Impact of estrogen depletion induced by 4-VinylcyClohexene Diepoxide (VCD) on the ERα and ERβ gene expression and ER oxidant /antioxidant on the female rats

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Abstract **-- Estrogen is integral to brain health, influencing neuroprotection, cognition, mood, and development. The neurological effects of exposure, in particular its possible neurotoxic effects on the brain, have drawn more and more attention in research The aim of this study is to detrain the effect of decrease of estrogen on the brain. A total of ten healthy four month of female rats are used in present work which was divided into two groups. The first group contains 5 rats will served as control group injected IM(intra muscular) normal saline. The second group contains 5 rats injected IM of 4-VinylcyClohexene Diepoxide (VCD) (80mg/kg) for 2 weekly . After the experiment was completed, blood was drawn from the animals and serum was extracted to test GSH(glutathione test), estrogen, and MD (Amalondialdehyde) . Brain samples were also taken to measure the gene expression of alpha and beta estrogen in the brain. The current study showed that there was a decrease in the amount of serum estrogen and GSH in the group exposed to the VCD when compared with the control group, and that there was an increase in the amount of Malondialdehyde in the group exposed to the VCD when compared with the control group.The results of gene expression showed that there was a decrease in the expression of the gene estrogen alpha in the group exposed to the VCD when compared with the control group, and that there was an increase in the gene expression of the gene estrogen beta in the group exposed to the VCD when compared with the control group.**

Through the current study conclude that the VCD decrease the estrogen inside the body, which led to a decrease in antioxidants and an increase in oxidizing substances, also damage to the ovaries which change in the production of both alpha and beta estrogen receptor in brain.

Keywords **— brain, ERα, ERβ, Gene expression, estrogen and antioxidant**

INTRODUCTION

Estrogen is a group of steroid hormones primarily associated with female reproductive health, but it plays critical roles in both sexes across multiple body systems. It influences development, metabolism, cardiovascular health, bone density and brain function (1).

4-Vinylcyclohexene diepoxide (VCD) is a synthetic chemical molecule that has been researched mainly because of its possible health consequences and role as an environmental toxin. Although VCD is frequently utilized in industrial processes including the synthesis of polymers and resins, its presence as an environmental contaminant raises concerns (2). It has been demonstrated that VCD impacts neurodevelopment and neurodegenerative processes, especially those related to the endocrine system and hormone regulation, which may in turn impact behavior and brain function (3) .Its capacity to modify the activity of estrogen, a hormone essential to cerebral growth and activity, is the main cause of its neurotoxic consequences. Many parts of the brain, including the cortex and hippocampus, which are important in mental functions like memory and learning, have high expression levels of estrogen receptors(4). Exposure to VCD can cause estrogenic interruption, which alters the neurochemical balance of the brain and may be a factor in neurological disorders including Parkinson's and Alzheimer's (5). Furthermore, it has been demonstrated that VCD influences neurogenesis, especially in the hippocampus, a portion of the brain that is essential for memory and learning. Chronic exposure has been shown in animal experiments to reduce brain stem cell division, which may affect retained memories and cognitive abilities (6). When evaluating a compound's possible long-term impacts on brain health, the combined effects of oxidative stress, hormone disruption, and decreased neurogenesis raise serious concerns. The processes of neurotoxicity, its effects on hormonal balance, and its

propensity to exacerbate neurological illnesses will be the main topics of this paper's exploration of the body of research on VCD's effects on the brain. Premature ovarian failure (POF), or the cessation of ovarian function, is known to result from infections and environmental variables such 4-vinylcyclohexene diepoxide (VCD), which damages the ovary's essential elements. All of these will ultimately result in infertility (7).

MATERIALS AND METHODS

In this investigation, a total of 10 femalerats each 4-months-old will be utilized, split into two groups of five rats each, with the control group receiving an intramuscular injection 80 mg/kg of IM of normal saline. 80 mg/kg of IM (VCD) was intramuscular injected to five rats daily for two weeks.In the second group whereas the control group was given regular meals and water . All research rats were put to death at the end of the study period by intramuscular injections of xyl heartbeat using a medical syringe, where five drops of blood were drawn and after leaving it to clot the serum was isolated using a centrifuge device at a speed of 5000 revolutions per minute azine and ketamine. Blood was drawn by a for ten minutes. The serum was kept at a temperature of minus 20 degrees Celsius until the tests were performed. Brain samples were taken and preserved using liquid nitrogen until cRNA extraction was performed. This was done using gene expression experiments to determine the extent to which both alpha-estrogen and beta-estrogen levels change in the brain.

