalent and intense. Regrettably, an extended length of distressing symptoms is anticipated with earlier start during the transition, and certain women may experience these uncomfortable symptoms for more than a decade. While it has been established that anti-Müllerian hormone (AMH) can assist in forecasting the duration preceding natural menopause, its predictive efficacy diminishes with advancing age**(Davis and Baber,2022).**

Individuals and ethnic groups may experience menopausal symptoms that varies significantly. Vasomotor symptoms are believed to be less widespread

among Asian women, although Caucasian women often report that these symptoms, particularly "hot flashes" and "night sweats," are the most common menopausal manifestations. Vasomotor syndrome is believed to affect over 75% of menopausal women, with around one-third experiencing severe symptoms. These symptoms often commence one to two years before to menopause, last for an average duration exceeding seven years, and in 20% of women, endure for as long as fifteen years **(Gatenby and Simpson, 2024).**

Menopausal women commonly experience symptoms such as negative emotions, heightened anxiety, "brain fog," difficulty in word retrieval, and increased forgetfulness. There is less evidence that estrogen therapy mitigates dementia, and the exact etiology of cognitive deficits linked to menopause remains incompletely understood. Symptoms of anxiety and depression often substantially affect patients' self-esteem both inside and outside the house**(Hamoda *et al* .,2020).**

2.7.2.Causes of menopause

Menopause is a pivotal occurrence in a woman's life characterized by the cessation of ovarian function and the conclusion of the reproductive period. Approximately 4% of women experience menopause prior to the age of 40, while the mean age of menopause in women is 51**(Zamaniyan *et al*.,2020).**

Women in impoverished nations experience menopause earlier than those in affluent nations **(SaeiGhareNaz *et al*.,2019).** Genetic factors, obesity, alcohol use, social status, ethnicity, education, diet, pesticide exposure, reproductive traits, and residential location have all been associated with menopause; however, the results are not uniform. The age at which menopause occurs has garnered significant attention recently due to its potential influence on a woman's subsequent health**(Langton *et al*.,2020).**

Early menopause is associated with an elevated risk of Alzheimer's disease, atherosclerosis, stroke, osteoporosis, and cardiovascular disease.

Conversely, late menopause correlates with a heightened risk of endometrial, ovarian, and breast malignancies**(Xiaoyan *et al*., 2020).**

2.7.3.Mechanism of menopause

Menopause is the natural biological process that signifies the conclusion of a woman's reproductive years, marked by the irreversible stop of menstruation and a substantial decline in ovarian hormone levels, chiefly estrogen and progesterone. The process of menopause is chiefly influenced by the reduction of ovarian follicles and the decrease in reproductive hormone production, governed by intricate interactions among the hypothalamus, pituitary gland, and ovaries. During the initial stages of menopause, generally occurring between the ages of 45 and 55, a woman's ovaries commence a decline in their capacity to generate eggs. **(Fux-Otta *et al* .,2025)**

This is primarily attributable to the decreasing quantity of primordial follicles, which are the oocytes present at birth. With the passage of time, the reservoir of accessible follicles diminishes, leaving only a limited number susceptible to follicle-stimulating hormone (FSH). Consequently, a diminished number of follicles are selected for maturity, leading to progressively erratic ovulation. The hypothalamic-pituitary-gonadal axis, which governs reproductive hormones, is pivotal in this process. During the initial phases of menopause, the reduction in ovarian follicles results in diminished estrogen synthesis. Estrogen regulates the menstrual cycle, sustains the endometrial lining, and supports several bodily functions, including bone health and cardiovascular function **(Sultania *et al*.,2025)**

With the decline in estrogen levels, the pituitary gland compensates by increasing the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) to boost ovarian estrogen production. However, as the ovaries can no longer respond to these signals, estrogen production remains diminished.

The progressive decline of estrogen furthermore leads to the termination of progesterone synthesis. Progesterone, synthesized by the corpus luteum post-ovulation, is crucial for regulating the menstrual cycle and for preparing the endometrium for possible imurticia dioicaation.As ovulation diminishes and ultimately ceases, progesterone synthesis declines, leading to irregularities and the eventual termination of menstruation**,(Jovičić and Plebani,2025)**

The decline in estrogen and progesterone levels during menopause leads to various physiological and psychological problems. These encompass hot flashes, nocturnal perspiration, vaginal atrophy, emotional fluctuations, and a reduction in bone density. The deficiency of estrogen results in heightened bone resorption, rendering bones more vulnerable to osteoporosis. Decreased estrogen levels impact the cardiovascular system, elevating the risk of heart disease in postmenopausal women. In summary, menopause transpires as a consequence of the depletion of ovarian follicles and the subsequent reduction in estrogen and progesterone synthesis**,(Spicer *et al*.,2025)**

The hypothalamic-pituitary-gonadal axis regulates hormonal changes by elevating FSH and LH levels in response to diminished ovarian function. The ovaries' failure to respond to these hormones results in the end of menstruation and the other symptoms linked with menopause **(Bonberg *et al* .,2025).**

2.7.4.Diagnosis of menopause

2.7.4.1.Perimenopause

Perimenopause is the transitional phase prior to menopause, characterized by gradual hormonal changes in a woman's body that indicate the conclusion of her reproductive years. This phase may commence many years prior to the final menstrual period—usually in the mid-to-late 40s—but can initiate sooner in certain women. The defining characteristic of perimenopause is the gradual

deterioration of ovarian function, namely the diminished sensitivity of ovarian follicles to gonadotropins, resulting in irregular ovulatory cycles. Consequently, estrogen and progesterone levels fluctuate erratically, resulting in a hormonal imbalance that presents numerous physiological and psychological symptoms. **(Moline and Clerke, 2023).**

A prevalent clinical manifestation of perimenopause is menstrual irregularity, characterized by alterations in cycle duration, flow intensity, and the occurrence of missed periods. The anomalies are chiefly attributable to variable ovulation. Variations in estrogen levels may result in vasomotor symptoms, including hot flashes, night sweats, and sleep problems, impacting a considerable percentage of women throughout this period. Furthermore, numerous individuals encounter mood fluctuations, anxiety, irritability, diminished libido, and cognitive impairments such as impaired focus or memory lapses, all associated with the neuroendocrine consequences of estrogen withdrawal. **(Martin-Key *etal*., 2024)**

follicle-stimulating hormone (FSH) levels increase during perimenopause as a result of diminished negative feedback from ovarian estrogen. Due to the irregularity of ovulation, the corpus luteum may not consistently develop, leading to inadequate progesterone synthesis. The disparity between estrogen and progesterone leads to endometrial instability, resulting in abnormal uterine bleeding observed in numerous women at this phase**, (Crowder, 2023).**

Perimenopause can persist for several years, often ranging from 4 to 8, until culminating in menopause, which is retrospectively defined after 12 continuous months of amenorrhea,The duration and intensity of perimenopausal symptoms differ markedly across individuals and are affected by genetic, behavioral, and environmental variables **(Hand *et al*.,2021).**

2.7.4.2.Postmenopause

Postmenopause denotes the phase in a woman's life commencing after twelve consecutive months of amenorrhea, signifying the definitive cessation of normal menstruation and reproductive capacity. This phase is marked by a prolonged reduction in ovarian hormone synthesis, especially estrogen and progesterone, leading to enduring physiological alterations and heightened vulnerability to numerous health issues. The hormonal milieu of postmenopausal women is characterized by diminished circulating estrogen levels, which profoundly affects several organ systems**,(Ambikairajah *et al*.,2022).**

A significant consequence of postmenopause is its impact on bone metabolism. Estrogen is essential for preserving bone density by suppressing osteoclast activity; its lack results in increased bone resorption and an elevated risk of osteoporosis and associated fractures. The cardiovascular system is impacted due to the loss of estrogen's protective role in regulating lipid metabolism and vascular function. Consequently, postmenopausal women exhibit an elevated risk of atherosclerosis, hypertension, and ischemic heart disease**. (Akbar *et al* .,2025)**

Alongside systemic alterations, postmenopausal women frequently encounter urogenital atrophy, characterized by vaginal dryness, dyspareunia, and recurrent urinary tract infections The symptoms stem from the atrophy of the vaginal epithelium and diminished blood circulation, both affected by lowered estrogen levels. Some women experience enduring vasomotor symptoms, including hot flashes and night sweats, although these generally diminish in severity with time**,(Sun *et al* .,2025)**

Postmenopause may be linked to mood changes, sleep disruptions, and diminished cognitive function in certain women, frequently intensified by aging and lifestyle influences **(Vincent *et al* .,2025).**

2.7.5.Management (Menopausal hormone therapy)

Menopause is a physiological phase of life. It is neither a sickness nor a disorder, hence it does not inherently necessitate medical intervention. In instances where the physical, psychological, and emotional repercussions of menopause are sufficiently severe to substantially impair a woman's life, palliative medical intervention may occasionally be warranted**(Duralde *et al*.,2023)**.

Menopausal hormone therapy (MHT), or hormone replacement therapy (HRT), comprises medications that include estrogen, with or without progesterone, and occasionally testosterone, to alleviate symptoms related to menopause. MHT is efficacious and is the predominant therapy for alleviating menopausal symptom **(Nappi, 2022).**

Hormone replacement treatment (HRT) may be appropriate for alleviating menopausal symptoms, including hot flashes. This is the most efficacious treatment modality, particularly when administered via a transdermal patch. Nonetheless, its utilization seems to elevate the risk of stroke and thrombosis. For menopausal symptoms, the global guideline stipulates that hormone replacement therapy (HRT) should be administered only when there are defined therapeutic effects and objectives for each individual woman **(Hamoda *etal*,.2020)**.

Hormone replacement therapy is useful in reducing bone loss and fractures associated with osteoporosis; nevertheless, it is typically advised only for women at elevated risk who are unsuitable for alternative treatments..**(Faubion *et al*,.2022)**

Hormone replacement therapy may be unsuitable for many women, particularly those with heightened risk of cardiovascular disease, elevated risk of thromboembolic disorders (such as women with obesity or a history of venous thromboembolism), or higher susceptibility to specific malignancies. This

treatment raises concerns regarding an increased risk of breast cancer. Women at heightened risk of cardiovascular disease and deep vein thrombosis may utilize transdermal estradiol, which seemingly does not elevate risk at low to moderate dosages**. (Davis,and,Baber,2022)**

Incorporating testosterone into hormone therapy positively influences sexual function in postmenopausal women, while excessive use may lead to hair growth or acne. Transdermal testosterone therapy, when administered at suitable dosages, is typically safe. **(Davis *et al*., 2019).**

2.8.Ovarian failure

Premature ovarian failure (POF) is a disorder that induces menopause, reduces estrogen levels, and elevates gonadotropin levels prior to the age of 40, with an incidence of 1 in 100 by age 40 and 1 in 1,000 by age 30. The prevalence of primary ovarian failure (POF) is 10%–28% in women with primary amenorrhea and 4%–18% in those with secondary amenorrhea. POF is rather prevalent, considering these incidence rates and the potential underreporting by women who do not identify the cessation of monthly blood as a medical issue**(Hu *et al* .,2024)**

Decreased ovarian function prior to age 40, along with monthly irregularities such as amenorrhea or irregular cycles, elevated gonadotropin levels, and diminished estrogen, are the clinical characteristics of premature ovarian insufficiency (POI). Apoptosis, rapid follicle depletion, a decrease in primordial follicle count, and an insufficient response to gonadotropin stimulation may all serve as underlying reasons of primary ovarian insufficiency at any point in time. Genetic, immunological, medicinal, inflammatory, etc. Established risk factors for POI exist. Nonetheless, the causes of nearly 50% of POI incidents remain unidentified**(Giri,2020)**

While the etiology of POI remains mostly unidentified in most clinical cases, the catalog of acknowledged causes has expanded in recent years. It is

essential to exclude the most common clinical causes, including autoimmune illnesses, fragile X mutations, and chromosomal abnormalities. **(He *et al*.,2024)**

2.9.The 4-phenylcyclohexene dioxide (vcd) model of menopause and peri-menopause

The chemical substance 4-phenylcyclohexene diepoxide (VCD) induces the depletion of tiny ovarian follicles (primary and primordial) in mice by hastening the natural process of atresia and can serve as a model for human menopause in rodent studies of the condition. The VCD mouse model of menopause is a follicle-depleted animal with preserved ovaries that more accurately reflects the natural human transition through the premenopausal and postmenopausal stages of life **(Fernandes, 2018).**

The predominant pathway for women entering menopause involves a gradual decline in ovarian function while retaining residual ovarian tissue, making the transition to a follicle-depleted state with intact ovaries more representative of the natural human progression through premenopausal and postmenopausal phases than that of an ovariectomized (OVX) animal**(Ahmad *et al*.,2025)**

The VCD rat model of menopause necessitates daily intraperitoneal injections of VCD (160 mg/kg/day in corn oil) to selectively induce the loss of primordial and primary ovarian follicles by inhibiting the autophosphorylation of the survival receptor c-kit on the oocyte plasma membrane. Within 15 days of the termination of daily treatment, VCD has exhausted all primordial follicles**(Cakir *et al*., 2024).**

In this phase of impending ovarian failure, the duration of the menstrual cycle lengthens, estrogen levels fluctuate until they decline significantly, and FSH levels increase as the inhibitory effects of estrogen diminish, resembling human perimenopause**,**The VCD model offers numerous physiological benefits for

examining sex differences. The primary objective is the conservation of remaining ovarian tissue. **(Methawasin *et al* .,2025).**Notwithstanding the depletion of estrogen-producing follicles, the residual theca cells in the ovaries of VCD-treated rats persist in androgen secretion, illustrating the preservation of the ovary's androgenic capability in the VCD paradigm. Like healthy women, throughout perimenopause, ovarian hormone show in figure (2-2) production progressively diminishes, leading to an increase in FSH and LH levels, which remain elevated until ovarian failure. This contrasts with the sudden decrease of estrogen in the OVX animal. A further advantage of the VCD model is the inclusion of the previously described premenopausal phase, enabling researchers to evaluate the influence of coming menopause on disease pathways,**(Gilmer *et al* .,2025).**

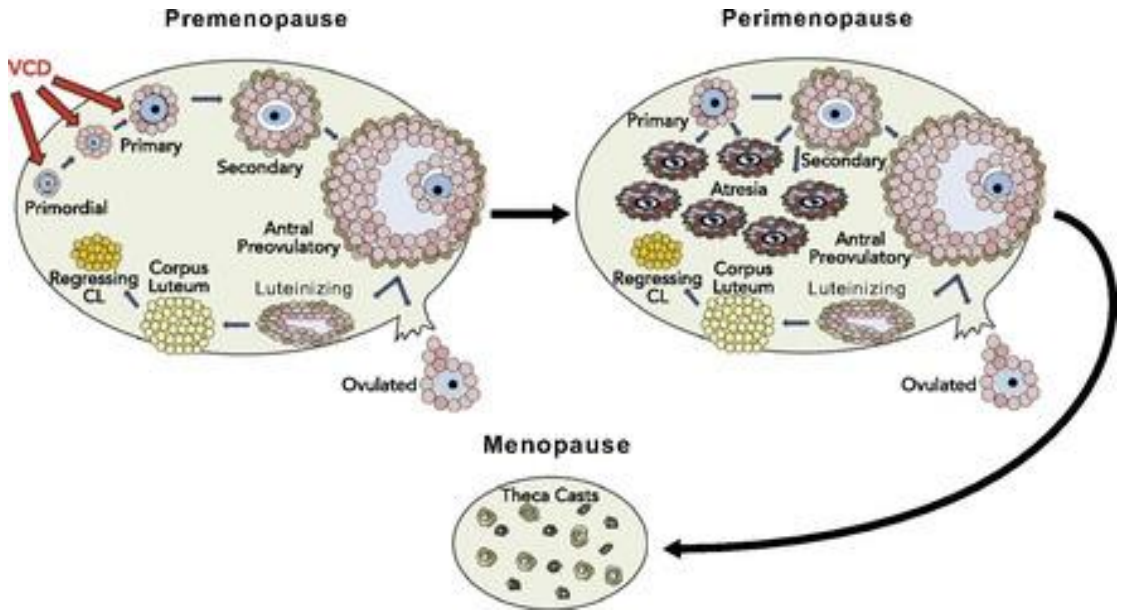


Figure (2-2) . The 4-phenylcyclohexene dioxide Rats Model of perimenopause (Marongiu,*et al*,.2025)

2.10.Biological activity of medicinal urticia dioica

Throughout history, medicinal urticia dioicas have been crucial to human health and wellness, serving as a vital source for the discovery of bioactive compounds. **(Mushtaq *et al*., 2018)**

Historically, traditional medicine has utilized pharmaceuticals sourced from natural materials, **(Gaber *et al* .,2020)**;**(El-Nashar *et al*., 2021) ;(Saied *et al*.,2021) and (Bhuia *et al*., 2023).** These drugs,characterized by extensive chemical diversity, intricate architectures, and unique biological action, are currently pivotal in drug development **(Laskar and Mazumder,2020**).Pharmaceuticals originating from natural sources often have remarkable efficacy, superior selectivity, satisfactory tolerance, and low toxicity **(Laskar and Mazumder,2020); (Rashad *et al* ., 2020);((Zhang *et al* .,2021);(El-Nashar *et al* .,2022)and(Elshaer *et al* .,2024).**

Numerous pharmacological agents currently available are inspired by or derived from botanical constituents. Identifying and developing innovative pharmaceuticals often requires investigating their potential for various biological effects. include urticia dioica-derived anti-inflammatory chemical constituents and their therapeutic applications **(Romano *et al*., 2021)** Alkaloids, flavonoids, terpenoids, and phenolics are among the numerous physiologically active metabolites produced by medicinal urticia dioicas that contribute to their therapeutic efficacy **(Adeleye *et al*., 2022).** These compounds possess antibacterial and antioxidant characteristics. **(Su *et al*., 2021);(Helmy *et al*.,2023).**

2.11.Urtica dioica

Urtica dioica, sometimes referred to as common nettle, burning nettle, or stinging nettle (not all specimens of this species include stinging properties), is a flowering herbaceous perennial belonging to the family Urticaceae,UD is abundant in minerals (calcium, sulfur, silicon, potassium, iron, copper, phosphorus, chromium, magnesium, cobalt, and zinc) and vitamins (A, B1, B2,

C, D, E, and K) It is indigenous to Europe, extensive regions of temperate Asia, and western North Africa, and is currently distributed globally **(Devkota *et al*.,2022).**

The species comprises six subspecies, five of which possess numerous hollow stinging hairs known as setae on their leaves and stems. These setae function like hypodermic needles, delivering histamine and other chemicals that elicit a stinging sensation upon contact, referred to as "contact urticaria," a type of contact dermatitis. The urticia dioica has longstanding history of utilization as a source of traditional medicine, food, tea, and textile raw materials in both ancient and contemporary countries **(Zade *et al*.,2023)**

Mice, dogs, and chickens served as animal models and cell cultures to assess the impact of urtica dioica on the regulation of inflammatory cytokines, clinical manifestations, immunological response, blood glucose levels, glucose transporter genes, and lipid peroxidation across several organs.**(Taheri *et al* .,2022**)

Urtica dioica effectively regulated morphological and histological alterations in polycystic ovaries and mitigated the problems associated with sex hormone metabolic syndrome in a mouse model of polycystic ovarian syndrome **(Bandariyan *et al*., 2021)**

2.11.1.Description

Urtica dioica is a perennial herbaceous urticia dioica in the Nettle family. This urticia dioica is characterized by its stinging hairs, known as setae show in figure (2-3)., which release histamine and other irritants upon contact, causing a temporary burning sensation on the skin. Nettle generally grows in moist, nutrient-rich soil and can reach a height of up to two meters. It features serrated leaves and small, green, densely clustered flowers **(Tiefenbrunner and Tiefenbrunner, 2025).**

The leaves and stems are densely covered with stinging hairs, and in most subspecies, also contain numerous barbs (spines), the tips of which detach upon contact, transforming the hairs into needles capable of injecting various chemicals that cause a painful sting or tingling sensation, hence the common names: stinging nettle, burning nettle, or burning nut **(Begić *et al*., 2020).**

Figure (2-3). Urtica dioica (Awad *et al* .,2025)

2.11.2.Taxonomy

Classification:

Kingdom: *Urticia dioicaae*

Phylum: *Streptophyta*

Class*: Equisetopsida*

Subclass*: Magnoliidae*

Order: *Rosales*

Family: *Urticaceae*

Genus: *Urtica*

Species: *Urtica dioica*

According to **(Alimoddin *et al* .,2024).**

2.11.3.Distribution and habitat

Urtica dioica, is extensively found in temperate and subtropical areas globally. Its native distribution encompasses a significant portion of Europe, Asia, North America, and North Africa, where it is frequently located in both wild and semi-cultivated environments,**(Zargar *et al*., 2024)** This urticia dioica has well adapted to diverse climates and is regarded as native to Eurasia, having also naturalized in numerous other regions, including sections of South America and New Zealand, owing to its ecological flexibility,**(Dhouibi *et al* .,2020).**

Urtica dioica generally flourishes in moist, nitrogen-abundant soils and prefers environments that range from partial shade to full sunlight. It is frequently located near riverbanks, woodland peripheries, roadways, ditches, meadows, and derelict agricultural sites**. (Danna, 2024).** Its presence frequently signifies fertile and disturbed soils, especially those augmented with organic matter or livestock waste, elucidating its prevalence in anthropogenically influenced settingsThe urticia dioica thrives in temperate temperatures, exhibiting optimal development in regions characterized by moderate precipitation and seasonal fluctuations. **(Spohn *et al*.,2025)**

2.11.4.Uses of Urtica dioica

The primary chemical constituents of Urtica dioica(UD), include volatile compounds, polysaccharides, sterols, proteins, tannins, flavonoids, isolectins, fatty acids, terpenes, vitamins, and minerals, which have been documented to

exhibit various pharmacological activities, including anti-inflammatory, antioxidant, and hepatoprotective effects. Urtica dioica commonly referred to as Urtica dioica is cultivated in various countries and has extensive applications in traditional medicine **(Al-Akash *et al*., 2022).**

Urtica dioica has been used for hundreds of years to treat painful muscles and joints eczema ,arthritis ,gout and anemia Anti-inflammatory is utilized to alleviate inflammation in ailments like arthritis, **(Hirabayashi *et al*., 2025)** It may alleviate the symptoms associated with benign prostatic hyperplasia in males, Allergies: It functions as a natural antihistamine to alleviate hay fever symptoms, Diuretic and detoxifying agent: It is utilized to enhance urine flow and purify the urinary system Arachidic acid, arachidonic acid, behenic acid, dodecenoic acid, uric acid, palmitic acid, palmitoleic acid, stearic acid, trichosanoic acid, lauric acid are the chemical composition (**Đurović *et al* ., 2024)**

2.11.5.Effects of urtica dioica on reproduction

The reproductive aging process in female animals is marked by a gradual shift from regular reproductive cycles to irregular cycles, followed by acyclic phases, culminating in a reduction or cessation of fertility at the conclusion of the reproductive period. Numerous studies have validated the inhibitory effects of estrogen on FSH and LH levels throughout the follicular period in females. **(Sabriyah and Salma,2025).** A deficiency in estrogen is recognized to influence female gonadotropin production by diminishing the release of GnRH from the brain. Substantial data indicates that reproductive hormones experience considerable alterations with advancing age in animals. Research indicates that the responses of LH and FSH to GnRH are diminished in older females relative to their younger counterparts**(Bandariyan *et al* .,2021)**

Estrogen exerts an inhibitory influence on FSH secretion and is significantly correlated with the aging process. Inhibitors that lower plasma

estrogen levels may be regarded as effective therapeutic alternatives for managing estrogen-related illnesses. The utilization of herbs and urticia dioica extracts as beneficial food additives in animal nutrition has grown over time owing to their diminished adverse effects and chemical additives**(Chira *et al* .,2025)**

Substantial data indicates that nettle (Urtica dioica) products inhibit aromatase and disrupt the conversion of testosterone to estrogen. Consequently, the administration of aromatase inhibitors may represent an innovative approach to normalize estrogen levels and subsequently enhance the FSH/LH ratio in elderly females by elevating LH levels, thereby enhancing fertility in animals,**(Du *et al* .,2023)**

2.12.physiology of gonadotropin-releasing hormone

Gonadotropin-releasing hormone (GnRH) is a hormone that stimulates the anterior pituitary gland to secrete FSH and LH. Gonadotropin-releasing hormone (GnRH) is a peptide hormone produced and secreted by GnRH neurons in the hypothalamus. Testosterone inhibits GnRH. This peptide is part of the gonadotropin-releasing hormone family and represents the initial phase of the hypothalamic-pituitary-gonadal axis. **(Casteel and Singh,2020).**

GnRH increases the production and secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the pituitary gland. The size and frequency of GnRH pulses, along with feedback from androgens and estrogens, regulate these processes. Low-frequency GnRH pulses are crucial for FSH secretion, but high-frequency GnRH pulses equally boost LH**(Casati *et al* .,2023)**

GnRH secretion differs between females and males. In males, GnRH is released in consistent pulses; in females, the pulse frequency fluctuates during the menstrual cycle, with a notable rise in GnRH occurring immediately prior to ovulation. Gonadotropin-releasing hormone (GnRH) secretion occurs in a

pulsatile manner across all vertebrates and is crucial for optimal reproductive function. Consequently, a singular hormone, GnRH1, regulates the intricate processes of follicle formation, ovulation, and corpus luteum maintenance in females, as well as spermatogenesis in males**(Lin *et al* .,2025)**

2.12.1.Gonadotropin-releasing hormone and regulation of the GnRH gene

GnRH is a peptide hormone consisting of 10 amino acids (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2). Gene expression initially produces the prepro-GnRH polypeptide, comprising a signal peptide, ten functional peptides, a signal amide/protein (Gly-Lys-Arg), and a GnRH-binding peptide **(Xiong *et al* .,2025).**Twenty-four GnRH variants have been found in neural tissues across vertebrates to protochordates, based on variations in amino acid sequences, locations, and embryonic origins. Notwithstanding the above noted distinctions, all these variations are decapeptides that exhibit fairly analogous structures. Typically, two or three isoforms of GnRH are present in the majority of vertebrate species,**(Lu *et al* .,2025)**

The predominant transcriptional regulators of GnRH genes are homeodomain proteins exhibiting diverse DNA-binding characteristics, and the majority are not only expressed in GnRH neurons. Specific interactions between transcriptional regulators and cofactors are necessary to attain precise promoter activity and tailored production of GnRH in GnRH neurons **(Wang *et al* .,2025)**.These cofactors can either facilitate or obstruct connections between homeodomain proteins and the transcriptional regulatory areas of the GnRH promoter and/or enhancer, hence regulating particular GnRH expression in GnRH neurons inside the hypothalamus and in adjacent tissues, such as the ovary,**(Turki and Ammar, 2024).**

2.12.2 Molecular biology of gonado tropin-releasing hormone (GnRH-1,GnRH-11)

Gonadotropin-releasing hormone (GnRH) serves as the primary neuroendocrine link in vertebrates between the brain and the reproductive axis; in certain species, as many as three different types of GnRH have been identified. It was formerly thought that non-human primates and humans exclusively produced one variant of GnRH (GnRH-I), but it is now established that they also produce a second variant (GnRH-II). GnRH-II, like GnRH-I, is highly successful in stimulating gonadotropin release; however, it is important to note that the neurons responsible for producing GnRH-I and GnRH-II are distinct**. (Dufour *et al.*,2020)**

Moreover, estradiol exerts a markedly different influence on GnRH-producing neurons compared to its effect on other GnRH-producing neurons. Estradiol enhances GnRH-II gene expression in the former and suppresses GnRH-I gene expression in the latter. Consequently, only the subpopulation of GnRH neurons expressing GnRH-I may mediate the negative feedback effect of estradiol, whereas the subpopulation expressing GnRH-II may facilitate the positive feedback effect,**(Bálint ,2018)**

The data indicate that human reproductive physiology is governed by two separate GnRH neural networks. The primary role of GnRH-II neurons is thought to be the generation of the preovulatory LH surge, whereas the regulation and sustenance of tonic pulsatile LH release is likely the major function of GnRH-I neurons. The neuroendocrine reproductive axis in primates operates independently, enabling targeted treatment of human reproductive disorders and precise fertility management,**(Mate *et al* .,2024)**

Mammals were formerly believed to have solely one classical variant of GnRH (GnRH-I). GnRH-I molecules exhibit identical amino acid sequences among mammals, with the exception of the guinea pig, which has substitutions

at the second and seventh amino acids. This gene in humans is situated on chromosome 8p11.2-p21, with four exons that comprise a 276-base-pair open reading frame (ORF) encoding a 92-amino-acid ancestral protein. Nonetheless, a second variant of GnRH (GnRH-II) has just been discovered. GnRH-II was initially extracted from the chicken brain, so it is designated as chicken GnRH-II. GnRH-II is encoded by a distinct gene and varies from GnRH-I in its amino acid sequence, **(Desaulniers *et al*., 2025)**

2.13.The Antioxidant Role of Urtica dioica

Urtica dioica (stinging nettle) is a medicinal urticia dioica rich in flavonoids, phenolic acids, carotenoids, and vitamins (C, A, and K), as well as essential minerals. These bioactive compounds possess strong antioxidant activity by scavenging free radicals and preventing lipid, protein, and DNA oxidation. Experimental studies have shown that Urtica dioica significantly reduces malondialdehyde (MDA) levels, enhances glutathione (GSH) concentration, and protects tissues—particularly ovarian and neural tissues—from oxidative stress–induced damage. Therefore, the antioxidant potential of Urtica dioica plays a protective role in maintaining cellular balance, reducing oxidative injury, and supporting reproductive function, suggesting its promise as a natural therapy for menopause-related complications.