RESULT & DISCUSSION

Serum estrogen concentration shows a significant ($p < 0.05$) decrease in the VCD group (10.35± 2.36) as compared with the control group (45.90 ±4.24) as shown in the fig (1).

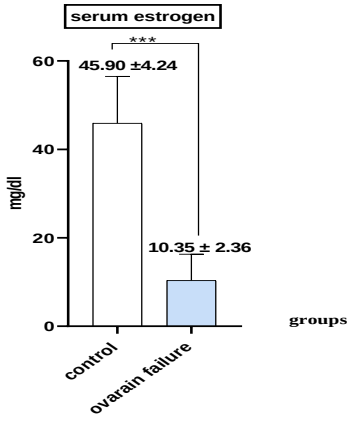

Figure 1. effect of ovarian failure induced by 80 mg/kg/daily/2 weeks Im injected of VCD in the serum estrogen in rats

VCD induces apoptosis in primordial and primary follicles via the activation of pro-apoptotic pathways such as caspase-3 and Bax/Bcl-2 dysregulation, as demonstrated in murine models(8). Granulosa cells within these follicles are critical for estrogen production, as they express aromatase (CYP19A1), the enzyme responsible for converting androgens like testosterone into estradiol. The depletion of these cells directly reduces aromatase activity, thereby diminishing estrogen synthesis. Multi-omics studies by (9) revealed that chronic VCD exposure in rat led to a 60–70% reduction in ovarian aromatase mRNA levels, correlating with a 50% decline in serum estradiol concentrations. This follicle loss is irreversible, as primordial follicles constitute the non-renewable ovarian reserve, making estrogen depletion a permanent consequence of prolonged VCD exposure (10).

In the current study there is a significant ($p < 0.05$) decrease in the GSH concentration within VCD (66.02 ±4.61) group as compared with the control group (119.19 ±3.46) as shown in figure (2).

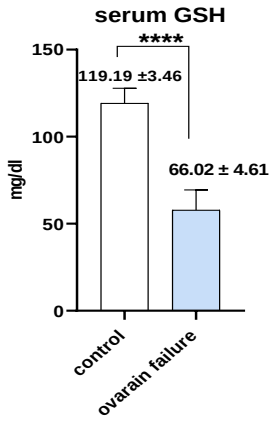

Figure 2.effect of ovarian failure induced by 80 mg/kg/daily/2 weeks Im injected of VCD in the serumGSH in rats

In the current study there is a significant ($p < 0.05$) increase in the MDA concentration within VCD group (94.70 ± 2.78) as compared with the control group (62.54± 2.9) as shown in figure (3)

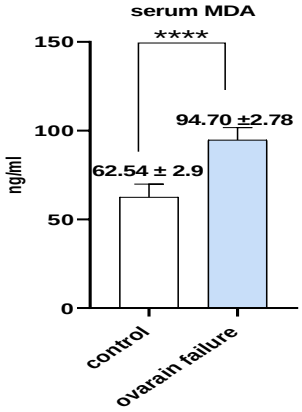

Figure 3. effect of ovarian failure induced by 80 mg/kg/daily/2 weeks Im injected of VCD in the serum MDA in rats

Oxidative stress plays a pivotal role in exacerbating VCD-induced estrogen decline. VCD's electrophilic diepoxide groups deplete glutathione (GSH), the primary intracellular antioxidant, through GST-mediated conjugation, leaving

granulosa cells vulnerable to reactive oxygen species (ROS) accumulation (11). Mitochondrial dysfunction, characterized by disrupted electron transport chains (ETC) in granulosa cells, further amplifies ROS production, particularly superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) (12). These ROS inhibit steroidogenic acute regulatory (StAR) protein and CYP19A1, key regulators of cholesterol transport and aromatase activity, respectively. Lipid peroxidation byproducts like malondialdehyde (MDA) exacerbate this damage by forming adducts with aromatase, rendering it inactive. In vitro studies on human granulosa cells (KGN cells) exposed to VCD showed a 40% reduction in aromatase activity and a parallel increase in MDA levels, underscoring the link between oxidative stress and impaired estrogen synthesis (13).