Chapter Three

Methodology

3.1. Materials:

3.1.1. Instruments and Equipment:

The instruments and equipment used in the current study's research are identified and summarized in table 3-1 below, listed and describe with their details (company, city, and country).

Table (3-1): The instruments and equipment used in the current study's research

No	Instruments	Manufacture	Country
1	Anatomical set (Scissors, Forceps, Scalpel)	Chemo lab	China
2	Beakers (250)	Chemo lab	China
3	Buchner vacuum filtration system	Olympus	Japan
4	Centrifuge	Sartorius	Mse (England)
5	Container	Chemo lab	China
6	Curve scissor	Hettich Roto fix11	Japan
7	Disposable syringes	Medical ject	S.A.R
8	Disposable syringes 1ml/cc 7g × 1/2 and 3ml/cc 23g× 1	Medical ject	S.A.R
9	ELISA	Sartorius	Mse (England)
10	Eppendorfs tube	Chemo lab	China
11	Erlenmeyer flask	Chemo lab	China
12	Fiter paoer wattman no.	Chemo lab	China
13	Freezer	Hitachi	Japan
14	Gel tube	Chemo lab	China
15	Herb grinder	Silver crest	Germany
16	Light microscope with camera	Olympus	Japan
17	Medical gauze	Enterpreneure	India

18	**Micropipettes**	**Chemo lab**	**China**
19	**Needle gavage (stomach tube)**	**Wego medical factory**	**Iraq**
20	**Oven**	**Memmert**	**Germany**
21	**Pipette tips (10-100 µl) volume**	**Chemo lab**	**China**
22	**Rotary evaporator**	**Olympus**	**Japan**
23	**Sensitive electronic balance**	**Citizen scale INC**	**U.S.A**
24	**Shaking inkubato**	**Olympus**	**Japan**
25	**Slide and cover slides**	**China MHECO**	**China**
26	**Spray dryer**	**Olympus**	**Japan**
27	**Sterile cotton swab**	**Enterpreneure**	**India**
28	**Test tube**	**Chemo lab**	**China**
29	**Water bath**	**Memmert**	**Germany**

3.1.2. Chemicals and Kits:

The chemical ingredients, purified reagents, and standard kits utilized in this study along with their origins shown in the tables (3-2).

Table (3-2): The chemical ingredients, purified reagents, and standard kits utilized in this study

NO	Chemical and Kits	Company
1	4-Vinylcyclohexene diepoxide (VCD)	Denlpharama, Germany
2	Alcoholic 70%	Sharlab spain
3	Anti mulleria hormone (AMH) kit	Biolab, China
4	Deionized distilled water	Al-Joud, Iraq

5	Estradiol kit	Biolab, China
6	Ethanol 95%	Sharlab spain
7	Formalin 10%	TEDIA company Inc.USA
8	Follicle-stimulating Hormone (FSH)kit	Biolab, China
9	Giemsa stain	Leica, germany
10	Gonadotropin-Releasing Hormone (GnRH1)	United States
11	Gonadotropin-Releasing Hormone GnRH2	United States
12	Glutathione (GSH)kit	Japan
13	Hematoxylin and Eosin stain	Merck, Germany
14	ketamine 0.5	Sharlab spain
15	Luteinizing Hormone (LH)kit	Biolab, China
16	Malondialdehyde (MDA) kit	Japan
17	Testosterone kit	Biolab, China
18	TransZol	Italy
19	xylazine 0.25	Sharlab spain

3.2. Examination Methods:

3.2.1:Urticia dioica Material

Urticia dioica of urtica dioica was purchased from local market in north Iraq, Voucher specimens was deposited to be identified and authenticated at University of Kerbala, College of Education for Pure Sciences Department of Life Sciences in **appendix I**

3.2.2 : Preparation of Ethanolic Extract of Urtica Dioica

The urticia dioica was cleaned and grounded by electrical grinder.,100 g from leves urticia dioica was placed into the thimble inserted in extraction unite

of Soxhlet apparatus to extracted with 70% ethanol at temperature of 45°C until a clear and colorless solvent appeared. The extract was then filtered and dried under vacuum for four hours by using a rotary evaporator at 40°C and 150 rotates per minute. Later, the crude extract of urticia dioica was concentrated in a glass petri dish by placed it in an incubator at 40°C until a semi-solid mass and thick appeared. All dried extract was collected, and kept in dark sterile glass container in the refrigerator at 4°C **(Chira *et al* .,2025)** the urticia dioica was chemically analyzed by using GCMs at Ibn al-Bitar Research.

3.2.3 Preparation 4-Vinylcyclohexene diepoxide (VCD)

For 20 days in a row, female rats were given intraperitoneal injections of either corn oil vehicle or VCD (Sigma, V3630) at a dosage of 160 mg/kg **(Troy,*et al*.,2024).** In order to identify the onset of menopause (ovarian failure), which was previously defined as 10 consecutive days of amenorrhea, vaginal cytology was studied every day **(Methawasin,*et al*.,2025).** The menopausal VCD paradigm is well-characterized and comparable to earlier research where VCD was given for 20 days in a row **(Mashimo,*et al* .,2025).**

3.2. 4Animals Experimental:

In this study 40 adult female rats are used at age 49 days and average weight 150 -200 grams and were housed and maintained in the animal house /College of Veterinary Medicine /University of kerbala with optimal conditions these rats were fed special formula (food pellets)and supplied by clean drinking water .all experimental animal were housed in a clean plastic cages which contained sawdust bedding that was changed twice a week to provide a clean environment which terms of constant temperature round (25 ±5 C°), as well as ventilation and light system was 14/10 hours light/dark cycle with a relative humidity of 50±5%.

3.2.5. Experimental Design:

Forty adult female rats at 49 days 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):-(Figure 3-1)

Group 1 : (the control group) was administered only normal saline

Group 2 : was administered (100 mg/kg) Urtica dioica ethanolic extract orally for 20 days **.(JOI ,2022)**

Group 3: was administered (160 mg/kg) VCD via IP for 20 days . **(Osse, 2024)**

Groupt 4 :was administrered (160 mg / kg) VCD via IP and (100 mg /kg) Urtica dioica ethanolic extract orally for 20 days

3.2.6.Dose preparation and administration

Adult female rats with an average body weight of 250 g were used for dose .calculations

3.2.6.1.VCD preparation

The -4Vinylcyclohexene diepoxide (VCD) was dissolved in corn oil to prepare a stock solution with a concentration of 128 mg/mL. Each rat received an intraperitoneal injection at a dose of 160 mg/kg body weight. Accordingly, a 250 g rat received 40 mg of VCD, equivalent to an injection volume of 0.31 mL.

3.2.6.2.Urtica dioica extract preparation

The crude ethanolic extract of Urtica dioica was dissolved in distilled water (or normal saline) to prepare the required stock concentration. Rats were treated with a dose of 100 mg/kg body weight administered via oral gavage. For a 250 g

rat, this corresponds to 25 mg of extract. The actual volume administered was adjusted according to the prepared stock concentration (e.g., 0.25 mL for a 100 mg/mL) solution, or 0.50 mL for a 50 mg/mL solution

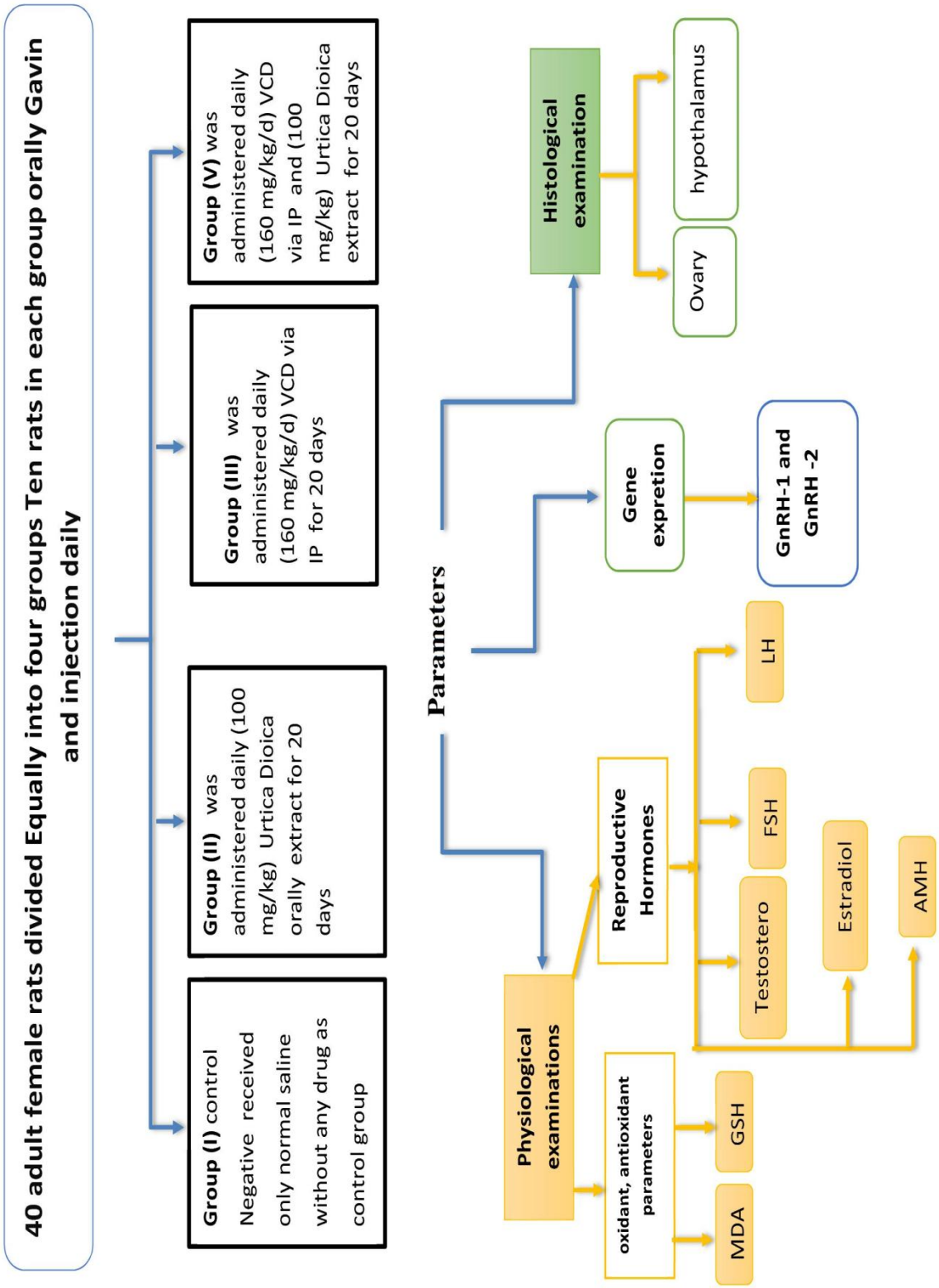


Figure (3-1) Experimental design

3.2.6.Estrus synchronization in rats

-synchronization of estrus mature female rats were be synchronized with a IP injection of PGF2α of dose (0.05mg/kg B.W),with single dose of progesterone (0.15 mg/kg B.W. S/C), between 03:00 to 04:00pm,three days apart, in same time the female rats were injected with finally single dose of PGF2α(0.05mg/kg B.W IP) **(Potapova *et al* ., 2025).**

3.2.7.Vaginal cytology

In experimental rats, vaginal cytology is a useful method for evaluating the estrous cycle and ovarian function. This is a detailed tutorial on using vaginal cytology to identify ovarian failure through microscopy **(Babaev *et al*.,2025).**
The estrous cycle of female rats was monitored and confirmed by vaginal cytology. Vaginal smears were obtained daily by flushing the vaginal canal with a small volume of normal saline (0.9%) using a micropipette. The collected fluid was placed on clean glass slides, air-dried, and examined under a light microscope. The identification of the estrous stages was based on the predominance of specific cell types: nucleated epithelial cells in proestrus, cornified epithelial cells in estrus, a mixture of cornified cells and leukocytes in metestrus, and leukocytes predominance in diestrus. This method allowed accurate determination of the estrous cycle stages and ensured synchronization of animals before the beginning of the experimental treatments. (**kim *et al* .,2022)**

3.3. Samples Collection:

3.3.1. Collection of Blood Samples:

Blood was collected over 3 specific periods of the 52-day experiment. Blood was collected in the first period on day 20 of the experiment. Blood was collected during the second period on day 35. Blood was collected in the third and final period on the last day of the experiment, day 52 of the experiment . all

animals anesthetized by (xylazine 0.25 -ketamine 0.5) sterile medical syringes of 3ml were accustomed to extract 3ml of cardiac blood .the blood than put into gel tube and the blood was centrifuged in a special gel tube that did not contain an anticoagulant at a speed of 4000 revolutions per minute for 5minutes .once the serum had been separated it was put in eppendrof tubes for storage and kept in the fridge at (-30 C) while the assays were working **(Daniel *et al* .,2025) UOK.VET. PH. 2024.097**

3.3.2. Collection of The Organs for Histological Section:

The animals were then dissected to remove sections (Ovary and Hypothalamus), and each of them was isolated separately. The organs were then kept in sterile plastic containers with a 10% formalin concentration after being numbered each group for use in the histological section, **(Wang,*et al*,.2025).**

3.4. Physiological Parameters Measurement:

3.4.1. Estimation of Reproductive Hormones:

3.4.1.1. Estimation Testosterone Hormone:

Serum testosterone hormone(T) concentration in the current study was measured by ELISA technique using commercial test kit .Uses a biotin double antibody sandwich technology-based enzyme-linked immunosorbent assay (ELISA) to measure Testosterones levels in samples (**Zerroug *et al* .,2025**). testing procedure was according the manufacturer's instruction as illustrated in **appendix II.**

3.4.1.2. Estimation Follicle Stimulating Hormone (FSH) Concentration

(mIU/ml).

Serum follicle stimulating hormone concentration in the current study was measured by ELISA technique using commercial test kit.Uses a biotin double antibody sandwich technology based enzyme-linked immunosorbent assay (ELISA) to measure FSH levels in samples **(Jinno, 2025).**testing procedure was according the manufacturer's instruction as illustrated in **appendix III**.

3.4.1.3. Estimation of Luteinizing Hormone (LH) Concentration (ng/ml).

Serum Luteinizing hormone concentration in the current study was measured by ELISA technique using commercial test kit.Uses a biotin double antibody sandwich technology-based enzyme-linked immunosorbent assay (ELISA) to measure LH levels in samples **(Chen,2025).**testing procedure was according the manufacturer's instruction as illustrated in **appendix IV**

3.4.1.4.Estimation OF Estradiol (E2) (pg/ml)

Serum Estradiol hormone concentration in the current study was measured by ELISA technique using commercial test kit.Uses a biotin double antibody sandwich technology-based enzyme-linked immunosorbent assay (ELISA) to measure E2 levels in samples **(Vicatos, 2022).**testing procedure was according the manufacturer's instruction as illustrated in **appendix IV**

3.4.1.5. Estimation OF Anti mulleria hormone (AMH)(ng/ml)

Serum Anti mulleria hormone hormone concentration in the current study was measured by ELISA technique using commercial test kit.Uses a biotin double antibody sandwich technology-based enzyme-linked immunosorbent assay (ELISA) to measure AMH levels in samples **(Oldfield,2021).**testing procedure was according the manufacturer's instruction as illustrated in **appendix** V

3.5. Estimation of Oxidant's and Antioxidants Concentrations:

3.5.1. Serum Malondialdehyde Measurement (MDA)(µmol/L)

The ability to accurately measure lipid peroxidation in disease states necessitates this method of assessing oxidative stress. MDA and 4 hydroxynonenal (4-HNE) are the natural bi-products of lipid peroxidation. One of the most commonly acknowledged methods for assessing oxidative damage is to measure the lipid peroxidation products. It is easy to use the MDA Microplate Assay Kit to detect MDA in a range of samples. Thiobarbituric Acid (TBA) reacts with MDA in the sample to form the MDA-TBA adduct. You may readily measure the MDA-TBA adduct using a colorimeter (λ= 532 nm). According to **(Omar and Al-Helaly ,2025)**,as illustrated in the **appendix** VI

3.5.2. Serum reduced glutathione concentration (GSH)(nmol/mL)

The quantification of reduced glutathione (GSH) content relies on its reactivity with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), commonly referred to as Ellman's reagent. This colorimetric approach involves GSH reducing DTNB to produce a yellow compound, 5-thio-2-nitrobenzoic acid (TNB), which is quantifiable spectrophotometrically at 412 nm. According to **(Abdulla *et al* .,2025)** The colour intensity generated is directly proportional to the

concentration of GSH in the sample. This assay enables the quantitative measurement of intracellular or extracellular GSH concentrations in biological fluids and tissue homogenates. shown in **appendix VII**

3.6. Estimation Gene expretion

3.6.1. Gonadotropin-Releasing Hormone-I (GnRH-I)

GNRH1

This RIA technique relies on a competitive binding response. Endogenous GnRH-I from tissue extracts competes with a constant quantity of radiolabeled ^{125}I-GnRH-I for a finite number of anti-GnRH-I antibody binding sites. The radioactivity associated with the antibody is inversely proportional to the concentration of GnRH-I in the sample. Following the separation of bound and free hormone, the radioactivity is measured, facilitating the quantification of GnRH-I levels. **by (Xie et al .,2025)** shown in **appendix IX**

By gene sequence

GNRH1

F: 5- GACTGTGTGTTTGGAAGGCTGC-3

R: 5-CCATTTGATCCTCCTCCTTGC-3 101 bp

3.6.2. Gonadotropin-Releasing Hormone -II (GnRH-II)

This Principle of the Assay This RIA technique relies on a competitive binding response. Endogenous GnRH-II peptides compete with a constant quantity of radiolabeled ^{125}I-GnRH-II for a finite number of particular antibody binding sites. The quantity of radioactivity associated with the antibody is inversely related to the concentration of GnRH-II in the sample. Following the separation of bound and free fractions, radioactivity is assessed, enabling the

determination of GnRH-II levels by **(Ubuka and Bentley,2024**) show in **appendix X**

By gene sequence

GNRH2

F: 5-TCCTTTCTGGTGGGCATTTAG-3

R: 5-GCTCTAGGTCATCCCTAACTTG-3 94 bp

3.7. Histological Section Method:

At the end of the current study, rats were sacrificed and organ specimens were immediately removed. For histopathological study according to **(Wang,et al,.2025)** as show in **(Appendix XI)**

3.8. Statistical Analysis:

Data were analyzed using the Statistical Package for the Social Sciences (SPSS). One-way analysis of variance (ANOVA) was performed to compare the means among groups at different time intervals. When significant differences were detected, the Least Significant Difference (LSD) post hoc test was applied to determine pairwise differences. Results were expressed as mean ± standard error (SE), and differences were considered statistically significant at $P \leq 0.05$. **(SPSS ,2019).**

Chapter Four
Results and Discussions

4. Results and Discussion:

4.1.Detection of active groups and compounds in Urtica Dioica

_Chemical tests:-

NO	Type of detection	Alcoholic extract
1	Tannins Test	+
2	Carbohydrate Test	+
3	Glycosides Test	+
4	Phenols Test	+
5	Resins Test	+
6	Flavonoid's Test	+
7	Saponin Test	_
8	Alkaloid Test	+
9	Protein Test	_
10	Coumarins Test	+
11	Terpenes Test	_
12	Steroids Test	_

4.2. Gas chromatography Mass Analysis (GCMS)

The bioactive compounds identified by GC-MS analysis are summarized

In **APPENDIX XI**

4.3. Hurmonal study

4.3.1 Testosterone

Effect of urtica dioica , VCD and Their combination on Serum Testosterone levels in female rats

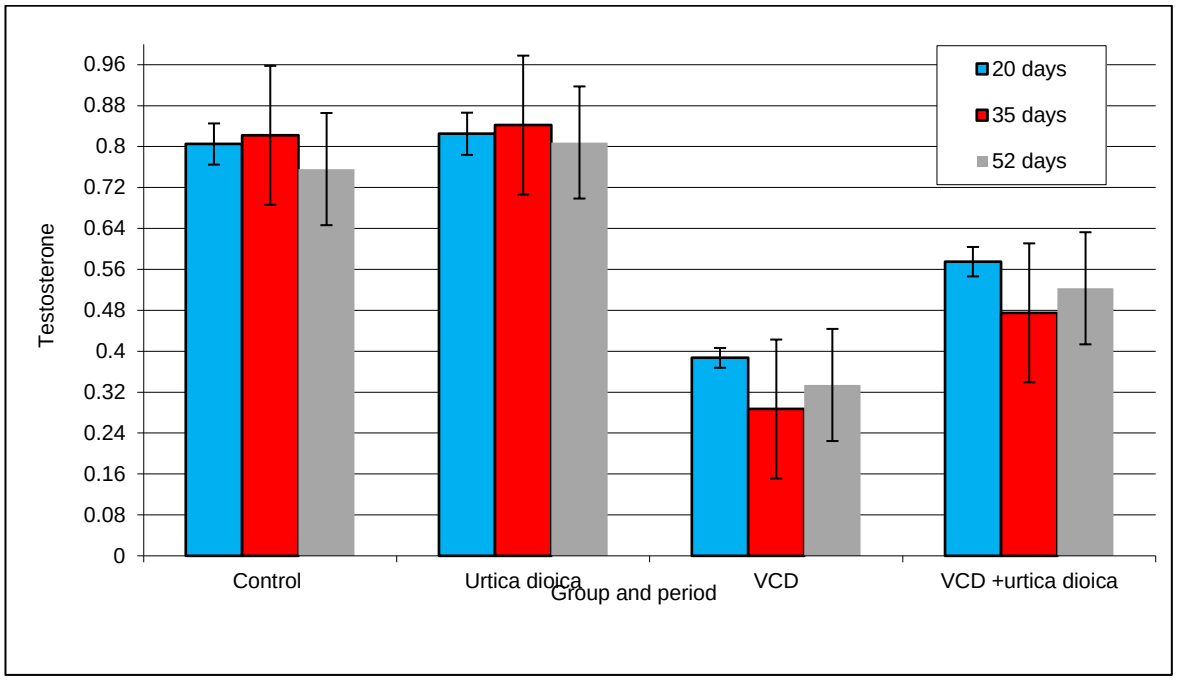


Figure (4.1): Comparison of serum Testosterone levels in the control,urtica dioica,VCD and VCD+ Urtica dioica groups in female rats at 20,35.52 days

The mean ± SD serum Testosterone level (ng/dl) were significantly (P≤0.05) lower in VCD group particulary on day 35 compared to control group and significantly (P≤0.05) higher in (VCD+ Urtica dioica) group compared to VCD group.

Table (4-1): comparsion of the serum Testosterone levels among the control,urtica dioica,VCD and VCD+ Urtica dioica groups in female rats

Group	Mean ±SE of Testosterone (T)			L.S.D. value
	20 days	35 days	52 days	
Control	0.805 ±0.02 **A ab**	0.822 ±0.02 **A a**	0.756 ±0.02 **A b**	0.064 *
Urtica dioica	0.825 ±0.01 **A a**	0.842 ±0.02 **A a**	0.808 ±0.01 **A a**	0.049 *
VCD	0.387 ±0.02 **C a**	0.287 ±0.02 **C b**	0.334 ±0.03 **C ab**	0.082 *
VCD +urtica dioica	0.575 ±0.03 **B a**	0.475 ±0.03 **B a**	0.523 ±0.03 **B a**	0.101 *
L.S.D. value	0.072 *	0.076 *	0.072 *	---
Means having with the different big letters in same column and small letters in same row differed significantly. * (P≤0.05).				

The results of this study that appears **in table (4-1)** revealed that Testosterone levels in the VCD group were significantly lower that the control group. Meanwhile, The testosterone levels were substantially higher in group treated with VCD+ Urtica dioica group compared to VCD group.

4-Vinylcyclohexene diepoxide (VCD) is recognised for inducing ovarian failure by preferentially obliterating tiny pre-antral and antral follicles in animals. This leads to a substantial decrease in steroidogenic cell activity, particularly in theca and stromal cells that produce androgens, including testosterone**.(Yu,*etal*,.2023)** study established that VCD exposure results in reduced serum testosterone levels in female rats, indicating disrupted ovarian steroidogenesis and a general hormonal imbalance typically observed in perimenopausal or premature ovarian failure models.**)** and diminished levels of testosterone The result described above corresponds to the research undertaken by **(Carolino,*etal*,.2019**) These alterations replicate normal menopause in humans, hence substantiating the model.

On the other hand pact of Urtica dioica on Testosterone Levels in Females Urtica dioica (stinging nettle) has exhibited potential hormone-regulating properties. Although predominantly examined in male subjects, current findings indicate that its extract may aid in restoring testosterone levels in female mice by improving the functionality of residual theca and interstitial cells while mitigating oxidative damage to ovarian tissue study**(Pestana,*etal*,.2018)**also the other study demonstrated that the ethanolic extract of Urtica dioica roots markedly enhanced testosterone levels and reproductive indicators in female albino rats, indicating its pro-androgenic and antioxidant properties**(Bandariyan *etal*., 2021)**

4.3.2. Follicle Stimulating Hormone (FSH)

Effect of urtica dioica , VCD and Their combination on Serum Follicle Stimulating Hormone levels in female rats

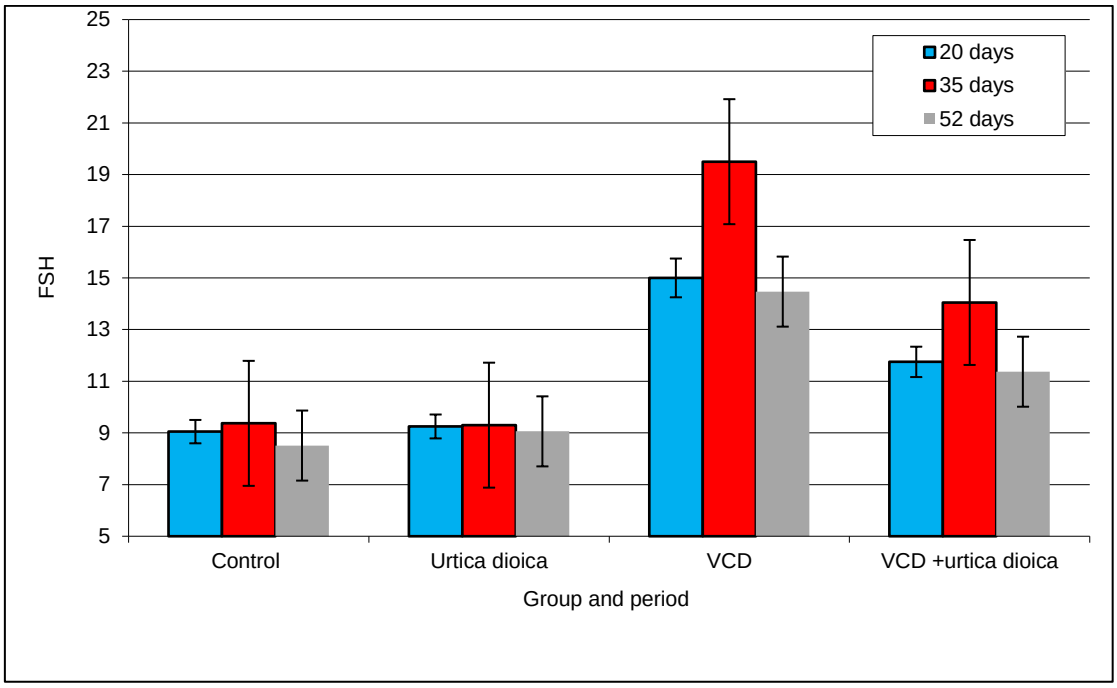


Figure (4.2) Comparison of Follicle Stimulating Hormone (FSH) levels in the control,urtica dioica,VCD and VCD+ Urtica dioica groups in female rats at 20,35.52 days

There was a significant ($P \leq 0.05$) increase in levels of FSH in VCD group ,peaking on day 35 compared to control and urtica dioica groups also there was reduction in (VCD+urtica dioica)group in FSH levels as compare to VCD group both, the control and urticia dioica groups maintained stable and normal levels

Table (4.2): Comparison of Follicle Stimulating Hormone (FSH) levels in the control,urtica dioica,VCD and VCD+ Urtica dioica groups in female rats

Group	Mean ±SE of Follicle Stimulating Hormone (FSH)			L.S.D. value
	20 days	35 days	52 days	
Control	9.05 ±0.21 **C ab**	9.37 ±0.17 **C a**	8.51 ±0.19 **C b**	0.623 *
Urtica dioica	9.25 ±0.10 **C a**	9.30 ±0.20 **C a**	9.06 ±0.10 **C a**	0.463 *
VCD	15.00 ±0.20 **A b**	19.50 ±0.64 **A a**	14.47 ±1.12 **A b**	2.430 *
VCD +urtica dioica	11.75 ±0.32 **B b**	14.05 ±0.21 **B a**	11.37 ±0.49 **B b**	1.153 *
L.S.D. value	0.690 *	1.124 *	1.927 *	---
Means having with the different big letters in same column and small letters in same row differed significantly. * (P≤0.05).				

As shown in the **table above (4.2),** the average levels of follicle-stimulating hormon (FSH) were elevated in the VCD group compared to the control and Urtica dioica groups, while there was decline in the levels of FSH in the (VCD + Urtica dioica) group as compare to VCD group

Follicle-stimulating hormone (FSH) is essential for promoting ovarian follicle development and estrogen synthesis. In a healthy ovary, increasing estrogen levels from mature follicles exert negative feedback on the hypothalamic-pituitary-gonad (HPG) axis, regulating FSH secretion. Administration of VCD (4-phenylcyclohexene diepoxide) selectively destroys young, developing follicles, particularly the preantral and antral follicles. This injury causes a significant decrease in estrogen synthesis, eliminating negative feedback on the pituitary gland, leading to a significant increase in FSH levels. This hormonal

response has been widely found in VCD-induced models of menopause or premature ovarian insufficiency. A study by **(Yang *et al* .,2024**). revealed that FSH levels were significantly elevated in female mice treated with VCD, corresponding to follicular depletion. Similarly, **(Li *etal*.,2023)** demonstrated the validity of persistent FSH elevation after VCD-induced ovarian failure.

The documented hormonal variations substantiate that VCD proficiently creates a perimenopausal condition by affecting ovarian follicles, as indicated by elevated gonadotropins (FSH) The result described above corresponds to the research undertaken by **(Kumari *etal*,.2024)**

On the other hand These observations suggest a potential stimulatory or protective influence of the urticia dioica extract on ovarian function. Herbal substances have demonstrated the ability to affect folliculogenesis via hormonal regulation **(Jasim *etal*,.2023)** . Nettle's regulatory effect on follicle-stimulating hormone (FSH) may extend beyond traditional estrogenic feedback. While the urticia dioica's phytoestrogens partially mimic estrogen, emerging evidence suggests that their effects on FSH levels may also involve modulating oxidative stress pathways, inflammatory cytokines, and hypothalamic signaling. **(Abdalkareem *etal* .,2023)**

4.3.3. Luteinizing Hormone (LH)

Effect of urtica dioica , VCD and Their combination on Serum Luteinizing Hormone levels in female rats

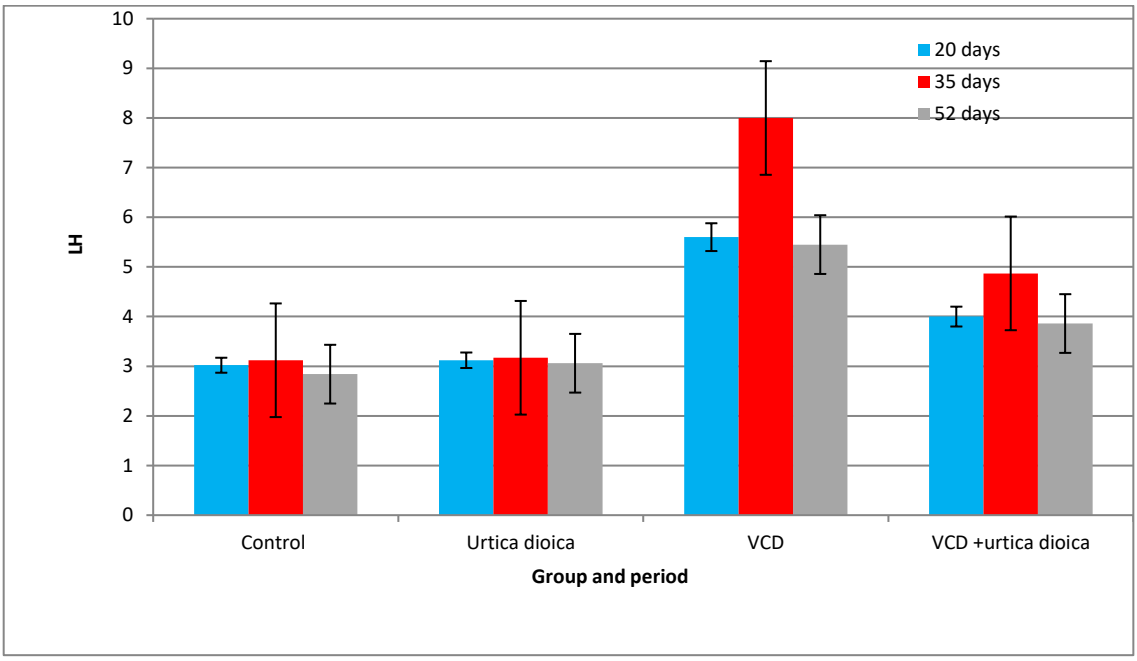


Figure (4.3) Comparison of Luteinizing Hormone (LH)levels in the control,urtica dioica,VCD and VCD+ Urtica dioica groups in female rats at 20,35.52 days

There was a significant (P≤0.05) increase in the mean LIH level in VCD group, Peaking on day 35 when compared to control and urtica dioica groups, while (VCD+urtica dioica) group had significantly decrease LH level in comparison to VCD group.

Table (4.3) : Comparison of Luteinizing Hormone (LH)levels in the control,urtica dioica,VCD and VCD+ Urtica dioica groups in female rats

Group	Mean ±SE of Luteinizing Hormone (LH)			L.S.D. value
	20 days	35 days	52 days	
Control	3.02 ±0.08 **C ab**	3.12 ±0.06 **C a**	2.84 ±0.08 **C b**	0.249 *
Urtica dioica	3.12 ±0.04 **C a**	3.17 ±0.08 **C a**	3.06 ±0.04 **BC a**	0.2005 *
VCD	5.60 ±0.16 **A b**	8.00 ±0.45 **A a**	5.45 ±0.56 **A b**	1.376 *
VCD +urtica dioica	4.00 ±0.08 **B b**	4.87 ±0.15 **B a**	3.86 ±0.24 **B b**	0.542 *
L.S.D. value	0.313 *	0.758 *	0.959 *	---
Means having with the different big letters in same column and small letters in same row differed significantly. * (P≤0.05).				

As noted in the **table (4.3)** , results reported the highest levels of LH in VCD group when Compared to control and Urtica dioica groups while (VCD+ Urtica dioica) group had significantly decrease in LH levels in comparison to VCD group

Luteinizing hormone (LH) is essential for regulating ovulation and corpus luteum function in females. Its secretion is tightly controlled by negative feedback from estrogen produced by ovarian follicles. Dysfunction of follicles leads to decreased estrogen production, resulting in the loss of negative feedback and a compensatory increase in LH levels. The present study demonstrated that mice treated with VCD exhibited significantly increased LH levels over different timescales. **(Pearson etal .,2025)** This results from the targeted destruction of preantral and early follicles by VCD, which impairs granulosa cell function and estrogen production. The decrease in circulating estradiol removes the inhibitory effect on the hypothalamic-pituitary-gonad axis, leading to increased gonadotropic secretion, including LH. These findings are consistent with previous research using VCD to induce ovarian insufficiency. **(Meyer *et al*.,2019)** documented an increase in luteinizing hormone (LH) levels in a VCD-induced perimenopausal rat model, demonstrating that VCD mimics the endocrine characteristics of menopause by impairing folliculogenesis and altering steroid hormone balance. Furthermore, **(Li *etal*., 2023)** revealed that prolonged exposure to VCD leads to reprogramming of hypothalamic-pituitary signaling, characterized by a persistent increase in LH levels due to chronic estrogen deficiency and follicular depletion **(Majeed *etal* ., 2025)**

Also the result 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**, (Orsi *etal***

.,2024) Following VCD-induced ovarian insufficiency, luteinizing hormone (LH) levels generally increase as a result of estrogen deficiency and the subsequent loss of negative feedback on the hypothalamic-pituitary axis. However, treatment with urtica dioica had a clear modulatory effect, drastically reducing LH levels in VCD-deficient mice. The reduced LH levels suggest a partial restoration of ovarian feedback regulation. The likely mechanism behind this effect lies in the phytochemical components of nettle, specifically flavonoids, sterols, and lignans, which exhibit phytoestrogenic properties. **(Bandariyan *etal* .,2021)** These chemicals can mimic natural estrogen and interact with estrogen receptors, restoring inhibitory feedback to gonadotropin-releasing hormone (GnRH) and ultimately reducing LH secretion. Furthermore, nettle possesses antioxidant and anti-inflammatory properties, which may enhance follicle viability and steroidogenic activity. The urticia dioica extract may enhance granulosa cell health, which promotes endogenous estrogen synthesis and facilitates hormonal rebalancing. Recent research has confirmed these findings studied the hormone-regulating properties of nettle, confirming its ability to normalize elevated luteinizing hormone (LH) levels. also demonstrated a significant decrease in LH levels in female rats treated with nettle extract, suggesting that this effect promotes ovarian function and hormonal modulation **(Sabriyah and Salma,2025)**

4.3.4.Estradiol (E2)

Effect of urtica dioioca, VCD and Their combination on Serum Estradiol Hormone levels in female rats

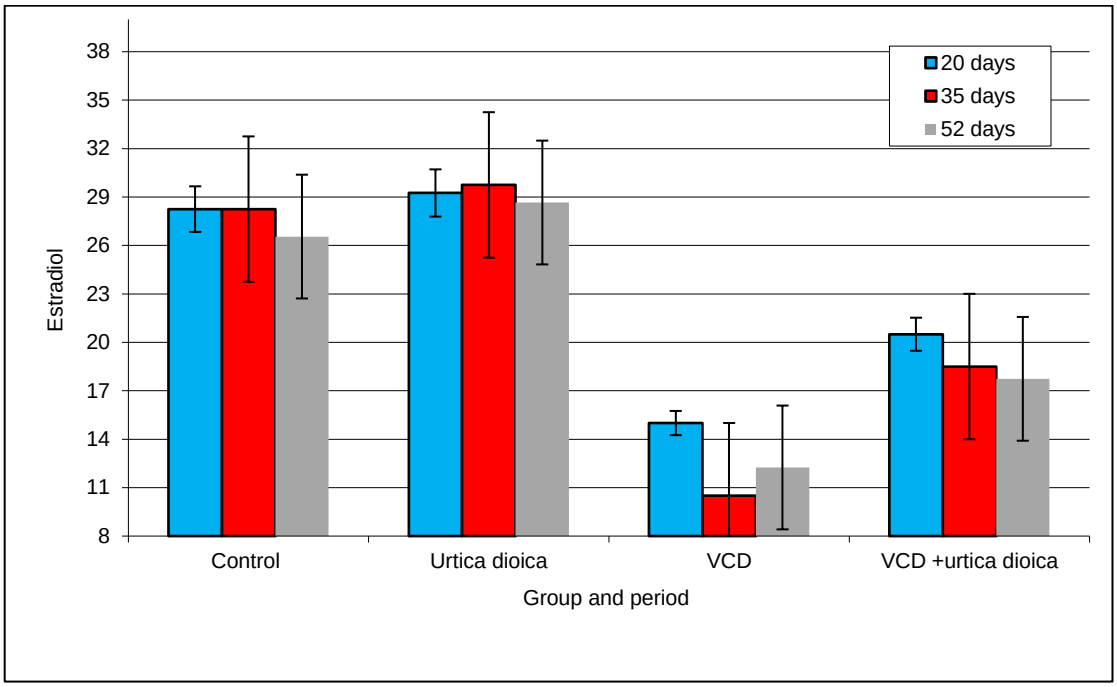


Figure (4.4) comparison of seram Estradiol (E_2) levels in the Control, urtica dioioca, VCD , and VCD + urtica dioica groups in female rats at 20,35.52 days

There was eva significant (P≤0.05) decrentes in the (E2) level in the VCD group Particalary on 35 day compared to control and urtica Dioica group Also significantly (P≤0.05) higher in (VCD + urtica dioica) group compared to VCD group

Table (4-4): comparison of seram Estradiol (E_2) levels in the Control, urtica dioioca, VCD , and VCD + urtica dioica groups in female rats

Group	Mean ±SE of Estradiol (E2)			L.S.D. value
	20 days	35 days	52 days	
Control	28.25 ±0.47 **A a**	28.25 ±0.63 **A a**	26.55 ±0.44 **A b**	1.680 *
Urtica dioica	29.25 ±0.47 **A a**	29.75 ±0.47 **A a**	28.66 ±0.46 **A a**	1.521 *
VCD	15.00 ±0.41 **C a**	10.50 ±0.64 **C b**	12.25 ±1.27 **C b**	2.583 *
VCD +urtica dioica	20.50 ±0.64 **B a**	18.50 ±0.64 **B ab**	17.74 ±0.58 **B b**	2.005 *
L.S.D. value	1.572 *	1.860 *	2.254 *	---
Means having with the different big letters in same column and small letters in same row differed significantly. * (P≤0.05).				

The results in **table (4.4**) revealed that E2 level in VCD group were decreaed compared to Control and Urtica dioica groups. Meanwhile, The E2 level were higher in (VCD + Urtica dioica) group comparted to VCD group

Estradiol (E2) is the most potent female estrogen and is essential for controlling the reproductive cycle, maintaining follicular growth, and modulating the hypothalamic-pituitary-gonadal axis. Administration of VCD in this study resulted in a significant decrease in estradiol levels, consistent with its known ovarian toxicity. ,**(Gilmer *etal* .,2025**). VCD selectively eliminates young, developing follicles, particularly preantral and early follicles, which are the primary sources of estradiol synthesis by granulosa cells. Decreased estradiol levels eliminate negative feedback on gonadotropin-releasing hormone (GnRH), resulting in elevated LH and FSH levels,

further disrupting reproductive hormonal balance**. (Methawasin *etal* .,2025)**

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 levels, The result described above corresponds to the research undertaken by **(Kargozar,*etal*,.2019)**

Urtica dioica treatment significantly increased estradiol levels in VCD-treated rats. This improvement is attributed to the urticia dioica's estrogenic phytochemicals—flavonoids and lignans—which mimic estrogen and activate its receptors. Furthermore, the urticia dioica's antioxidant and anti-inflammatory properties protect ovarian tissue from further damage, promote granulosa cell viability, and may increase endogenous estrogen synthesis. As a result, estradiol levels rise, and the negative feedback mechanism to the pituitary gland is partially restored**(Chira *et al* .,2025)**; **(Sharokhyan Rezaee *etal* .,2022)**

4.3.5. Anti-Müllerian Hormone (AMH)

Effect of urtica dioica , VCD and Their combination on Serum Anti-Müllerian Hormone levels in female rats

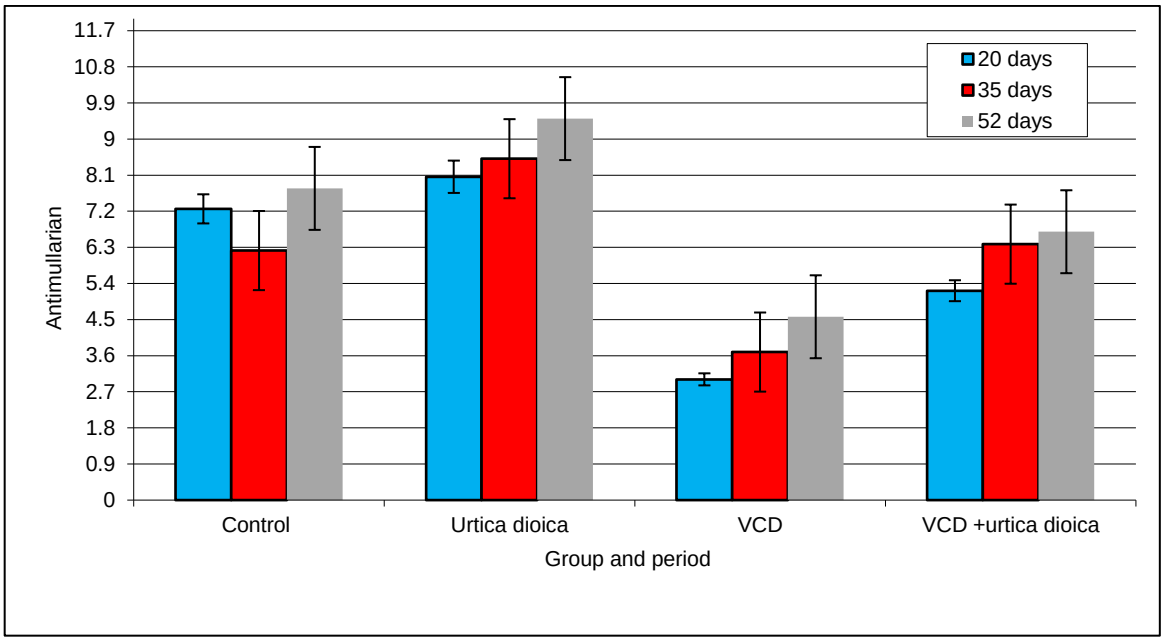


Figure (4.5): Comparsion of Anti-Müllerian Hormone (AMH) level in the Control, urtica dioioca, VCD , and VCD + urtica dioica groups in female rats at 20,35.52 days

The mean AMH level was significantly (P≤0.05) lower in VCD group compared to control and urtica Diocid groups Also There was significantly (P≤0.05) Higher in (VCD + urticia dioica) group compared to VCD group

Table (4-5): Comparsion of Anti-Müllerian Hormone (AMH) level in the Control, urtica dioioca, VCD , and VCD + urtica dioica groups in female rats

Group	Mean ±SE of Antimullarian (AMH)			L.S.D. value
	20 days	35 days	52 days	
Control	7.26 ±0.35 **A ab**	6.22 ±0.68 **B b**	7.77 ±0.32 **B a**	1.546 *
Urtica dioica	8.06 ±0.50 **A b**	8.51 ±0.36 A ab	9.51 ±0.42 A a	1.391 *
VCD	3.01 ±0.66 **C b**	3.69 ±0.33 **C ab**	4.57 ±0.31 **C a**	1.487 *
VCD +urtica dioica	5.22 ±0.34 **B b**	6.38 ±0.47 **B ab**	6.69 ±0.52 **B a**	1.448 *
L.S.D. value	1.490 *	1.494 *	1.247 *	---
Means having with the different big letters in same column and small letters in same row differed significantly. * (P≤0.05).				

The level of AMH were significantly lower in **table (4-5)** of VCD group compared to control and urtica dioica groups ,while in (VCD+ urtica dioica)group There was the level of AMH higher Compared to VCD group

Anti-Müllerian hormone (AMH) is a primary indicator of ovarian reserve, secreted by the granulosa cells of small preantral follicles. AMH levels directly indicate the quantity and function of the developing follicular reserve. Therefore, any disruption to folliculogenesis or granulosa cell function is expected to significantly affect AMH secretion. In the current study, VCD (4-phenylcyclohexene diepoxide) administration significantly decreased AMH levels at all measured time points. by **(Karur *etal* .,2021)** This decrease is closely related to the established mechanism of action of VCD—the selective removal of small and developing follicles, particularly the primary and secondary follicles, where AMH is synthesized. Depletion of these follicles results in a concomitant decrease in granulosa cell activity and, consequently, in

AMH secretion. These findings are consistent with previous research indicating that AMH is among the earliest and most sensitive markers of VCD-induced ovarian damage. indicated that AMH levels decreased significantly within weeks of VCD exposure in mice, before complete disruption of the estrous cycle. Similarly**,(Wilker, 2024)** demonstrated that AMH is a reliable early indicator of VCD-induced ovarian follicle loss in premenopausal animals

The 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 **(Grigorova,*etal*,.2022)**) AMH is a proxy for ovarian reserve, produced by granulosa cells in early-stage follicles. Following VCD-induced ovarian injury, AMH levels generally decline due to the eradication of these young, developing follicles. In this study, treatment with Urtica dioica extract significantly enhanced AMH levels, suggesting a protective and regenerative effect on folliculogenesis. The increase in AMH suggests that Urtica dioica may help maintain or stimulate early-stage follicle development by protecting granulosa cells from oxidative damage. **(Davis and Baber,2022).** The urticia dioica's known antioxidant, anti-inflammatory, and phytochemical properties likely help maintain follicular health and enhance granulosa cell function, thereby facilitating AMH formation. Recent studies have confirmed these observations. **(**demonstrated that urtica dioica improved the morphology and function of ovarian follicles in mice, leading to a significant increase in AMH levels. indicated that nettle alleviates oxidative stress in ovarian tissue, indirectly enhancing granulosa cell viability and hormone release**(Lykoudi *etal* .,2020)**

4.4. Estimation of Oxidant's and Antioxidants Concentrations.

4.4.1.Serum Glutathione (GSH) Activity

Effect of urtica dioica , VCD and Their combination on Serum Glutathione levels in female rats

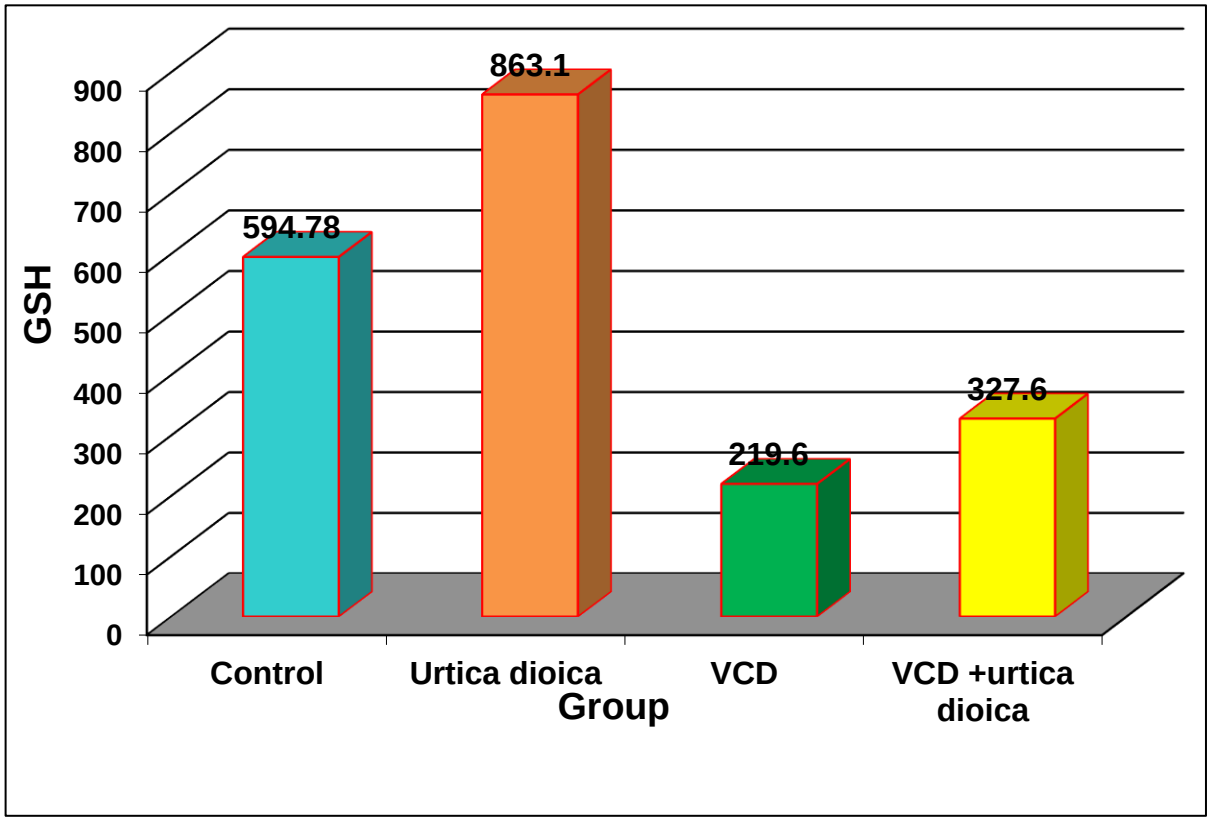


Figure (4.6) comparison of Glutathione (GSH) concentration in the control , urtica dioioca, VCD , and VCD + urtica dioica groups in female rats

GSH concentration was significantly (P≤0.05) decreased in VCD group as compare to control and urtica dioica group ,on the other hand (VCD + urtica dioioca) group had shown significantly (P≤0.05) increase in GSH concentration as compare (table4.6) to VCD group

Table (4-6): comparison of Glutathione (GSH) concentration in the control , urtica dioioca, VCD , and VCD + urtica dioica groups in female rats

Group	Means ±SE	
	GSH ( )	MDA ( )
Control	594.78 ±22.33 **b**	91.04 ±3.93 **c**
Urtica dioica	570.10 ±5.94 **a**	83.25 ±1.37 **c**
VCD	219.60 ±4.64 **d**	271.99 ±5.95 **a**
VCD +urtica dioica	327.60 ±4.65 **c**	183.24 ±3.93 **b**
L.S.D. value	37.018 *	12.733 *
Means having with the different letters in same column differed significantly. * (P≤0.05).		

Statistical of **table (4.6)** showed that levels of GSH were decresed in VCD group as compare to control and urtica dioica groups, on the other hand (VcD+ urtica dioioca) group had shown significatly increase in GSH concentration as compare to VCD group

Exposure to VCD caused a notable reduction in GSH levels, signifying heightened oxidative stress and lipid peroxidation, which are characteristic of ovarian failure and premenopause **(Song *et al*.,2023).** These alterations indicate a compromised antioxidant defence mechanism, potentially resulting from the overproduction of reactive oxygen species (ROS) during follicular depletion and hormonal dysregulation **(Alam *et al* .,2021)**

Treatment with Urtica dioica markedly reinstated GSH levels The observed effects can be ascribed to the urticia dioica's rich phytochemical profile, comprising flavonoids, carotenoids, and polyphenols, recognized for their capacity to directly neutralize free radicals and augment endogenous antioxidant enzymes **[(Devkota *etal*.,2022) (Du *etal*.,2023)]** Antioxidants and anti-

inflammatory compounds produced from urticia dioicas may mitigate glial activation, hence promoting brain health and potentially enhancing neuroendocrine signaling. The result described above corresponds to the research undertaken **(Du *etal* .,2023)** The findings indicate that the urticia dioica extract may possess a restorative or protective function against VCD-induced ovarian damage, potentially via antioxidant, anti-apoptotic, and hormone-modulating pathways. The result described above corresponds to the research undertaken **(Li *etal* .,2025)** 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 **(Carolino *etal* ., 2019)**

4.4.2. Malondialdehyde (MDA) Activity

Effect of urtica dioica , VCD and Their combination on Serum Malondialdehyde levels in female rats

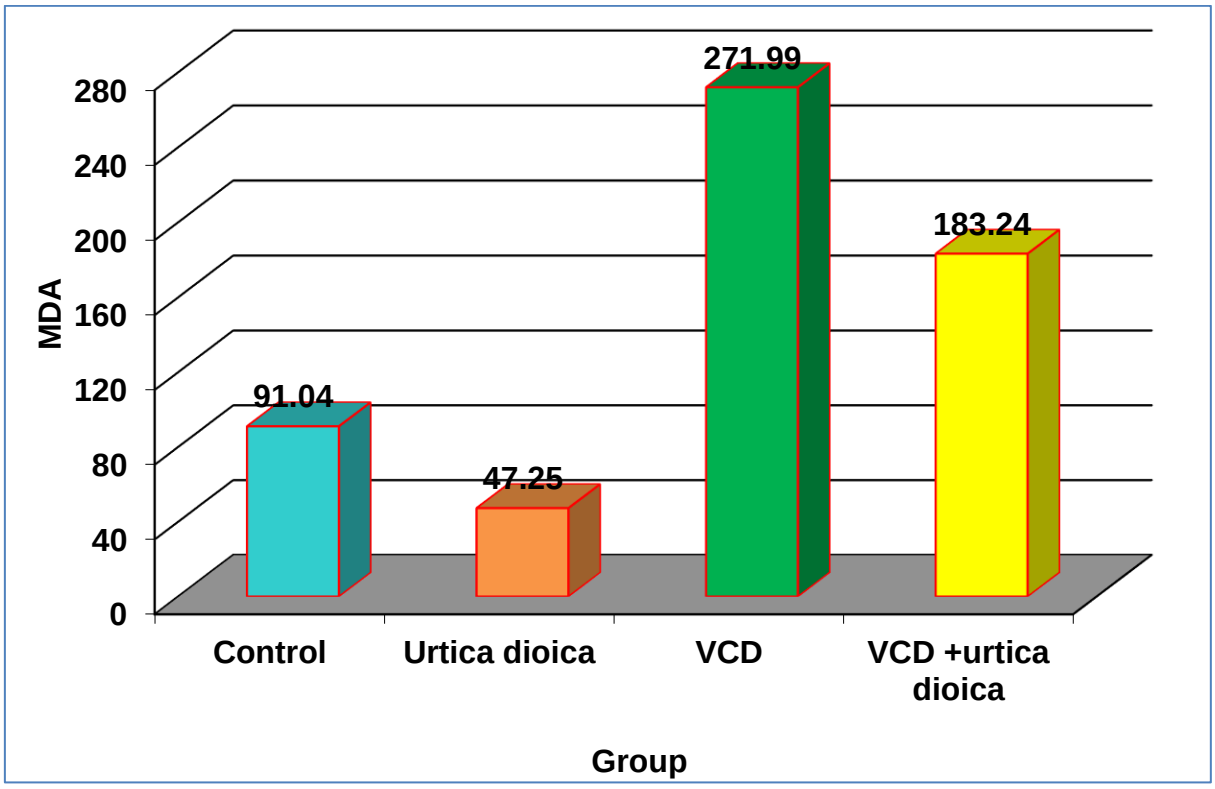


Figure (4.7) comparison of Malondialdehyde (MDA) level in the control , urtica dioioca, VCD , and VCD + urtica dioica groups in female rats

Data of MDA level is represented in **table(4.6)** significantly (P≤0.05) higher in VCD group ,as compare to control and urtica dioica groups .Also MDA levels were significantly reduced in (VCD + urtica dioioca) group as compare to VCD group

The level of MDA in **table (4-6)** were siginifcatly higher in VCD group as compare to control and urtica dioica group, while MDA levels were Significatly reduced in (VCD+ urtica dioioca) group as compere to VCD group

These findings endorse the neuroprotective capacity of the urticia dioica extract, maybe via its capability to regulate neuroinflammatory pathways and facilitate neuronal regeneration

Malondialdehyde (MDA) is an important end product of lipid peroxidation and is valued as a sensitive indicator of oxidative stress. This research demonstrated that VCD exposure significantly enhanced MDA levels, indicating increased lipid membrane breakdown, possibly due to an overabundance of reactive oxygen species (ROS). Similar elevations in MDA have been documented in models of rheumatoid arthritis and cardiovascular disease, where its levels show a close association with inflammation and tissue damage **(Yan *etal* .,2022)** The VCD-induced increase in MDA confirms its function as a measure of oxidative damage and a potential trigger of cell death and mitochondrial dysfunction in ovarian tissue. **(Timóteo-Ferreira *etal* .,2021)**

Urtica dioica treatment markedly reduced MDA amounts. The observed effects can be ascribed to the urticia dioica's abundant phytochemical constituents, including as flavonoids, carotenoids, and polyphenols, recognised for their capacity to directly neutralise free radicals and enhance endogenous antioxidant

enzyme activity **[(Devkota *etal* .,2022), (Skalska-Kamińska *etal* .,2023)]**. Comparable antioxidant activities of nettle have been previously documented in different models of reproductive and hepatic oxidative injury **(Muceniece *et al*., 2025)**

Prior research has recorded comparable antioxidant effects of nettle in diverse models of reproductive and hepatic oxidative damage Nettle's capacity to modulate redox indicators indicates its potential to preserve redox equilibrium throughout perimenopause, hence postponing tissue damage and neuroendocrine decline**(Vajic *et al*., 2024)**

4.5. Gene expression

4.5.1 Estimation of gonadotropin-releasing hormone I (GnRH-1)

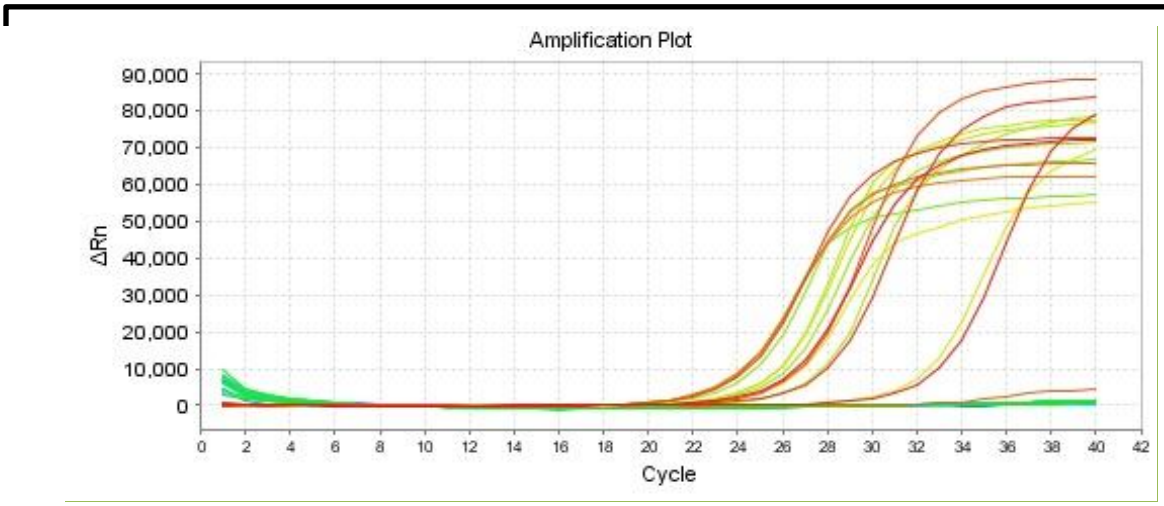


Figure (4.8) Amplification plot on (GnRH-1) gene expression (fold chang) in female rats

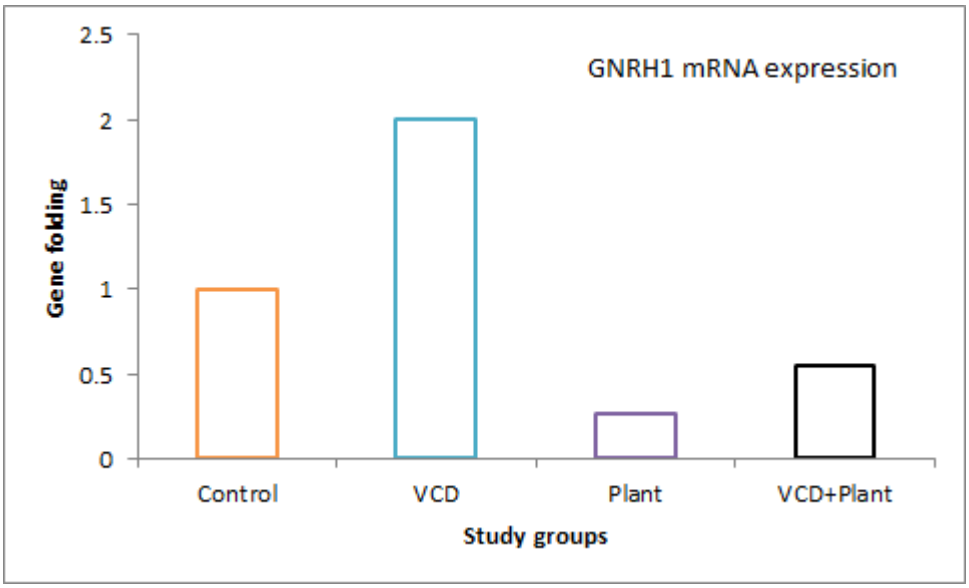


Figure (4.9) Reverse transcriptase real time (PCR)on (GnRH-1) mRNA expression in the control ,VCD, urticia dioica and VCD+ urticia dioica group in female rats

reverse transcriplase real-time PCR results shown that (GnRH-1) mRNA expression level two fold increate in VCD group compared to control group ($p \leq 0.05$) Also VCD + urtica dioica group the expression of GnRH-1 was decreased ($p \leq 0.05$) by (0.55 fold) Compared to VCD group .but urtica dioica group alone reduced the expression to (0.26 fold)

Table (4.7) Amplification plot on (GnRH-1) gene expression (fold chang) in female rats

Group	Sample	Ct (GnRH1)	Ct (Housekeeping)	ΔCt (GnRH1)
Control	1	24.5	20.2	4.3
Control	2	25.0	19.8	5.2
VCD	1	23.5	20.1	3.4
VCD	2	24.0	19.9	4.1
VCD + Urticia dioica	1	25.5	20.3	5.2
VCD + Urticia dioica	2	26.0	20.0	6.0
Urticia dioica	1	26.5	20.4	6.1
Urticia dioica	2	27.0	19.7	7.3

Gene Expression of GnRH-I Gene expression study revealed that VCD treatment markedly increased the mRNA levels of both GnRH-I, indicating hyperactivity of the hypothalamic-pituitary axis. This rise is posited to reflect a compensatory neuroendocrine response to VCD-induced ovarian follicular depletion and estrogen deficiency. **(Dai, *etal*.,2020)** The hypothalamus, due to inadequate negative feedback from ovarian hormones, increases GnRH gene transcription to boost gonadotropin output in an effort to reestablish reproductive equilibrium.

GnRH-I, the principal isoform accountable for the release of pituitary gonadotropins, exhibited a twofold elevation in expression subsequent to VCD exposure These findings corroborate prior research illustrating the estrogenic effects of nettle phytochemicals, indicating a direct influence on the hypothalamus neurons that regulate GnRH. These data indicate that nettle has a dual purpose—both an antioxidant and a neuroendocrine regulator—rendering it an effective option for alleviating menopausal symptoms and counteracting the detrimental effects of ovarian toxins such as VCD **(Moayeri *etal*., 2018).**

Collectively, these data underscore the dual role of nettle as both an antioxidant and a neuroendocrine modulator. Its capacity to restore oxidative equilibrium and normalize GnRH gene expression offers compelling evidence of its therapeutic effectiveness in addressing premenopausal diseases, including hormonal imbalance and oxidative damage **(Giannini, *et al*., 2021).** This rise may signify a compensatory reaction to reduced ovarian hormone levels, especially oestradiol, which usually provides negative feedback on the hypothalamic-pituitary-gonadal axis Nettle administration notably decreased GnRH-I indicating a possible regenerative influence on neuroendocrine signalling pathways. The fundamental process may involve reducing oxidative stress, which is recognised to influence gene transcription in the hypothalamus and neuronal excitability Nettle may restore sensitivity to hormonal feedback by diminishing reactive oxygen species and inflammation, hence regulating GnRH gene activity to healthy levels **(Şişli, *et al*., 2022)**

4.5.2. Estimation of gonadotropin-releasing hormone -II (GnRH-II)

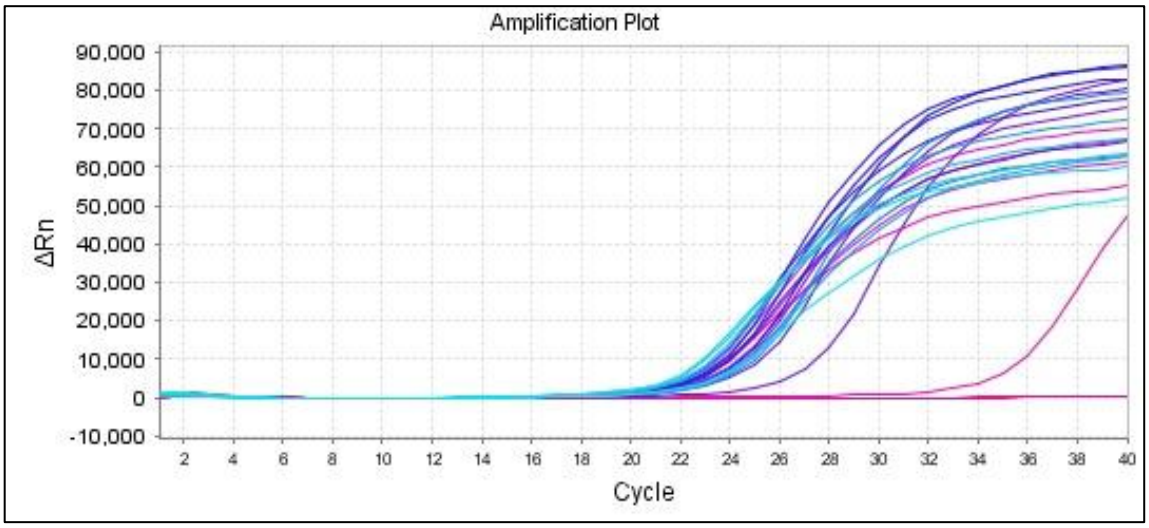


Figure (4.10) Amplification plot on (GnRH-2) gene expression (fold chang) in female rats

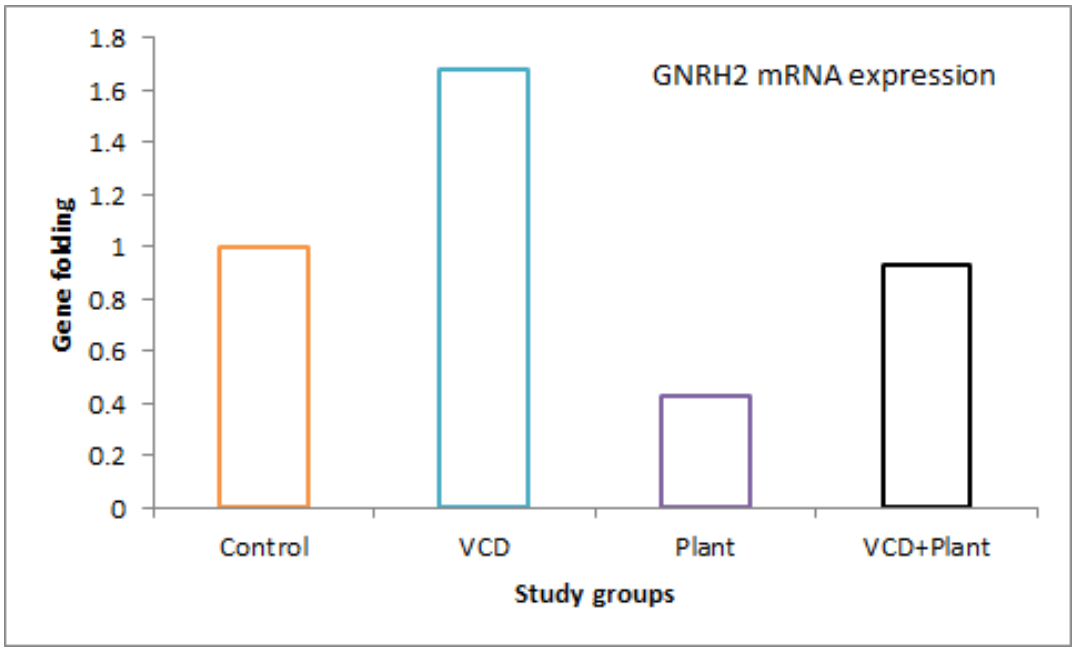


Figure (4.11) Reverse transcriptase real time (PCR)on (GnRH-2) mRNA expression in the control ,VCD, *urticia dioica* and VCD+ urticia dioica group in female rats

Reverse transcriptase real-time PCR results shown that (GnRH-II) mRNA expression level increased (1.68 fold) in VCD group compared with control group ($p \leq 0.05$)

Also in VCD + urticia dioica group the expression of GnRH-II was decreased to(0.93 fold) approaching normal levels ($p \leq 0.05$)Compared to the vCD group But urticia dioica group alone reduced the expression to(0.43 Fold)

Table (4.8) Amplification plot on (GnRH-2) gene expression (fold chang) in female rats

Group	Average ΔCt	ΔΔCt (vs. Control)	Relative Expression (2^-ΔΔCt)
Control	1.5	0	1.0
VCD	0.75	0.75 - 1.5 = -0.75	20.75=1.6820.75=1.68

VCD + urticia dioica	**1.6**	**1.6 - 1.5 = 0.1**	**2−0.1=0.932−0.1=0.93**
urticia dioica	**2.7**	**2.7 - 1.5 = 1.2**	**2−1.2=0.432−1.2=0.43**

.GnRH-II, linked to the modulation of reproductive behavior and extra-pituitary functions, exhibited a considerable rise These modifications indicate that both isoforms engage with the fluctuating hormonal milieu during perimenopause, Significantly, the combination treatment with urtica dioica markedly decreased the gene expression of both GnRH-I and GnRH-II, reinstating their levels to normalcy. This indicates that nettle may affect gene expression in the hypothalamus, maybe via its estrogenic phytochemicals that replicate estrogen's effects and partially reinstate feedback inhibition in the hypothalamic-pituitary axis **(Namazi *et al.*, 2018).** The extract alone markedly decreased the expression of the GnRH gene, hence augmenting its neurohormonal regulating functions.

Moreover, phytochemicals in nettle have demonstrated the ability to interact with oestrogen receptors and regulate gene transcription in hormone-sensitive organsThis may partially elucidate the normalisation of gene expression reported, emulating estrogen-like effects without the attendant hazards of hormone replacement treatment (HRT) **(Bandariyan *et al.*, 2021)**

Collectively, these findings validate the dual role of nettle as both an antioxidant and a neuroendocrine modulator. Its capacity to restore oxidative equilibrium and normalise GnRH gene expression offers substantial evidence of its therapeutic promise in addressing premenopausal problems, such as hormonal imbalance and oxidative damage **(Desaulniers *et al.*, 2025)**

4.6 . Histological Findings

The control group had active folliculogenesis, characterized by a normal germinal layer and ovarian stroma (Fig4.12)

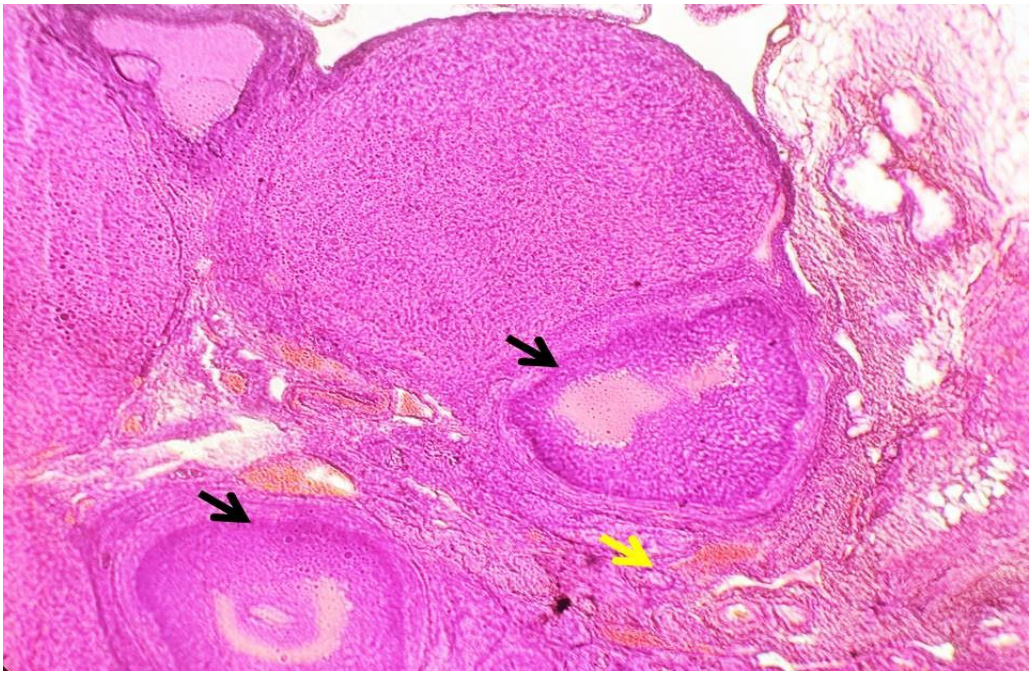


Figure (4.12): Photography of ovary (control group) show normal and appearance highly activity germinal layer. Stromal ovarian tissue appearance normal state(yellow arrow) with numerous of follico-genesis (black arrows). H&E stain. 100X

The control group demonstrated ovaries with a normal structure, characterised by an active germinal epithelium and a substantial quantity of healthy follicles at several developmental stages. This indicates the physiological integrity of the ovaries under standard hormonal management **(Orsi *et al*.,2024)**

The Urtica dioica Group had improved follicular growth and little degeneration, showing the favorable benefits of the extract alone (Fig4.13)

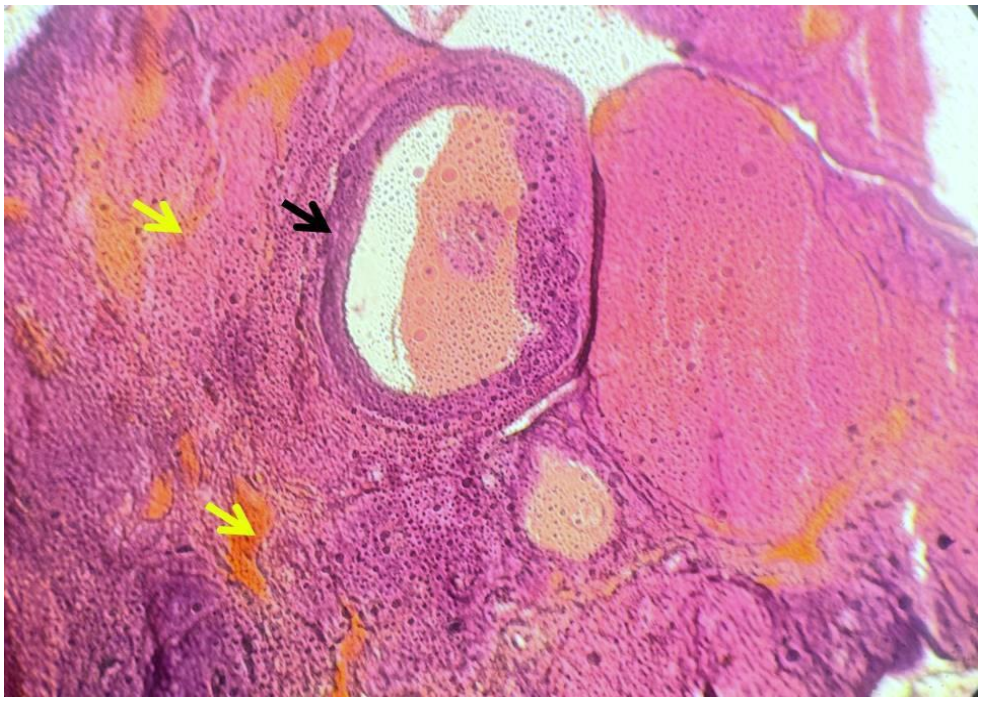
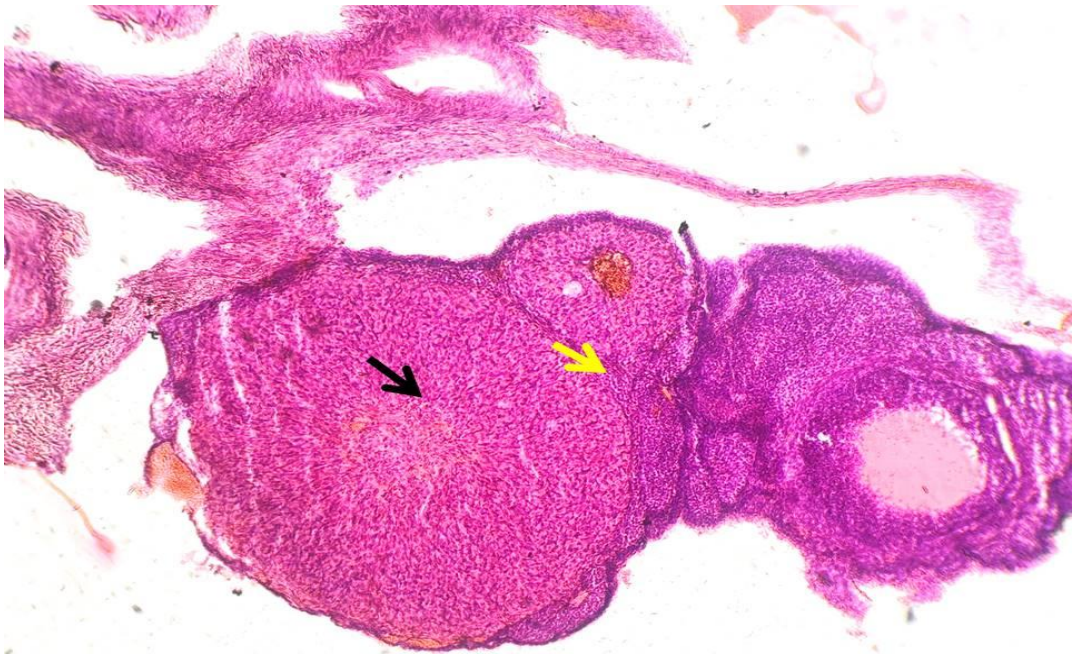


Figure (4.13): Photography of ovary (urticia dioica group) show the rise in the variety of mature follicle (black arrow) and the ovarian cortex should show well-functioning in (yellow arrows). H&E ..stain.100X

The group treated with the urticia dioica exhibited in ovarian tissue, characterised by a greater quantity of mature follicles and maintained stromal cell architecture, indicating that the urticia dioica extract may facilitate folliculogenesis and safeguard against cellular degeneration**. (Li *et al*., 2025)**

Ovaries of VCD Group: exhibited atresia, fibrotic stromal alterations, and reduced follicular count. (Fig 4.14) demonstrating perimenopausal

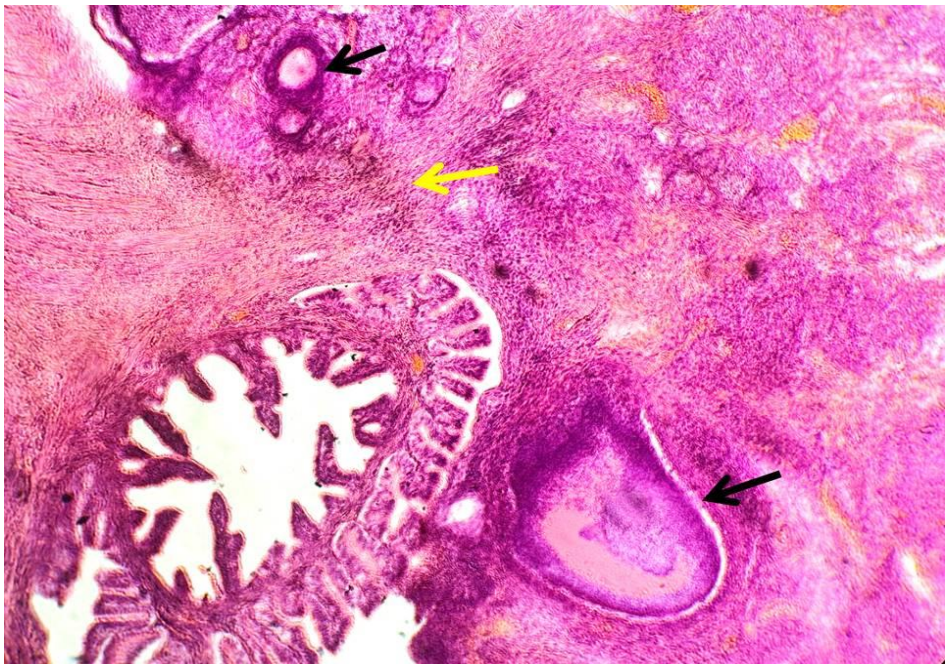


Figure(4.14): Photography of Ovary (VCD group) show which is seen as a reduction in healthy follicles(black arrow) and an increase in atretic follicles. VCD can lead to changes in the stromal cells, causing fibrosis or thickening of the ovarian stroma (yellow arrow).H&E stain.100X

The VCD-treated group demonstrated significant histological abnormalities. Ovarian tissue exhibited a significant reduction in the quantity of healthy follicles and an elevation in fibrotic follicles, together with fibrotic thickening of the connective tissue. These results are indicative of VCD-induced ovarian toxicity, which affects primordial and primary follicles, resulting in premature ovarian failure **(Niu *et al*.,2025**)

These histological alterations are indicative of chemically caused ovarian damage. (4-vinylcyclohexene diepoxide) VCD is recognized for its affinity for primordial and primary follicles, resulting in premature ovarian failure **(Ahmad *et al*.,2025)**

Histological analysis (VCD + urticia dioica Group) indicated restoration of follicular architecture, a rise in viable follicles, normalization of stroma, and evidence of corpus luteum development (Fig4. 15)



Figure(4.15): Photography of Ovary (VCD + urticia dioica group) show Increased number of mature follicles (black arrow). Normalization of ovarian stroma and architecture and Potential maintenance of corpus luteum and healthy oocyte development. H&E stain.100X

The VCD + urticia dioica group exhibited remarkable histological healing in both tissues. Ovarian sections demonstrated rejuvenated follicular growth and normalised stromal architecture This corroborates the idea that the urticia dioica extract alleviates the cytotoxic effects of VCD, potentially via the control of oxidative stress, inflammation, and apoptotic pathways **(Yuan *et al* .,2020)**

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(Fig4.16)

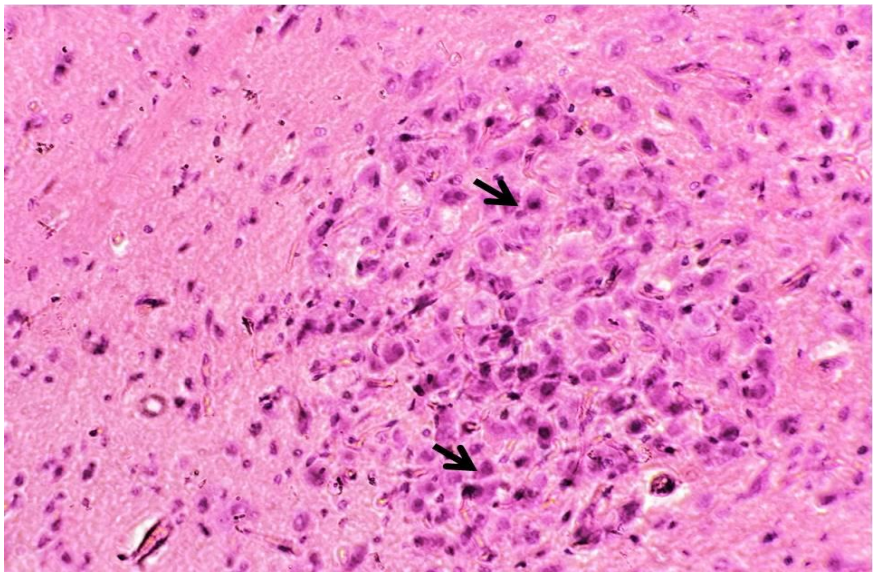


Figure (4.16): Photography of Hypothalamus (control group) show the hypothalamus would indicate a normal cellular arrangement. (black arrows).H&E stain.400X

The hypothalamus demonstrated a well-structured neuronal and glial architecture, signifying a steady neuroendocrine milieu crucial for reproductive function. **(Armocida *et al* .,2025)**

The hypothalamic tissue exhibited diminished glial activation and reactivity, decrease, indicating a decrease in neuroinflammatory processes relative to the control group(Fig4.17)

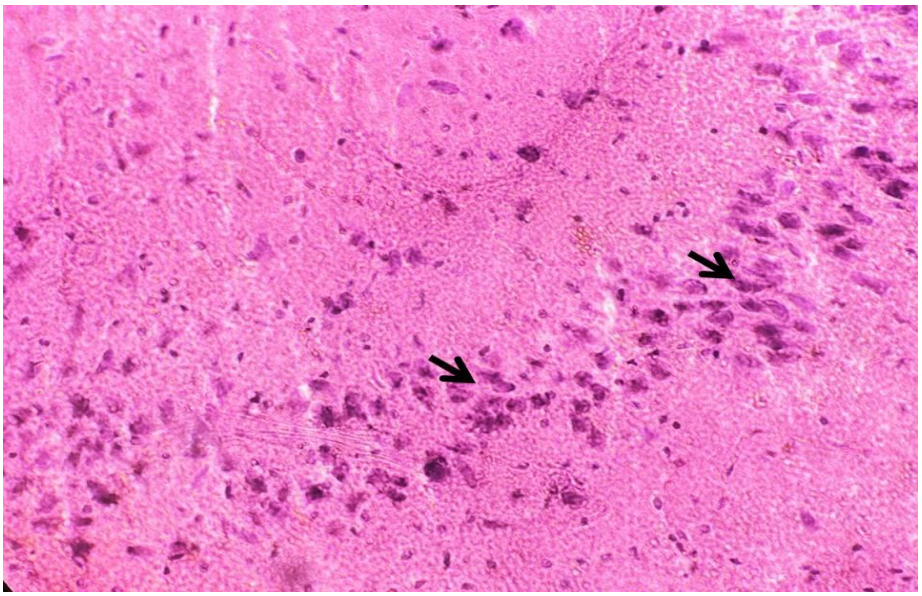
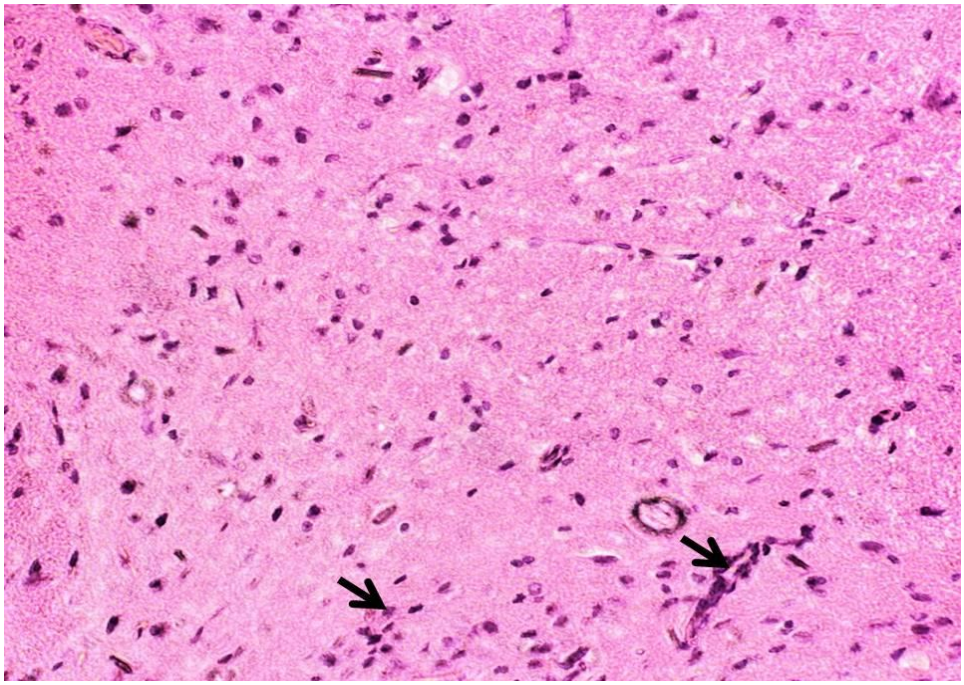


Figure (4.17): Photography of hypothalamus (urticia dioica group) show The cells in the hypothalamus and pituitary were observed to be in an almost normal state (black arrows),Urtica dioica helped maintain the structural integrity of the gland and prevented degenerative changes. H&E stain. 400X

The diminished glial reactivity and maintained neuronal integrity in the hypothalamus indicate neuroprotective and anti-inflammatory benefits, possibly attributable to the antioxidant phytochemicals in the urticia dioica extract, which support brain health and may improve neuroendocrine signalling. **(Du *et al*.,2023)**

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(Fig4.18)



Figure(4.18) 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

The hypothalamus in this cohort had neurodegeneration, glial activation, and apoptotic remains, indicating that ovarian failure may compromise hypothalamic integrity, potentially via modified feedback signals along the hypothalamic-pituitary-gonad (HPG) axis. **(Carolino *et al*.,2019)**

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(Fig4.19)

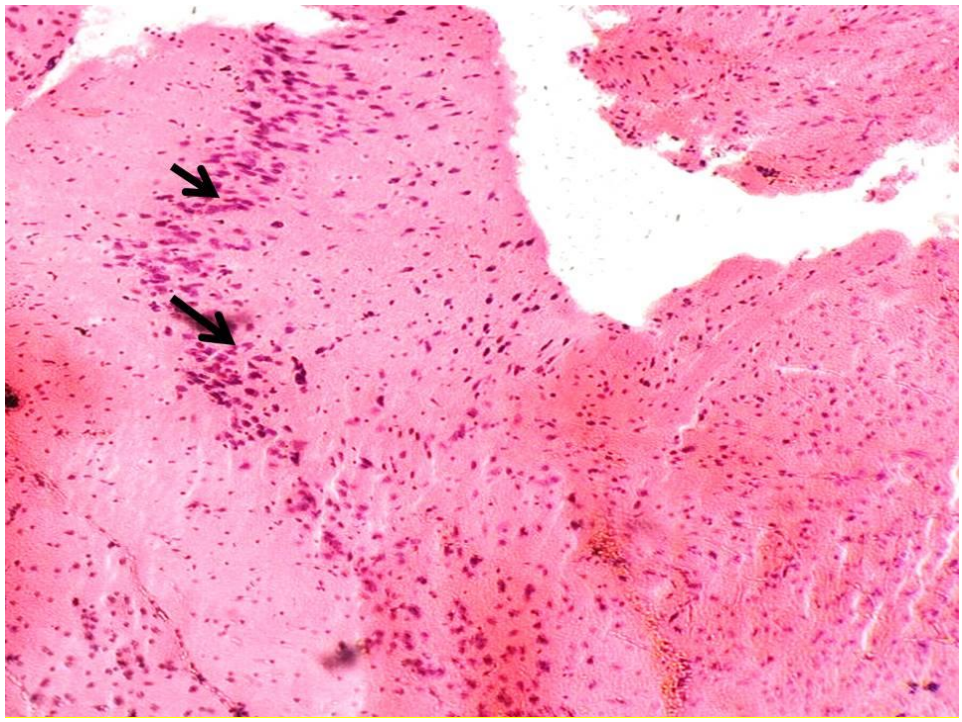


Figure (4.19): Photography of hypothalamus (VCD +Urticia dioica group) show Improved neuronal morphology, with some repair of damaged neurons Possible restoration of some normal neuronal functions and cellular architecture with Reduced glial activation compared to the VCD group/ (Black arrow).H&E stain.400X

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 **(Armocida *et al* .,2025)** In the nettle group, improved follicle growth was observed, suggesting that the urticia dioica extract may offer protective properties against VCD-induced toxicity.

Histologically, the VCD + nettle group showed improved neuronal morphology and decreased microglial activity in the hypothalamus, suggesting the potential

for restoring normal neuronal function. This finding is consistent with the findings of **(Semwal *et al* .,2023**)who documented similar neuroprotective benefits of nettle in models of oxidative stress. The decreased microglial activity suggests that nettle may be beneficial

hypothalamus tissues displayed enhanced neuronal morphology and reduced glial activation **(Yuan *et al*., 2020)**

Chapter Five
Conclusions and Recommendation

5.1. Conclusions:

I. Conclusions:

1-Urtica dioica extract ameliorated the adverse effects of VCD in female rats
2-Improved reproductive hormone balance (FSH, LH, E2, AMH, testosterone)
Reduced oxidative stress (↓ MDA, ↑ GSH)
3-Alleviated histological damage in ovary and hypothalamus
4-Modulated GnRH-I and GnRH-II gene expression, restoring levels toward .normal
5-Suggests a protective and modulatory role of Urtica dioica as a potential .natural alternative for menopausal complications
6-Further clinical studies are recommended to validate efficacy and safety.

II. Recommendations:

1. long-term studies are needed to confirm the safety and dose of Urtica dioica
2Research should explore how Urtica dioica works on estrogen receptors and hormone Balance.
3. Compare the efficacy of Urtica dioica with other herbal and conventional therapies to establish its relative therapeutic value.
4. Initiate clinical trials in women to validate the experimental findings and determine appropriate human dosages.

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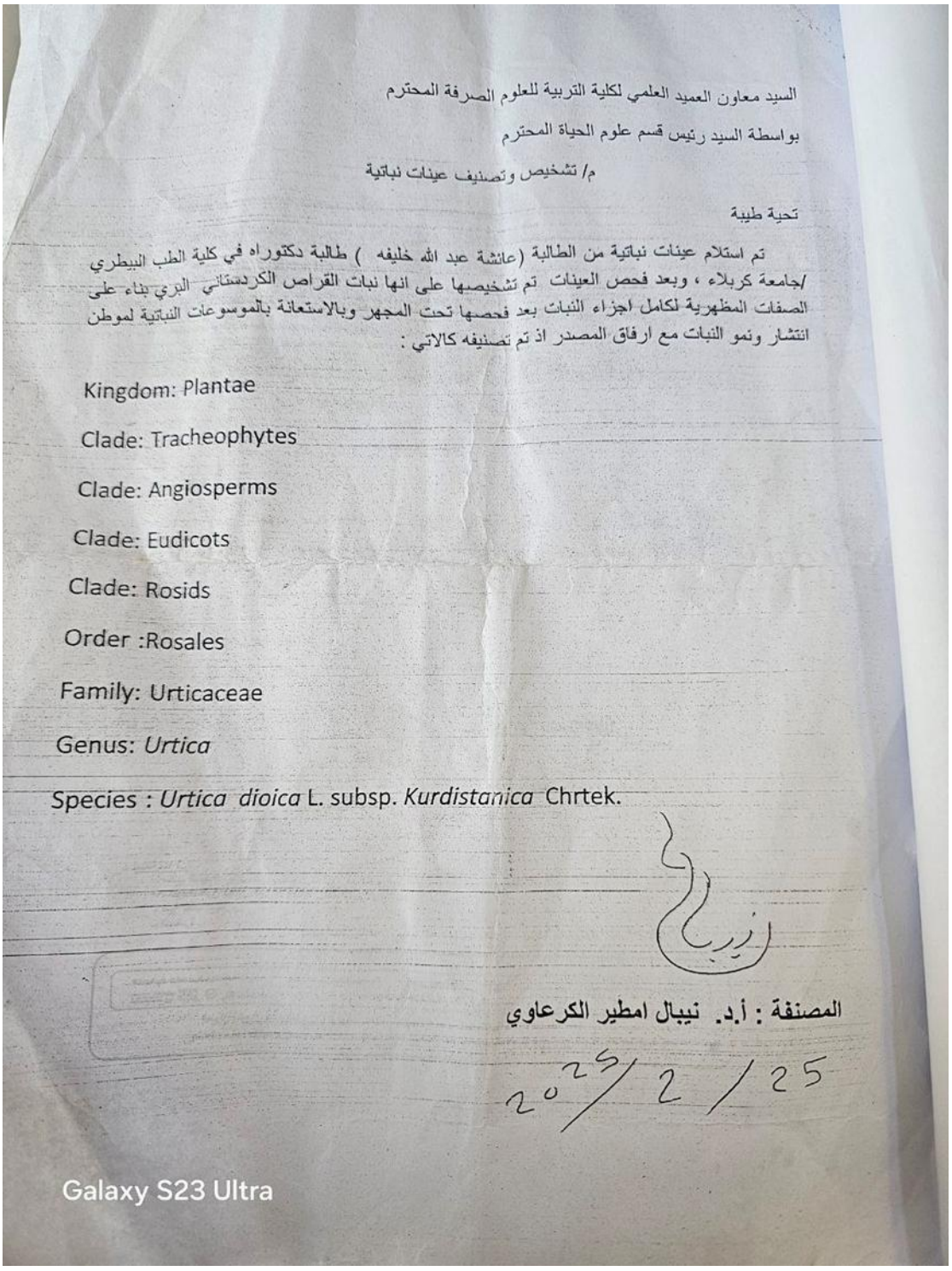
Appendix

APPENDIX I



السيد معاون العميد العلمي لكلية التربية للعلوم الصرفة المحترم

بواسطة السيد رئيس قسم علوم الحياة المحترم

م/ تشخيص وتصنيف عينات نباتية

تحية طيبة

تم استلام عينات نباتية من الطالبة (عائشة عبد الله خليفه) طالبة دكتوراه في كلية الطب البيطري /جامعة كربلاء ، وبعد فحص العينات تم تشخيصها على انها نبات القراص الكردستاني البري بناء على الصفات المظهرية لكامل اجزاء النبات بعد فحصها تحت المجهر وبالاستعانة بالموسوعات النباتية لموطن انتشار ونمو النبات مع ارفاق المصدر اذ تم تصنيفه كالاتي :

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order :Rosales

Family: Urticaceae

Genus: *Urtica*

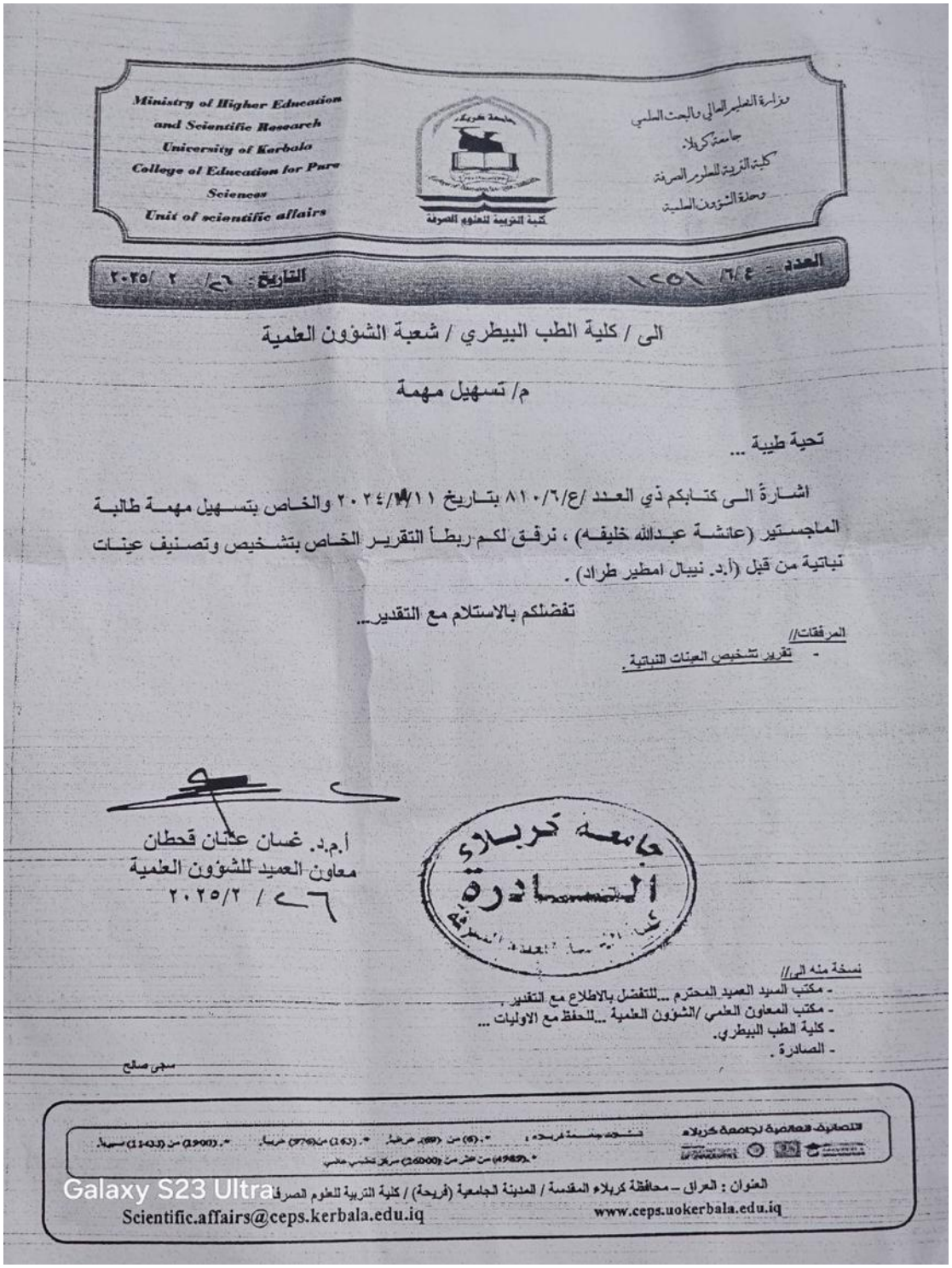
Species : *Urtica dioica* L. subsp. *Kurdistanica* Chrtek.

المصنفة : أ.د. نيبال امطير الكرعاوي

2025/2/25

Galaxy S23 Ultra

Appendix



Ministry of Higher Education and Scientific Research
University of Karbala
College of Education for Pure Sciences
Unit of scientific affairs

وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية التربية للعلوم الصرفة
وحدة الشؤون العلمية

كلية التربية للعلوم الصرفة

العدد: ٤/٦/ ٨٥٦ / ٢

التاريخ: ٢٦/ ٢ /٢٠٢٥

الى / كلية الطب البيطري / شعبة الشؤون العلمية

م/ تسهيل مهمة

تحية طيبة ...

اشارةً الى كتابكم ذي العدد /ع/٦/٨١٠ بتاريخ ١١/١٢/٢٠٢٤ والخاص بتسهيل مهمة طالبة الماجستير (عائشة عبدالله خليفه) ، نرفق لكم ربطاً التقرير الخاص بتشخيص وتصنيف عينات نباتية من قبل (أ.د. نيبال امطير طراد) .

تفضلكم بالاستلام مع التقدير...

المرفقات//
- تقرير تشخيص العينات النباتية.

أ.م.د. غسان عدنان قحطان
معاون العميد للشؤون العلمية
٢٠٢٥/٢ / ٢٦

جامعة كربلاء
الصادرة

نسخة منه الى//
- مكتب السيد العميد المحترم ...للتفضل بالاطلاع مع التقدير .
- مكتب المعاون العلمي /الشؤون العلمية ...للحفظ مع الاوليات ...
- كلية الطب البيطري.
- الصادرة .

يتبع صفحة

العنوان : العراق – محافظة كربلاء المقدسة / المدينة الجامعية (فريحة) / كلية التربية للعلوم الصرفة
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APPENDIX II

Kit for Testosterone ELISA

Type of Sample: Serum

Principle:

The sample's testosterone competes with a predetermined quantity of enzyme-labeled testosterone for binding to particular antibodies. The amount of testosterone present is inversely correlated with the colour intensity.

Procedure:

1. Each well received 50 µL of the sample or standard.

2. The plate was incubated after 50 µL of HRP-conjugated testosterone was added.

3. Five washings were performed.

4. After adding and incubating the TMB substrate, it was halted.

5. At 450 nm, the optical density was recorded.

Calculation:

Inverse correlation was utilised to determine testosterone levels using a standard curve. The results were given in ng/mL.

APPENDIX III

ELISA Kit for Follicle Stimulating Hormone (FSH)

Type of Sample: Serum

Principle:

The quantitative detection of FSH in rat serum is the purpose of this sandwich ELISA kit. The microplate wells are previously coated with an FSH-specific monoclonal antibody. An HRP-labeled secondary antibody subsequently detects the target FSH in the sample once it binds to the immobilised antibody. A colour shift that is proportionate to the amount of FSH present in the sample is the outcome of the enzymatic reaction with the TMB substrate.

Procedure:

1. The reagents and samples were brought to room temperature.

2. Each well received 50 µL of the standard solution or serum sample.

3. After adding 50 µL of HRP-conjugate, the plate was incubated for 60 minutes at 37°C.

4. Wash buffer was used to wash each well five times.

5. 50 µL of each of the chromogens A and B were added.

6. The plate was incubated for 15 minutes at 37°C in the dark.

7. A 50 µL stop solution was used to halt the reaction.

8. Within ten minutes, the absorbance at 450 nm was measured.

Calculation:

A standard curve was created that plotted the OD against the FSH concentration. The 4PL regression approach was used to compute the sample concentrations, which were then reported in ng/mL

APPENDIX IV

Luteinizing Hormone (LH) ELISA Kit

Type of Sample: Serum

Principle:

The double-antibody sandwich ELISA technique is used in this experiment. The LH in the serum sample binds to a microtiter plate that has been pre-coated with a monoclonal antibody specific to rat LH. To produce an immunological combination, streptavidin-HRP and a biotinylated detecting antibody are gradually added. after the TMB substrate is added, a blue tint appears, which changes to yellow after the reaction stops. The colour intensity, which is measured at 450 nm, is directly proportional to the LH content.

Procedure:

1- Every sample and reagent was brought up to room temperature.

2- Each well received fifty microlitres of the standard or serum sample. 3- 50 µL of HRP-conjugated reagent was added right away.

4- For one hour, the plate was incubated at 37°C.

5- The provided wash buffer was used five times to rinse five wells.

6- Each well received 50 µL of Chromogen A and 50 µL of Chromogen B

7- After that, incubation took place for 15 minutes at 37°C without light.

8- Each well received fifty microlitres of stop solution.

9- In ten minutes, optical density was measured at 450 nm using a microplate reader.

Calculation:

Using the known concentrations of LH standards included in the kit, a standard curve was created. To determine the LH concentration, the optical density measurements of the samples were compared to the curve. Nanogrammes per millilitre (ng/mL) were used to display the results. Every sample was measured twice, and the final computation used the mean optical density value. A logistic curve fitting method with four parameters was used.

APPENDIX V

ELISA Kit for Estradiol (E2)

Type of Sample: Serum

Principle :

The assay's basic idea is to use a particular antibody that has been coated onto microplate wells to bind the oestradiol in the sample. An immunological complex is created when the bound hormone interacts with a secondary enzyme-linked antibody. When TMB substrate is introduced, the sample's E2 level determines how much colour develops.

Procedure:

1. Each well was pipetted with 50 µL of the standard or serum sample.

2. After adding 50 µL of HRP-conjugated reagent, the mixture was

incubated for 60 minutes at 37°C.

3. The plates were properly cleaned five times.

4. Chromogen A and B were added in 50 µL each.

5. After that, it was incubated for 15 minutes at 37°C in the dark.

6. The stop solution was used to end the reaction.

7. At 450 nm, the OD was measured.

Calculation:

Using the standard curve, the concentration of oestradiol was calculated and expressed in pg/mL

APPENDIX IV

ELISA Kit for Anti-Müllerian Hormone (AMH)

Type of Sample: Serum

Principle:

A double-antibody sandwich ELISA was used to assess AMH. AMH-specific capture antibody and a second HRP-labeled detection antibody are used in the kit. The chromogenic substrate was TMB.

Procedure:

The same as previously described (typical sandwich ELISA format). The manufacturer's instructions were followed for incubation, washing, and detection.

Calculation:

To find the sample concentration in ng/mL, standard curves were created

APPENDIX V

ELISA Kit for Malondialdehyde (MDA)

Type of Sample: Serum

Principle:

Competitive ELISA serves as the foundation for the MDA assay. The sample's MDA and HRP-labeled MDA fight with one another to attach to particular antibodies coated on the plate. The MDA concentration has an inverse relationship with colour intensity.

Procedure:

Competitive binding and colour development at 450 nm were applied to the samples and reagents in accordance with the kit's instructions.

Calculation:

By comparing absorbance results to a standard curve, sample MDA levels were determined and reported in pg/mL.

APPENDIX VI

Assay Kit for Glutathione Peroxidase (GSH)

Type of Sample: Serum

Principle:

The capacity of decreased GSH to react with DTNB (Ellman's reagent) to create a yellow-colored chemical (TNB) that can be detected at 412 nm is the basis for this colorimetric assay. The color's intensity indicates the amount of GSH

present. Method: Working reagent was added to serum samples, which were then incubated and their absorbance was determined using spectrophotometry.

Calculation:

GSH levels were expressed in µmol/L and were ascertained by comparing them to a standard curve

.

APPENDIX VII

GnRH-I, or gonadotropin-releasing hormone I

Expression of Genes

Sample Type: Tissue from the hypothalamus preserved in TRIzol reagent

Principle:

Real-time quantitative PCR (qRT-PCR) was used to assess GnRH-I gene expression. TRIzol reagent was used to extract total RNA from hypothalamus tissue samples, and complementary DNA (cDNA) was then created by reverse transcription.

Procedure:

The amplification procedure made use of certain primers that target the GnRH-I gene. The intercalating dye utilised was SYBR Green, and a thermal cycler was used to measure amplification in real time.

Initial Sequences:

5′-GACTGTGTGTTTGGAAGGCTGC-3′ is the forward (F)

5′-CCATTTGATCCTCCTCCTTGC-3′ is the reverse (R).

Appendix

Size of Product: 101 base pairs (bp)

Summary of the Procedure:

1. In accordance with the manufacturer's instructions, hypothalamic tissues were homogenised in TRIzol reagent and RNA was extracted

. 2. A Nanodrop spectrophotometer was used to measure the concentration and purity of RNA.

3. To create cDNA, reverse transcription was carried out using [insert kit name].

4. Gene-specific primers and SYBR Green Master Mix were used for qRT-PCR.

5. To maximise primer efficiency, amplification was carried out using a temperature profile that included denaturation, annealing, and extension cycles.

Calculation:

Using the 2^–ΔΔCt method, normalisation to a housekeeping gene (such as GAPDH or β-actin) was used to determine the relative expression level of GnRH-I. Every sample was performed three times, and [insert software name] was used to statistically analyse the findings .

APPENDIX IX

GnRH-II, or gonadotropin-releasing hormone II

Expression of Genes

Sample Type: Tissue from the hypothalamus preserved in TRIzol reagent

Principle:

After extracting total RNA from rat hypothalamus tissue, GnRH-II mRNA expression was measured using real-time PCR. Following cDNA synthesis, qPCR was carried out with GnRH-II gene-specific primers, and SYBR Green fluorescence was used to detect amplification. For relative quantification, the expression level was normalised against a reference gene.

Initial Sequences:

5′-TCCTTTCTGGGGGGCATTTAG-3′ is the forward (F)

5′-GCTCTAGGTCATCCCTAACTTG-3′ is the reverse (R).

94 base pairs (bp) make up the product size.

Summary of the Procedure:

1. TRIzol reagent was used to extract total RNA, and spectrophotometric analysis was used to determine purity.

2. High-quality cDNA was produced through reverse transcription utilising [insert kit name].

3. GnRH-II primers and SYBR Green Master Mix were used to set up the qPCR experiment.

4. Forty cycles with an ideal annealing temperature were part of the amplification requirements.

5. To verify the amplification's specificity, melt curve analysis was employed.

Calculation:

The 2^–ΔΔCt method was used to evaluate relative gene expression. Fold changes were computed in relation to control group expression levels by comparing the Ct values of GnRH-II to the internal control gene.

APPENDIX X

Histological examination

At the conclusion of the experimental period, the animals were sedated via intramuscular injection of ketamine (75 mg/kg) and xylazine (10 mg/kg). After administering anesthetic, a midline abdominal incision was executed to reach the abdominal cavity, and the ovaries were meticulously removed. The skull was opened to reveal and isolate the hypothalamus region of the brain.

The harvested ovarian and hypothalamus tissues were promptly washed in normal saline to eliminate blood and debris, thereafter fixed in 10% neutral buffered formalin for 48 hours at ambient temperature.

Tissues were subsequently dehydrated using a graded series of ethanol (70%, 80%, 90%, and 100%), cleaned with xylene, and embedded in paraffin wax.

Paraffin blocks were subsequently sectioned to a thickness of 5 µm utilizing a rotary microtome. The tissue sections were affixed to glass slides, desiccated

in an incubator at 37°C, and stained with hematoxylin and eosin (H&E) for standard histological analysis.

The microscopic assessment concentrated on:

1-Quantification and morphology of developing

2- atretic follicles in the ovary Integrity of the ovarian

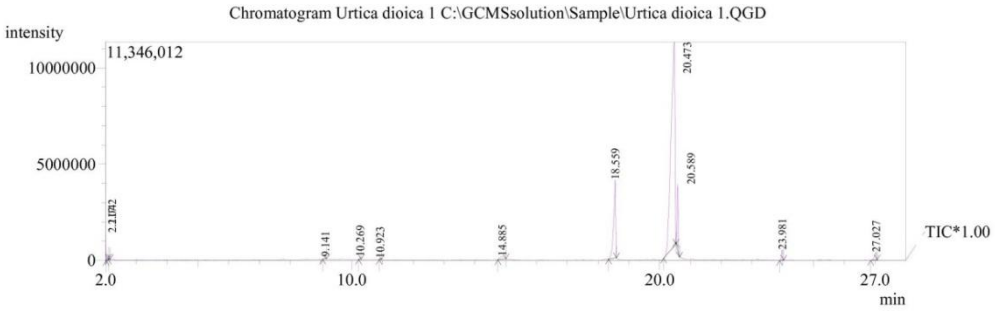
3- cortex and stromal architecture

4- Neuronal organization and glial cell localization inside the hypothalamus

APPENDIX XI

Gas chromatography Mass Analysis (GCMS)

GCMS Analysis Results
مركز بحوث البيئة والمياه
قسم البحوث البيئية
Esam



Peak Report TIC

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2	2.117	2.092	2.150	313183	0.25	234752	1.19	1.34	MI	
3	9.141	9.058	9.175	354435	0.28	104009	0.53	3.40	MI	
4	10.269	10.225	10.308	251442	0.20	119287	0.60	2.10	MI	
5	10.923	10.892	10.967	114990	0.09	60181	0.30	1.92	MI	
6	14.885	14.750	15.008	1495614	1.19	152280	0.77	9.75	MI	
7	18.559	18.342	18.600	18167885	14.47	4064297	20.53	4.47	MI	
8	20.473	20.133	20.533	94467245	75.26	10604079	53.56	8.90	MI	
9	20.589	20.525	20.650	7173645	5.71	3393685	17.14	2.12	MI	
10	23.981	23.900	24.033	1273740	1.01	534603	2.70	2.38	MI	
11	27.027	26.858	27.067	1633456	1.30	396325	2.00	4.11	MI	
				125527259	100.00	19797709	100.00			

Library
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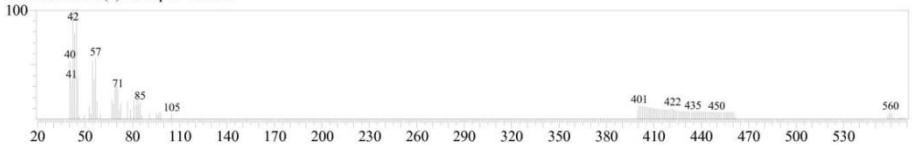

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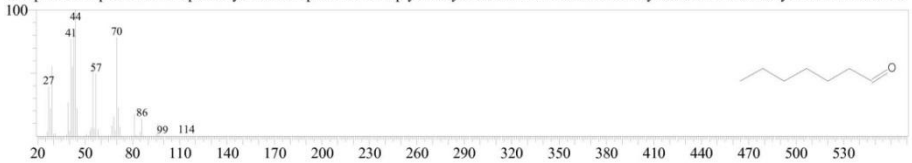

Appendix

<< Target >>
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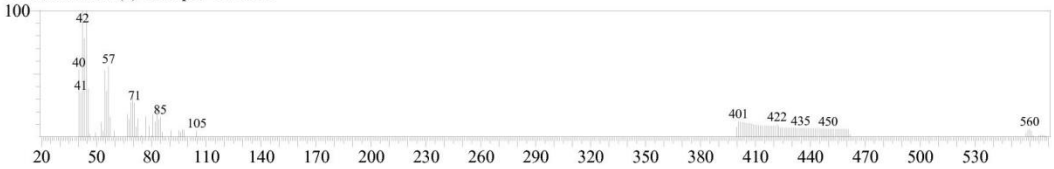
BG Mode:2.017(3) Group 1 - Event 1



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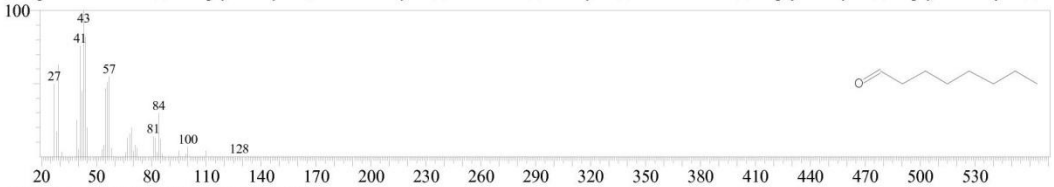

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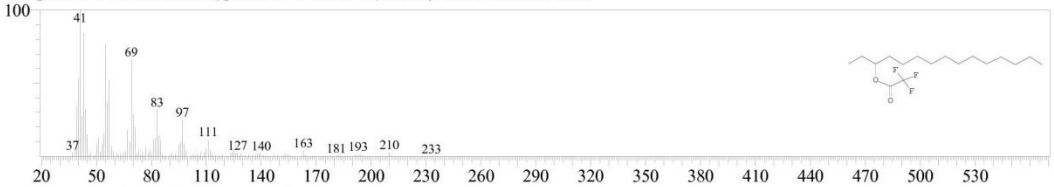

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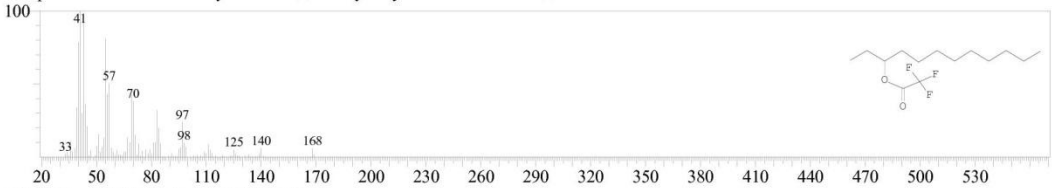

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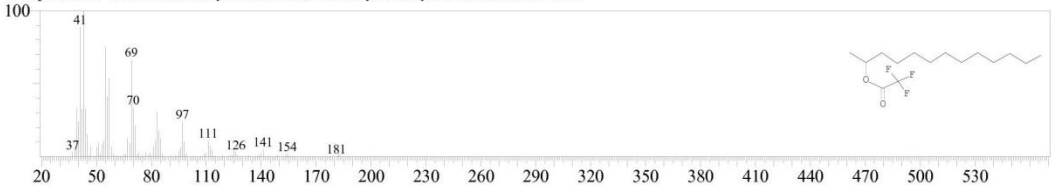

Appendix

<< Target >>
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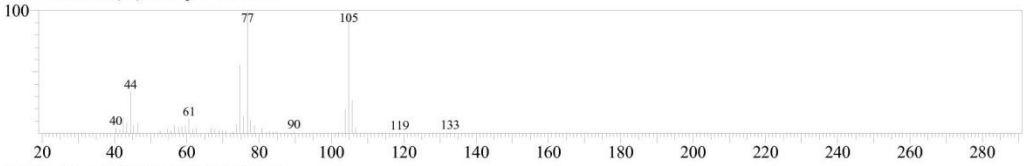

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SI:75 Formula:C4H12O2Si CAS:1112-39-6 MolWeight:120 RetIndex:481
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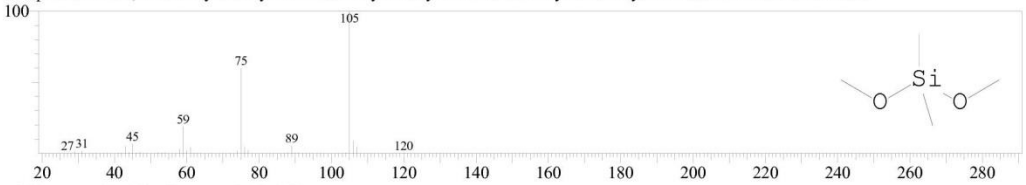

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SI:74 Formula:C13H13N5OS CAS:0-00-0 MolWeight:287 RetIndex:2530
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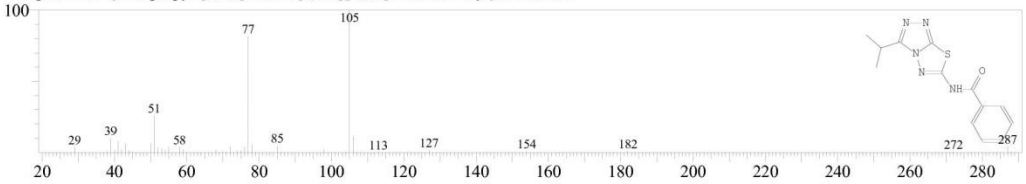

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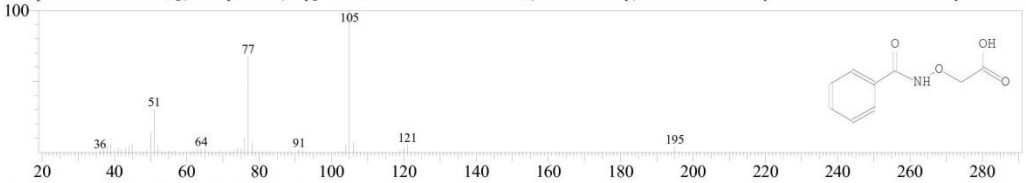
CompName:Acetic acid, [(benzoylamino)oxy]- $$ Benzadox $$ Acetic acid, (benzamidooxy)- $$ Benzamidooxyacetic acid $$ Benzamidoxyacetic ac



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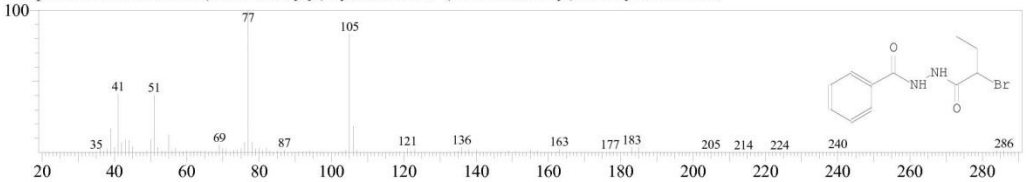

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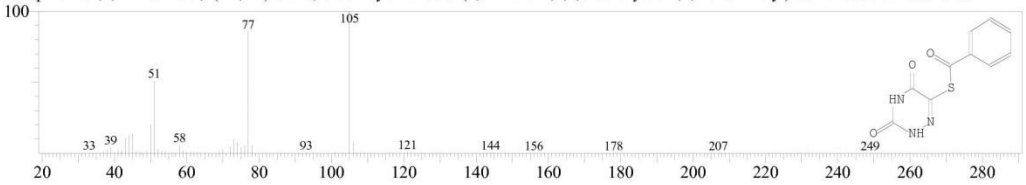
SI:73 Formula:C10H7N3O3S CAS:24168-34-1 MolWeight:249 RetIndex:2215
CompName:1,2,4-Triazine-3,5(2H,4H)-dione, 6-benzoylthio- $$ S-(3,5-Dioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl) benzenecarbothioate # $$



Appendix

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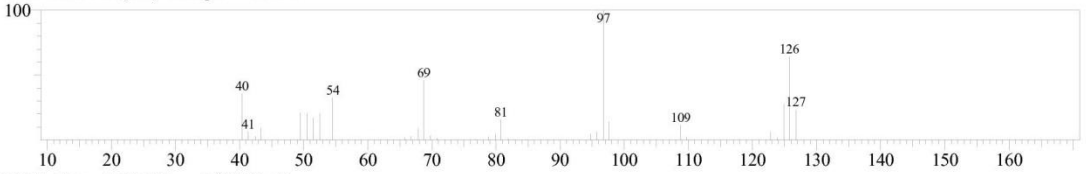

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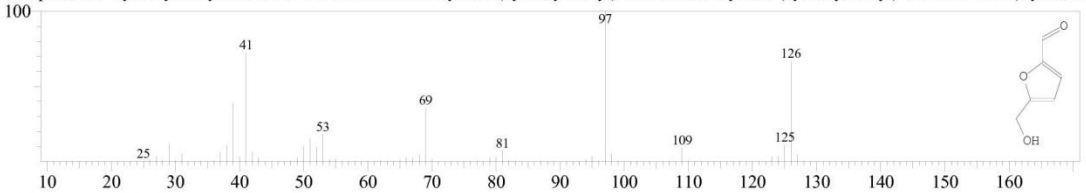

Hit#:2 Entry:6929 Library:NIST20-1.lib
SI:78 Formula:C6H10N2O CAS:63634-44-6 MolWeight:126 RetIndex:1147
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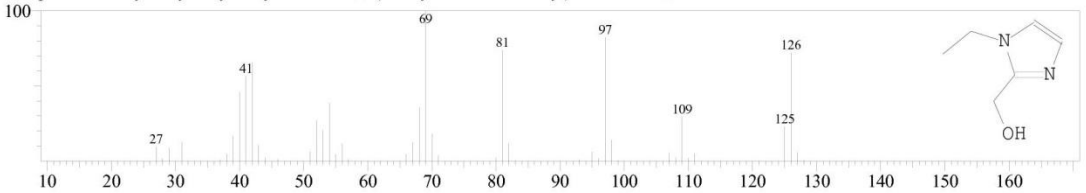

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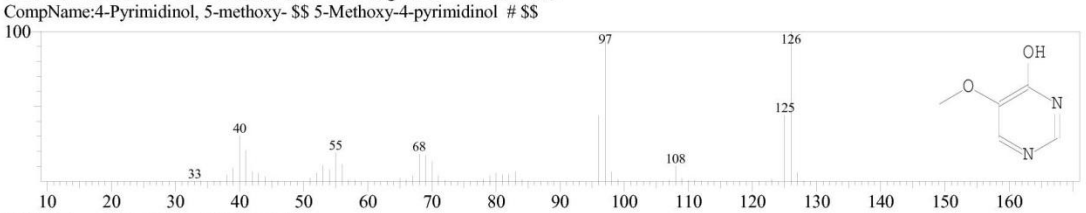

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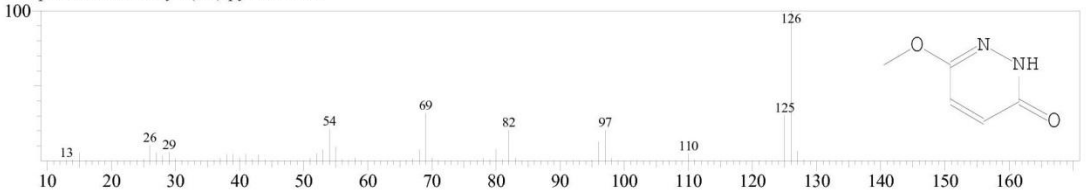

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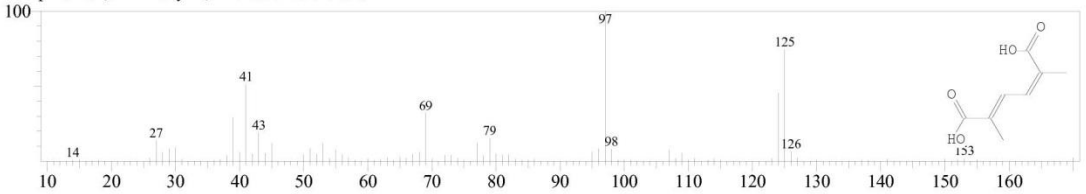

Appendix

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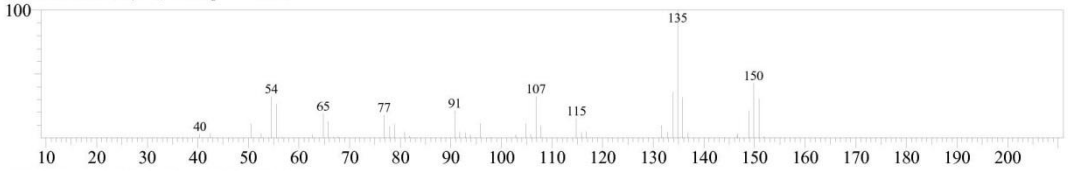

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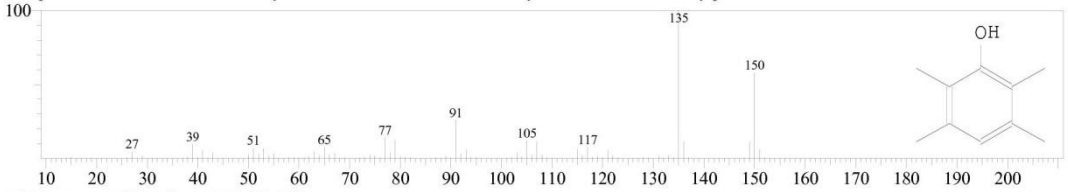

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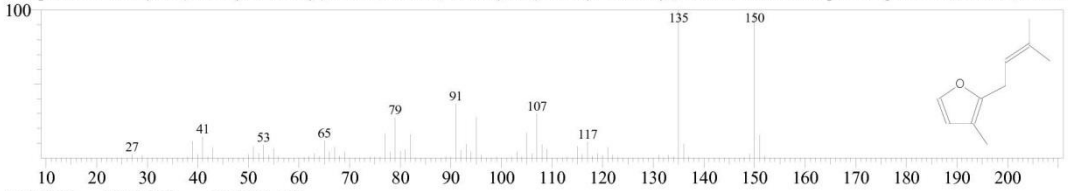

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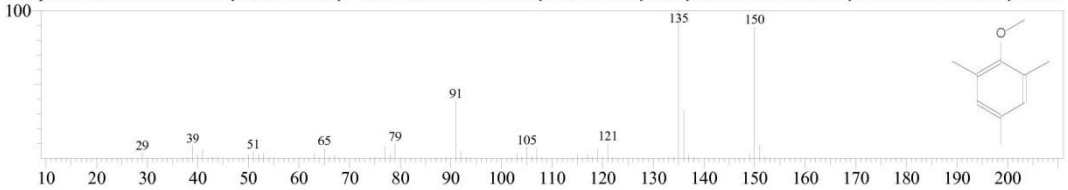

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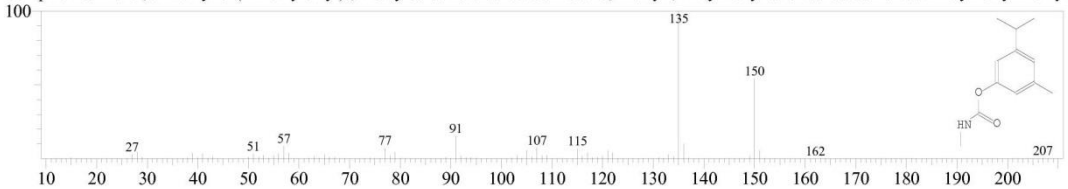
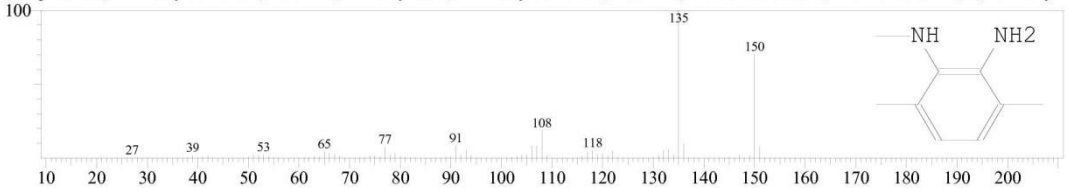
SI:71 Formula:C12H17NO2 CAS:2631-37-0 MolWeight:207 RetIndex:1620
CompName:Phenol, 3-methyl-5-(1-methylethyl)-, methylcarbamate $$ Carbamic acid, methyl-, m-cym-5-yl ester $$ Carbamult $$ m-Cym-5-yl methy

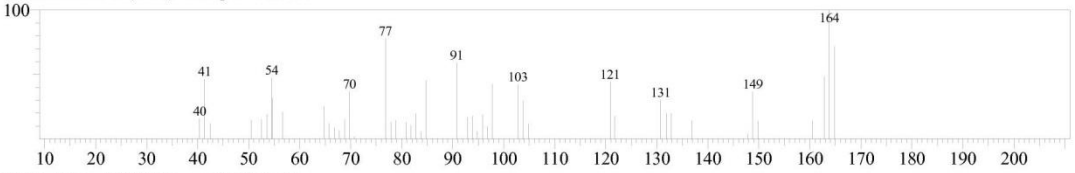


Hit#:5 Entry:17055 Library:NIST20-1.lib
SI:71 Formula:C9H14N2 CAS:0-00-0 MolWeight:150 RetIndex:1530
CompName:3,6-Dimethylbenzene-1,2-diamine, N-methyl $$ 3,6-Dimethylbenzene-1,2-diamine, Me derivative $$ 1,2-Benzenediamine, 3,6-dimethyl-

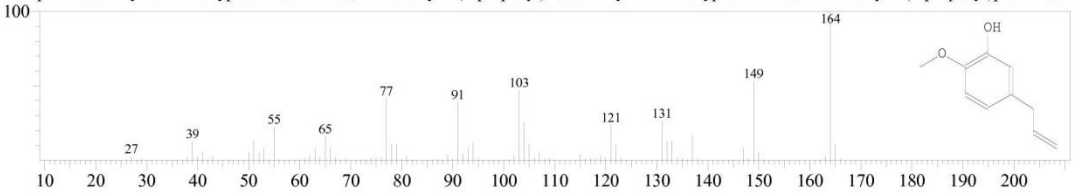


Appendix

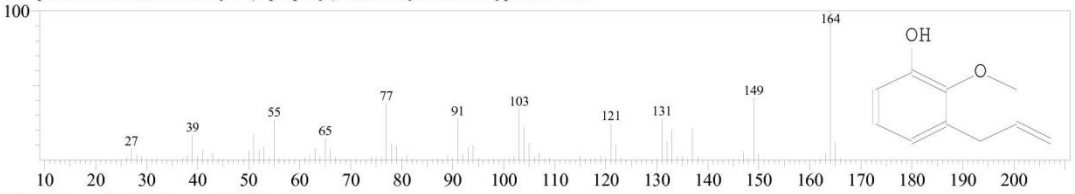
<< Target >>
Line#:5 R.Time:10.925(Scan#:1072) MassPeaks:47
RawMode:Averaged 10.875-10.983(1066-1079) BasePeak:163.80(1984)
BG Mode:10.975(1078) Group 1 - Event 1



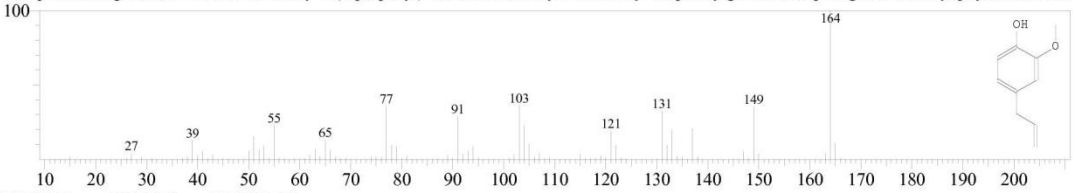
Hit#:1 Entry:14640 Library:NIST20s.lib
SI:69 Formula:C10H12O2 CAS:501-19-9 MolWeight:164 RetIndex:1392
CompName:3-Allyl-6-methoxyphenol $$ Phenol, 2-methoxy-5-(2-propenyl)- $$ 5-Allyl-2-methoxyphenol # $$ 2-Methoxy-5-(2-propenyl)phenol $$



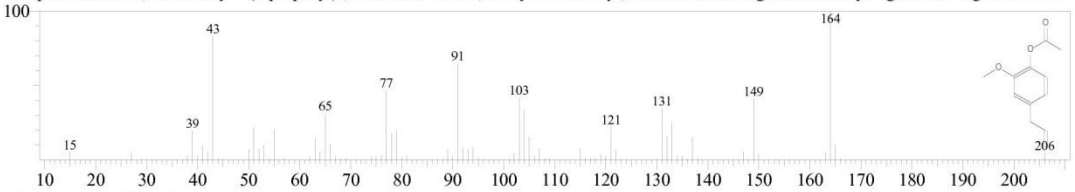
Hit#:2 Entry:25439 Library:NIST20-1.lib
SI:68 Formula:C10H12O2 CAS:1941-12-4 MolWeight:164 RetIndex:1392
CompName:Phenol, 2-methoxy-3-(2-propenyl)- $$ 3-Allyl-2-methoxyphenol # $$



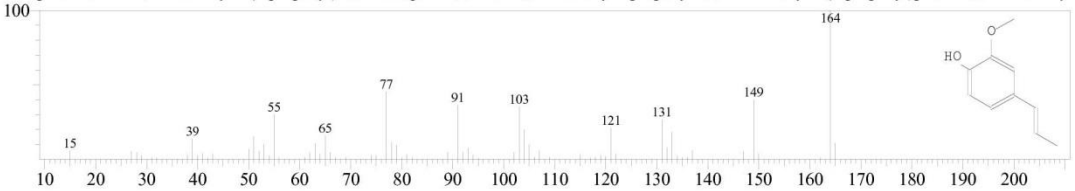
Hit#:3 Entry:25430 Library:NIST20-1.lib
SI:67 Formula:C10H12O2 CAS:97-53-0 MolWeight:164 RetIndex:1392
CompName:Eugenol $$ Phenol, 2-methoxy-4-(2-propenyl)- $$ Phenol, 4-allyl-2-methoxy- $$ p-Allylguaiacol $$ p-Eugenol $$ Caryophyllic acid $$



Hit#:4 Entry:23995 Library:NIST20s.lib
SI:67 Formula:C12H14O3 CAS:93-28-7 MolWeight:206 RetIndex:1552
CompName:Phenol, 2-methoxy-4-(2-propenyl)-, acetate $$ Phenol, 4-allyl-2-methoxy-, acetate $$ Aceteugenol $$ Acetyleugenol $$ Eugenol acetate

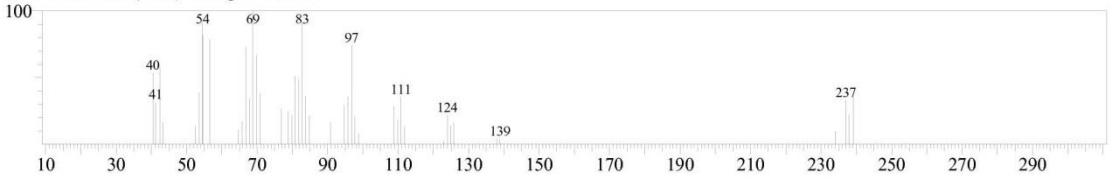


Hit#:5 Entry:25426 Library:NIST20-1.lib
SI:67 Formula:C10H12O2 CAS:97-54-1 MolWeight:164 RetIndex:1410
CompName:Phenol, 2-methoxy-4-(1-propenyl)- $$ Isoeugenol $$ Phenol, 2-methoxy-4-propenyl- $$ 2-Methoxy-4-(1-propenyl)phenol $$ 2-Methoxy

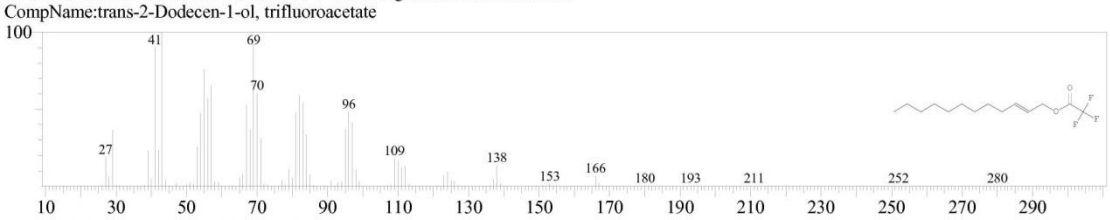


Appendix

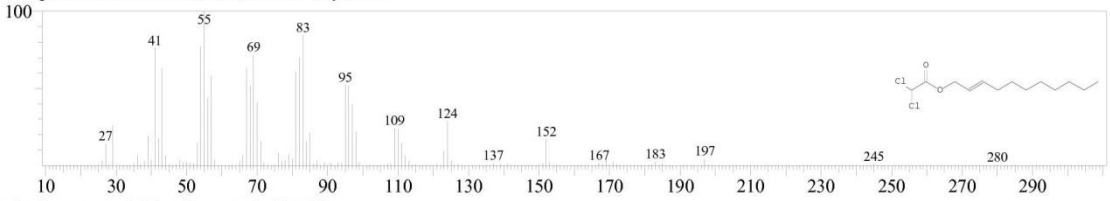
<< Target >>
Line#:6 R.Time:14.883(Scan#:1547) MassPeaks:44
RawMode:Averaged 14.733-15.025(1529-1564) BasePeak:68.80(4672)
BG Mode:15.017(1563) Group 1 - Event 1



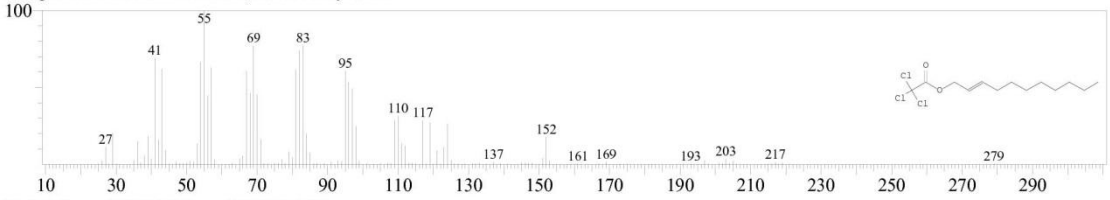
Hit#:1 Entry:139002 Library:NIST20-1.lib
SI:82 Formula:C14H23F3O2 CAS:0-00-0 MolWeight:280 RetIndex:1422
CompName:trans-2-Dodecen-1-ol, trifluoroacetate



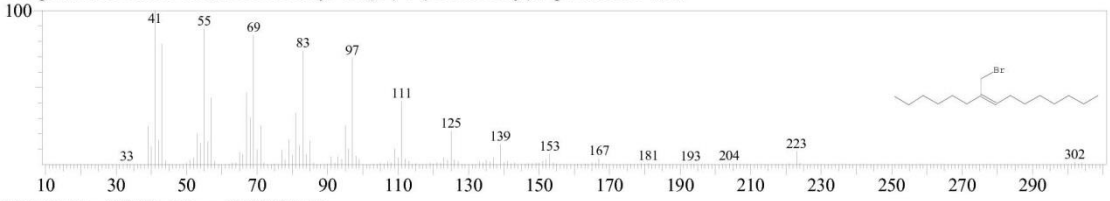
Hit#:2 Entry:138849 Library:NIST20-1.lib
SI:80 Formula:C13H22Cl2O2 CAS:0-00-0 MolWeight:280 RetIndex:1834
CompName:Dichloroacetic acid, undec-2-enyl ester



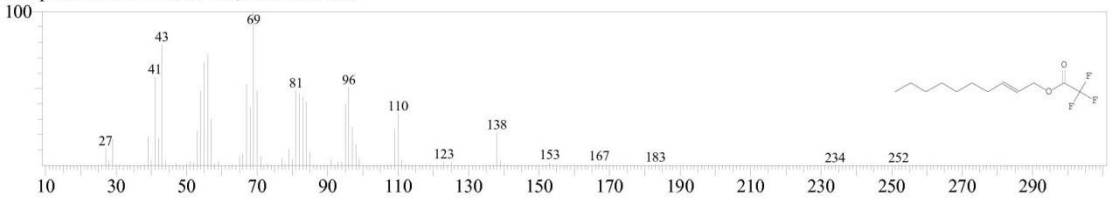
Hit#:3 Entry:179418 Library:NIST20-1.lib
SI:79 Formula:C13H21Cl3O2 CAS:0-00-0 MolWeight:314 RetIndex:1876
CompName:Trichloroacetic acid, undec-2-enyl ester



Hit#:4 Entry:165934 Library:NIST20-1.lib
SI:79 Formula:C16H31Br CAS:0-00-0 MolWeight:302 RetIndex:1893
CompName:Pentadec-7-ene, 7-bromomethyl- $$ (7Z)-7-(Bromomethyl)-7-pentadecene # $$

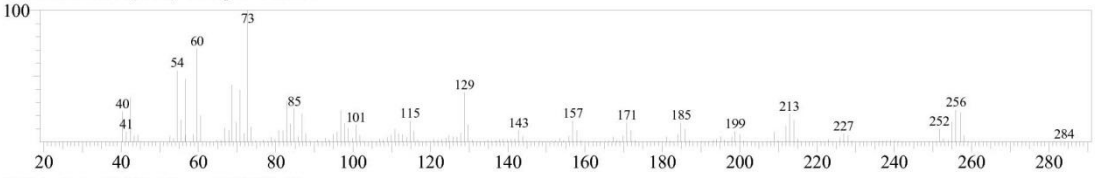


Hit#:5 Entry:106789 Library:NIST20-1.lib
SI:78 Formula:C12H19F3O2 CAS:0-00-0 MolWeight:252 RetIndex:1224
CompName:trans-2-Decen-1-ol, trifluoroacetate

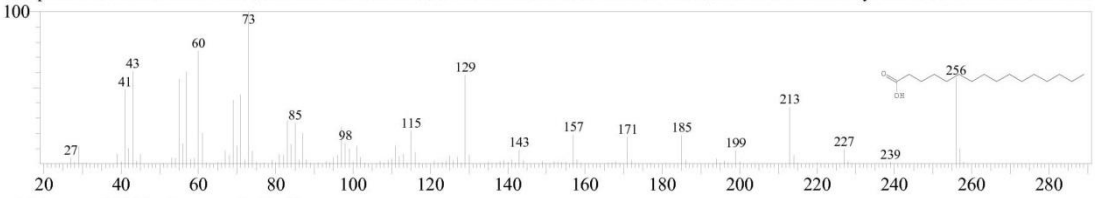


Appendix

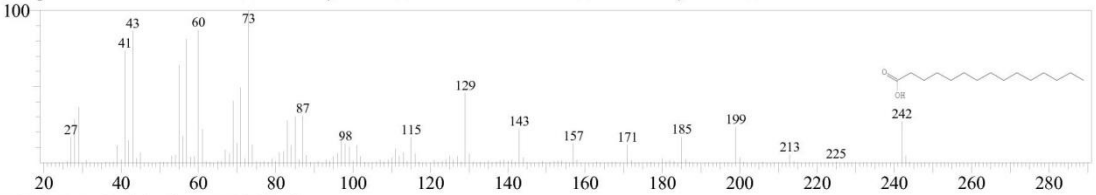
<< Target >>
Line#:7 R.Time:18.558(Scan#:1988) MassPeaks:187
RawMode:Averaged 18.317-18.658(1959-2000) BasePeak:72.75(69559)
BG Mode:18.617(1995) Group 1 - Event 1



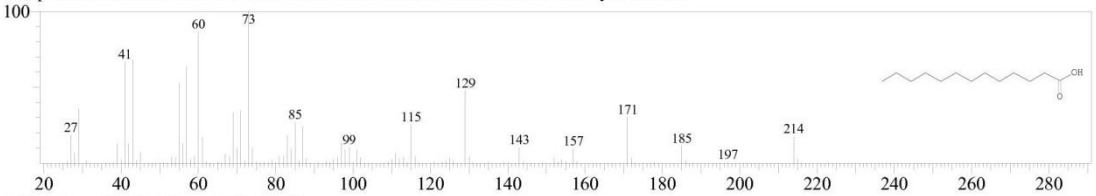
Hit#:1 Entry:31607 Library:NIST20s.lib
SI:79 Formula:C16H32O2 CAS:57-10-3 MolWeight:256 RetIndex:1968
CompName:n-Hexadecanoic acid $$ Hexadecanoic acid $$ n-Hexadecoic acid $$ Palmitic acid $$ Pentadecanecarboxylic acid $$ 1-Pentadecanecarb



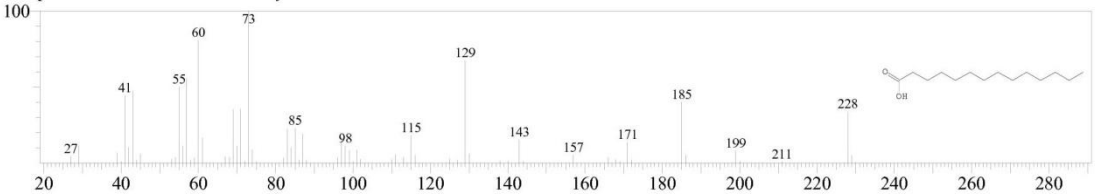
Hit#:2 Entry:29886 Library:NIST20s.lib
SI:76 Formula:C15H30O2 CAS:1002-84-2 MolWeight:242 RetIndex:1869
CompName:Pentadecanoic acid $$ Pentadecylic acid $$ n-Pentadecanoic acid $$ n-Pentadecylic acid $$



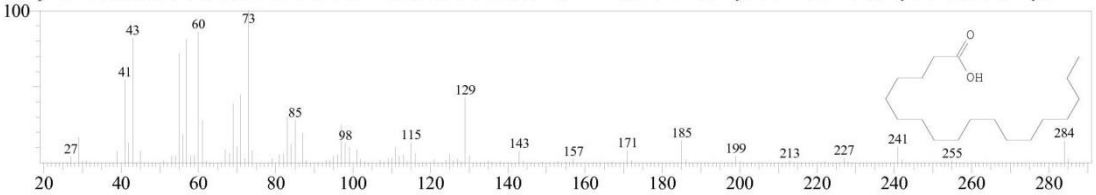
Hit#:3 Entry:25638 Library:NIST20s.lib
SI:75 Formula:C13H26O2 CAS:638-53-9 MolWeight:214 RetIndex:1670
CompName:Tridecanoic acid $$ n-Tridecanoic acid $$ n-Tridecoic acid $$ Tridecylic acid $$



Hit#:4 Entry:28007 Library:NIST20s.lib
SI:74 Formula:C14H28O2 CAS:544-63-8 MolWeight:228 RetIndex:1769
CompName:Tetradecanoic acid $$ Myristic acid $$ n-Tetradecanoic acid $$ n-Tetradecoic acid $$ Neo-Fat 14 $$ Univol U 316S $$ 1-Tridecanecarb

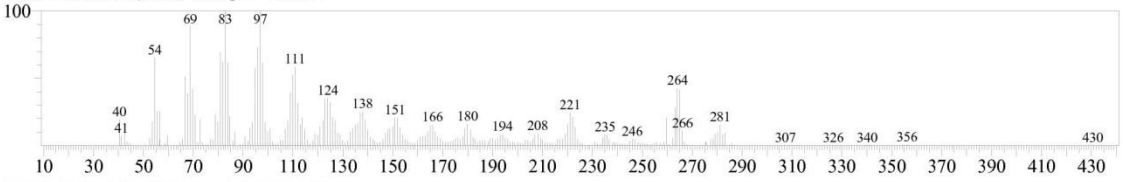


Hit#:5 Entry:144767 Library:NIST20-1.lib
SI:73 Formula:C18H36O2 CAS:57-11-4 MolWeight:284 RetIndex:2167
CompName:Octadecanoic acid $$ Stearic acid $$ n-Octadecanoic acid $$ Humko Industrene R $$ Hydrofol Acid 150 $$ Hystrene S-97 $$ Hystrene

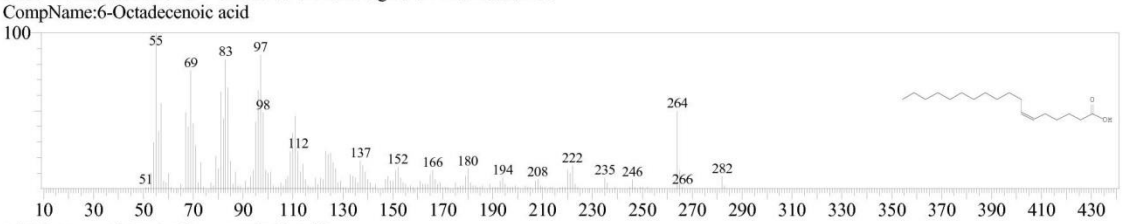


Appendix

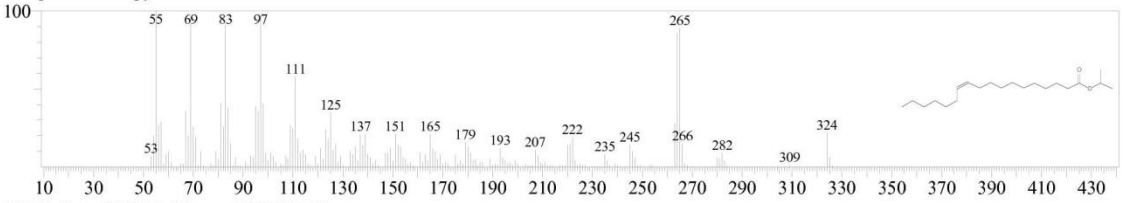
<< Target >>
Line#:8 R.Time:20.475(Scan#:2218) MassPeaks:260
RawMode:Averaged 19.950-20.583(2155-2231) BasePeak:82.80(71932)
BG Mode:20.525(2224) Group 1 - Event 1



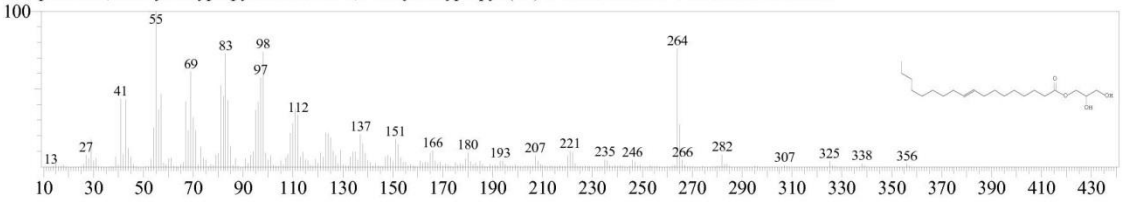
Hit#:1 Entry:142131 Library:NIST20-1.lib
SI:86 Formula:C18H34O2 CAS:0-00-0 MolWeight:282 RetIndex:2175
CompName:6-Octadecenoic acid



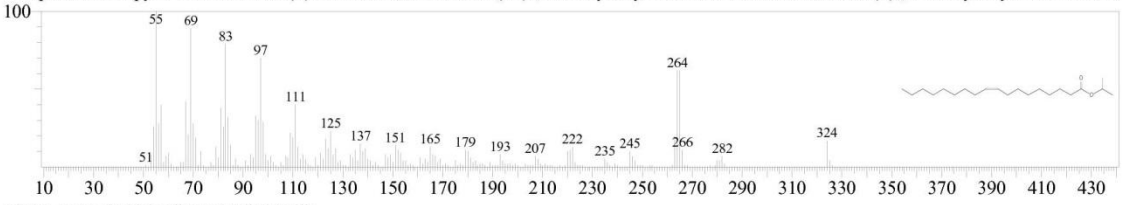
Hit#:2 Entry:191774 Library:NIST20-1.lib
SI:78 Formula:C21H40O2 CAS:0-00-0 MolWeight:324 RetIndex:2220
CompName:i-Propyl 11-octadecenoate



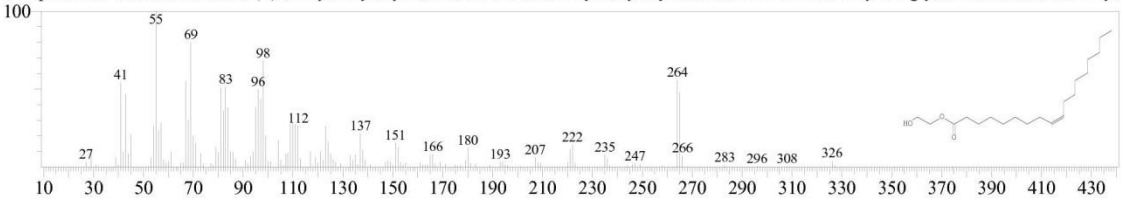
Hit#:3 Entry:226141 Library:NIST20-1.lib
SI:77 Formula:C21H40O4 CAS:2716-53-2 MolWeight:356 RetIndex:2689
CompName:2,3-Dihydroxypropyl elaidate $$ 2,3-Dihydroxypropyl (9E)-9-octadecenoate # $$ Monoelaidin $$



Hit#:4 Entry:37816 Library:NIST20s.lib
SI:77 Formula:C21H40O2 CAS:112-11-8 MolWeight:324 RetIndex:2220
CompName:i-Propyl 9-octadecenoate (Z) $$ 9-Octadecenoic acid (9Z)-, 1-methylethyl ester $$ 9-Octadecenoic acid (Z)-, 1-methylethyl ester $$ Oleic

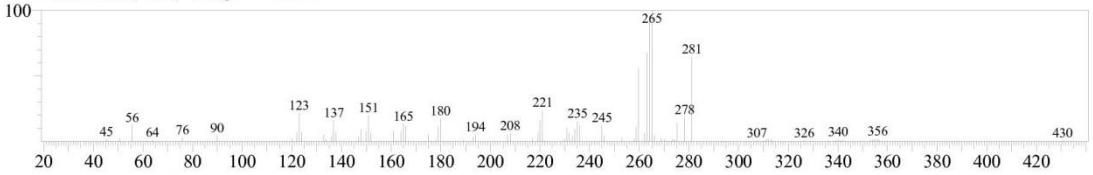


Hit#:5 Entry:37936 Library:NIST20s.lib
SI:76 Formula:C20H38O3 CAS:4500-01-0 MolWeight:326 RetIndex:2427
CompName:9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester $$ Oleic acid, 2-hydroxyethyl ester $$ Cithrol A $$ Ethylene glycol monooleate $$ 2-Hyc

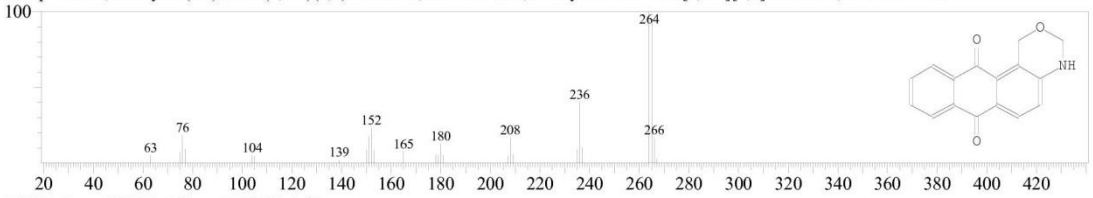


Appendix

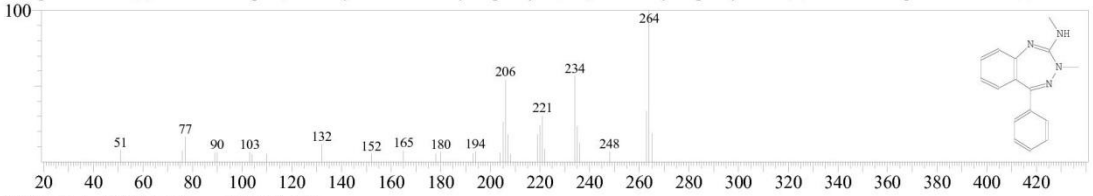
<< Target >>
Line#:9 R.Time:20.475(Scan#:2218) MassPeaks:91
RawMode:Averaged 20.458-21.025(2216-2284) BasePeak:265.10(8615)
BG Mode:20.583(2231) Group 1 - Event 1



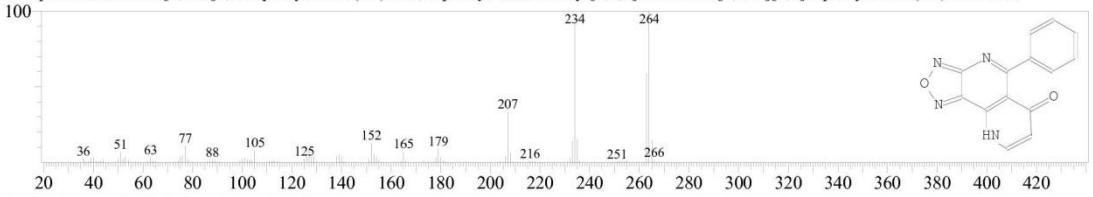
Hit#:1 Entry:121826 Library:NIST20-1.lib
SI:51 Formula:C16H11NO3 CAS:0-00-0 MolWeight:265 RetIndex:2454
CompName:1,2-Dihydro(4H)anthra(1,2-d)(1,3)oxazine-7,12-dione $$ 3,4-Dihydro-1H-anthra[2,1-d][1,3]oxazine-7,12-dione # $$



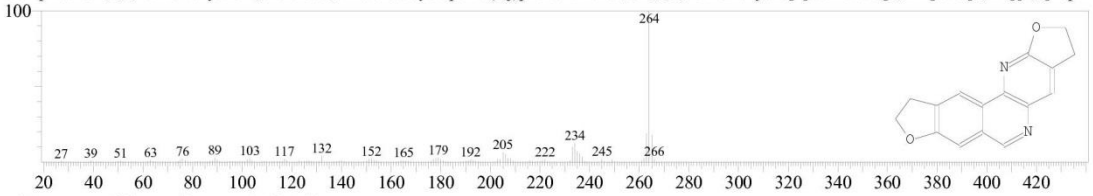
Hit#:2 Entry:120900 Library:NIST20-1.lib
SI:50 Formula:C16H16N4 CAS:121364-85-0 MolWeight:264 RetIndex:2391
CompName:3H-1,3,4-Benzotriazepine, 2-methylamino-3-methyl-5-phenyl- $$ N,3-Dimethyl-5-phenyl-3H-1,3,4-benzotriazepin-2-amine # $$



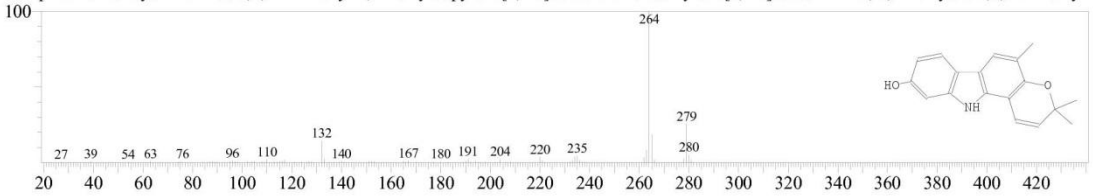
Hit#:3 Entry:120473 Library:NIST20-1.lib
SI:50 Formula:C14H8N4O2 CAS:296245-19-7 MolWeight:264 RetIndex:2471
CompName:Furazano[3,4-H]1,6-naphthyridin-6(9H)-one, 5-phenyl- $$ 5-Phenyl[1,2,5]oxadiazolo[3,4-H][1,6]naphthyridin-6(9H)-one # $$



Hit#:4 Entry:120881 Library:NIST20-1.lib
SI:50 Formula:C16H12N2O2 CAS:0-00-0 MolWeight:264 RetIndex:2357
CompName:2,3,9,10-Tetrahydro-1,8-dioxa-7,12-diazadicyclopenta(b,j)phenanthrene $$ 2,3,9,10-Tetrahydro[1]benzofuro[6,5-H]furo[2,3-b][1,5]napht

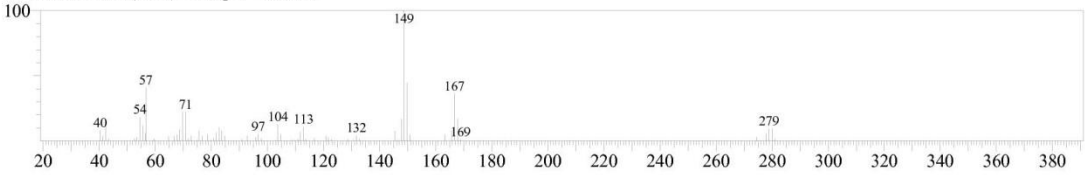


Hit#:5 Entry:138377 Library:NIST20-1.lib
SI:49 Formula:C18H17NO2 CAS:134779-17-2 MolWeight:279 RetIndex:2442
CompName:Murrayamine A $$ 3,3,5-Trimethyl-3,11-dihydropyrano[3,2-a]carbazol-9-ol $$ Pyrano[3,2-a]carbazol-9-ol, 3,11-dihydro-3,3,5-trimethyl-

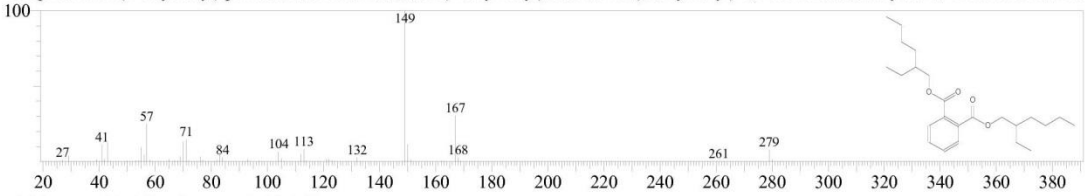


Appendix

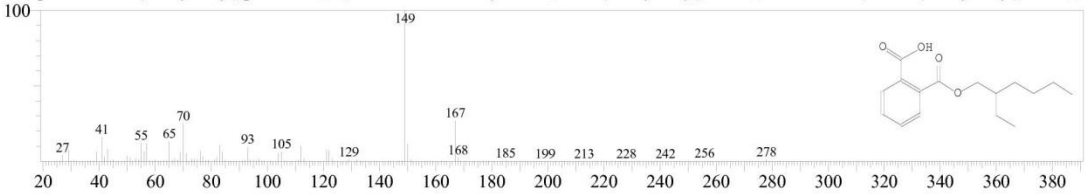
<< Target >>
Line#:10 R.Time:23.983(Scan#:2639) MassPeaks:69
RawMode:Averaged 23.800-24.125(2617-2656) BasePeak:148.70(10187)
BG Mode:24.033(2645) Group 1 - Event 1



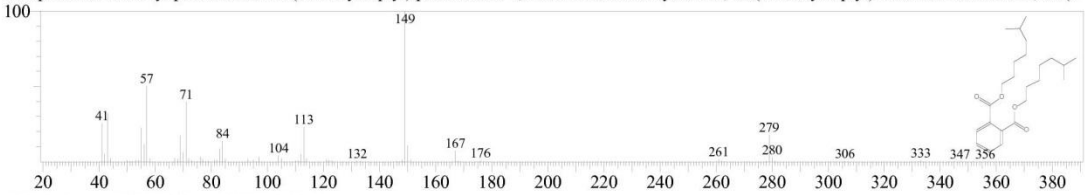
Hit#:1 Entry:41271 Library:NIST20s.lib
SI:79 Formula:C24H38O4 CAS:117-81-7 MolWeight:390 RetIndex:2704
CompName:Bis(2-ethylhexyl) phthalate $$ Phthalic acid, bis(2-ethylhexyl) ester $$ Bis(2-ethylhexyl) 1,2-benzenedicarboxylate $$ Bisoflex 81 $$ Co



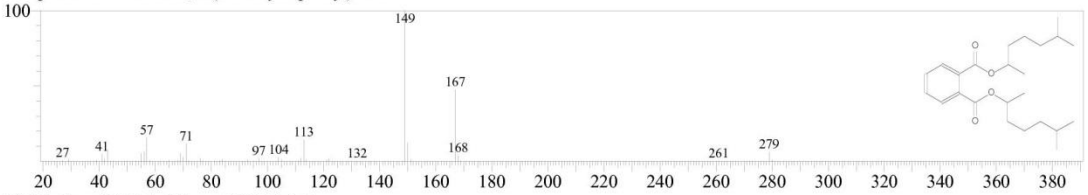
Hit#:2 Entry:137149 Library:NIST20-1.lib
SI:76 Formula:C16H22O4 CAS:4376-20-9 MolWeight:278 RetIndex:2162
CompName:Mono(2-ethylhexyl) phthalate $$ 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester $$ Phthalic acid, mono-(2-ethylhexyl) ester $$



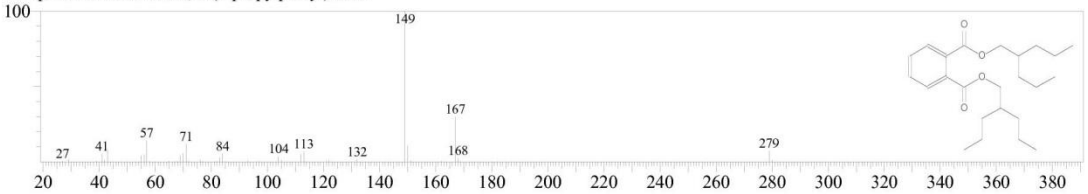
Hit#:3 Entry:254459 Library:NIST20-1.lib
SI:76 Formula:C24H38O4 CAS:131-20-4 MolWeight:390 RetIndex:2704
CompName:Diisooctyl phthalate $$ Bis(6-methylheptyl) phthalate $$ 1,2-Benzenedicarboxylic acid, bis(6-methylheptyl) ester $$ Phthalic acid, bis(6-



Hit#:4 Entry:254475 Library:NIST20-1.lib
SI:75 Formula:C24H38O4 CAS:0-00-0 MolWeight:390 RetIndex:2575
CompName:Phthalic acid, di(6-methylhept-2-yl) ester

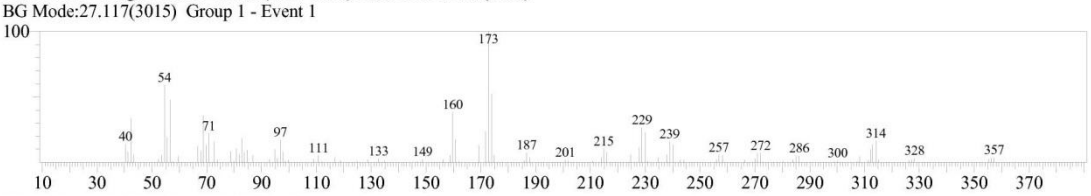


Hit#:5 Entry:254474 Library:NIST20-1.lib
SI:75 Formula:C24H38O4 CAS:0-00-0 MolWeight:390 RetIndex:2704
CompName:Phthalic acid, di(2-propylpentyl) ester

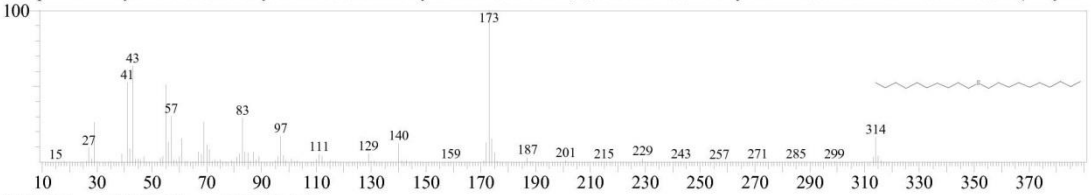


Appendix

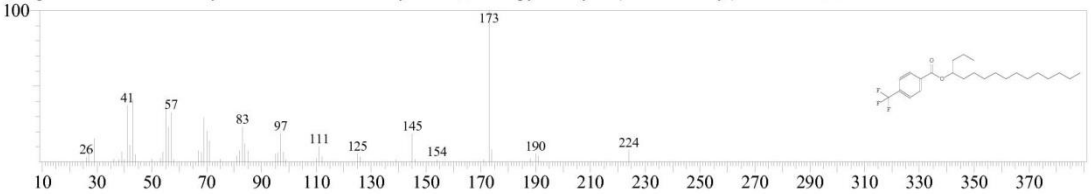
<< Target >>
Line#:11 R.Time:27.025(Scan#:3004) MassPeaks:100
RawMode:Averaged 26.825-27.208(2980-3026) BasePeak:172.75(7839)
BG Mode:27.117(3015) Group 1 - Event 1



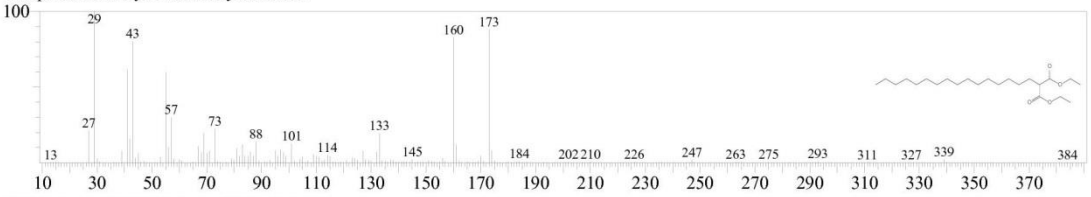
Hit#:1 Entry:37078 Library:NIST20s.lib
SI:62 Formula:C20H42S CAS:693-83-4 MolWeight:314 RetIndex:2260
CompName:Decyl sulfide $$ n-Decyl sulfide $$ di-n-Decyl sulfide $$ Decane, 1,1'-thiobis- $$ Didecyl sulfide $$ 11-Thiaheneicosane $$ 1-(Decylsul



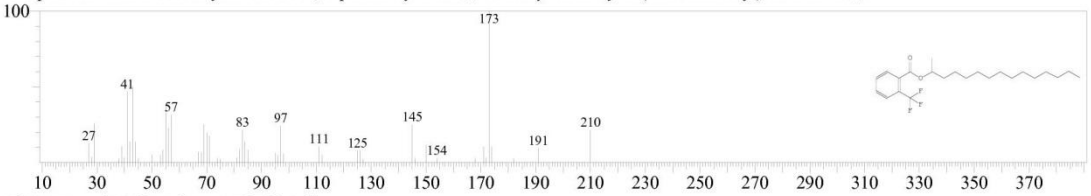
Hit#:2 Entry:268393 Library:NIST20-1.lib
SI:58 Formula:C24H37F3O2 CAS:0-00-0 MolWeight:414 RetIndex:2435
CompName:4-Trifluoromethylbenzoic acid. 4-hexadecyl ester $$ 1-Propyltridecyl 4-(trifluoromethyl)benzoate # $$



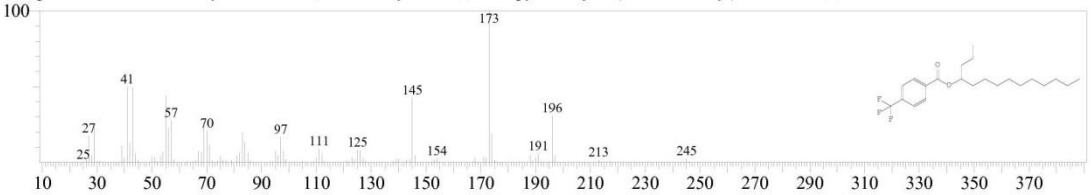
Hit#:3 Entry:249893 Library:NIST20-1.lib
SI:58 Formula:C23H44O4 CAS:0-00-0 MolWeight:384 RetIndex:2578
CompName:Diethyl n-hexadecylmalonate



Hit#:4 Entry:260804 Library:NIST20-1.lib
SI:57 Formula:C23H35F3O2 CAS:0-00-0 MolWeight:400 RetIndex:2335
CompName:2-Trifluoromethylbenzoic acid, 2-pentadecyl ester $$ 1-Methyltetradecyl 2-(trifluoromethyl)benzoate # $$



Hit#:5 Entry:251485 Library:NIST20-1.lib
SI:57 Formula:C22H33F3O2 CAS:0-00-0 MolWeight:386 RetIndex:2236
CompName:4-Trifluoromethylbenzoic acid, 4-tetradecyl ester $$ 1-Propylundecyl 4-(trifluoromethyl)benzoate # $$



Method

[Comment]

===== Analytical Line 1 =====

[AOC-20i]
# of Rinses with Presolvent :0
# of Rinses with Solvent(post) :5
# of Rinses with Sample :2

Plunger Speed(Suction) :High
Viscosity Comp. Time :0.2 sec
Plunger Speed(Injection) :Middle
Syringe Insertion Speed :High
Injection Mode :Normal
Pumping Times :5
Inj. Port Dwell Time :0.3 sec
Terminal Air Gap :No
Plunger Washing Speed :High
Washing Volume :8uL
Syringe Suction Position :0.0 mm
Syringe Injection Position :0.0 mm
Use 3 Solvent Vial :1 vial

[GC-2010]
Column Oven Temp. :80.0 °C
Injection Temp. :280.00 °C
Injection Mode :Split
Flow Control Mode :Pressure
Pressure :100.0 kPa
Total Flow :11.8 mL/min
Column Flow :1.46 mL/min
Linear Velocity :44.5 cm/sec
Purge Flow :3.0 mL/min
Split Ratio :5.0
High Pressure Injection :OFF
Carrier Gas Saver :OFF
Splitter Hold :OFF
Oven Temp. Program

Rate	Temperature(°C)	Hold Time(min)
-	80.0	2.00
10.00	280.0	6.00

< Ready Check Heat Unit >
Column Oven : Yes
SPL1 : Yes
MS : Yes
< Ready Check Detector(FTD) >
< Ready Check Baseline Drift >
< Ready Check Injection Flow >
SPL1 Carrier : Yes
SPL1 Purge : Yes
< Ready Check APC Flow >
< Ready Check Detector APC Flow >
External Wait :No
Equilibrium Time :3.0 min

[GC Program]

[GCMS-QP2010 Plus]
IonSourceTemp :200.00 °C
Interface Temp. :250.00 °C
Solvent Cut Time :2.00 min
Detector Gain Mode :Relative
Detector Gain :0.00 kV
Threshold :1000

[MS Table]
--Group 1 - Event 1--
Start Time :2.00min
End Time :28.00min
ACQ Mode :Scan
Event Time :0.50sec
Scan Speed :1666
Start m/z :40.00
End m/z :800.00

Sample Inlet Unit :GC

[MS Program]
Use MS Program :OFF

الخلاصة

أُجريت الدراسة الحالية في كلية الطب البيطري/جامعة كربلاء، وهدفت إلى تقييم الخصائص المضادّة للأكسدة والتأثيرات الوقائية لنبات القراص ((Urtica dioicaعلى الحالة الفسيولوجية للمبايض في إناث الجرذان بعد سن اليأس. كما تم تقييم تأثير نبات القراص على نمط التعبير الجيني لهرمون GnRH

استُخدم في التجربة 40 جرذاً أنثى بالغة ولمدة 52 يوماً، حيث استمرت المعاملات الدوائية لمدة 20 يوماً. قُسمت الحيوانات إلى أربع مجموعات (10 جرذان لكل مجموعة):مجموعة السيطرة: أعطيت ماءً مقطراً فموياً يومياً لمدة 52 يوماً ,مجموعة القراص: أعطيت 100 ملغم/كغم فموياً لمدة 20 يوماً,مجموعة VCD (4-vinylcyclohexene):أعطيت 160 ملغم/كغم داخل الغشاء البريتوني لمدة 20 يوماً ,مجموعة VCD+القراص: أعطيت 160 ملغم/كغم داخل الغشاء البريتوني و100 ملغم/كغم فموياً لمدة 20 يوماً.

تم جمع عينات الدم لقياس هرمونات التستوستيرون والـ FSH، إضافةً إلى قياس LH، الإستراديول، وهرمون AMH، فضلاً عن مؤشرات الإجهاد التأكسدي: الكلوتاثيون المختزل ((GSHوالمالونديالدهيد (.(MDAكما تم تقييم التعبير الجيني لـ GnRH-I و GnRH-II في منطقة الهيبوثالامس. أُجريت الفحوصات النسيجية لدراسة التغيرات في المبايض وتحت المهاد.

أظهرت نتائج مجموعة VCD زيادة معنوية في مستويات MDA وFSH وLH، مع انخفاض في GSH والإستراديول والتستوستيرون و .AMHكما سببت زيادة في التعبير الجيني لكل من GnRH-I وGnRH-II، مع حدوث أضرار نسيجية واضحة مثل ضمور الجريبات، تليّف السدى، وتنكس الخلايا العصبية. في المقابل، أدى العلاج المشترك مع نبات القراص إلى تحسين هذه التغيرات من خلال استعادة التوازن التأكسدي، تعديل مستويات الهرمونات، تقليل التعبير الجيني لـ GnRH، وتحسين البنية النسيجية للمبايض وتحت المهاد

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء

كلية الطب البيطري

فرع الفسلجة والكيمياء الحياتية والأدوية

التأثيرات التعديلية لمستخلص أوراق القراص على التعبير الجيني لهرمون GnRH-I و GnRH-II والوظائف التناسلية في الجرذان التي تعاني من انقطاع الطمث المستحث

رسالة مقدمة الى مجلس كلية الطب البيطري / جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في علوم الطب البيطري / الفسلجة

من قبل

عائشه عبدالله خليفه

باشراف

أستاذ دكتور

وفاء كاظم جاسم

أستاذ دكتور

مريم عبدالحسين كاظم

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