Oxidative stress arises when reactive oxygen species (ROS) production overwhelms cellular antioxidant defenses. GSH, a tripeptide (γ-glutamyl-cysteinyl-glycine), serves as the cell's primary redox buffer, neutralizing ROS directly and supporting antioxidant enzymes like glutathione peroxidase (GPx) and glutathione reductase (GR) (14). Conversely, MDA, generated during lipid peroxidation, reflects oxidative damage to cell membranes. Elevated MDA levels correlate with impaired cellular integrity and are predictive of chronic diseases, including ovarian dysfunction (15).

Glutathione S-transferases (GSTs), especially GSTP1 isoforms, catalyze the conjugation of VCD with GSH, forming non-toxic VCD-GSH adducts. While this detoxifies VCD, it irreversibly depletes cellular GSH reserves. Recent studies in ovarian granulosa cells demonstrated that VCD exposure reduces intracellular GSH by 40–50% within 24 hours, impairing redox homeostasis (16).

GSH synthesis depends on glutamate-cysteine ligase (GCL), the rate-limiting enzyme composed of catalytic (GCLC) and modifier (GCLM) subunits. RNA sequencing of VCD-exposed murine ovaries revealed significant downregulation of Gclc and Gclm transcripts, reducing GSH production capacity [17]. This suppression is exacerbated by ROS accumulation, which inhibits nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor regulating antioxidant gene expression [18].

Metabolize VCD into intermediates that generate H_2O_2 via NADPH oxidase activation. Reactive Excess H_2O_2 reacts with Fe^{2+} via Fenton reactions, producing hydroxyl radicals (•OH), which abstract hydrogen atoms from PUFAs, initiating lipid peroxidation (19).

VCD disrupts mitochondrial electron transport chains (ETC) in ovarian granulosa cells, increasing electron leakage and superoxide ($O_2^{\bullet-}$) production. Mitochondrial ROS (mtROS) trigger lipid peroxidation cascades, particularly in mitochondria-rich ovarian follicles (20).

Reduced GSH disrupts thioredoxin and peroxiredoxin systems, sensitizing follicles to ROS-induced apoptosis. Caspase-3 activation and cytochrome c release are hallmarks of VCD-induced follicular atresia (21).

Inflammatory pathways further contribute to estrogen suppression. VCD-induced mitochondrial ROS activate the NLRP3 inflammasome in ovarian stromal cells, triggering caspase-1-mediated pyroptosis and the release of pro-inflammatory cytokines like IL-1β and IL-18 (22). Chronic inflammation promotes fibrosis and theca cell hyperplasia, disrupting the follicular microenvironment necessary for steroidogenesis. Additionally, IL-1β directly suppresses CYP19A1 expression via NF-κB signaling, as evidenced in bovine granulosa cell models, highlighting a cytokine-driven mechanism for estrogen reduction (23).

Estrogen normally inhibits gonadotropin-releasing hormone (GnRH) secretion, modulating follicle-stimulating hormone (FSH) and luteinizing hormone (LH) release. However, VCD-induced estrogen decline disrupts this feedback, leading to elevated FSH levels in rodent studies (24).

In the current study there was a significant decrease in ERα gene expression in the VCD group compared with the control group as shown in figure (4 and 5)

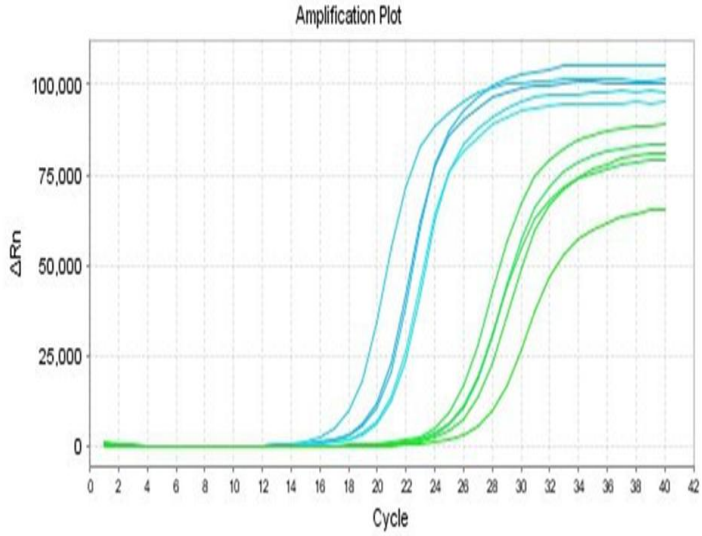

Figure 4: amplification curve of the tested samples represented the ERα gene. This indicates a successful RNA extraction and cDNA synthesis

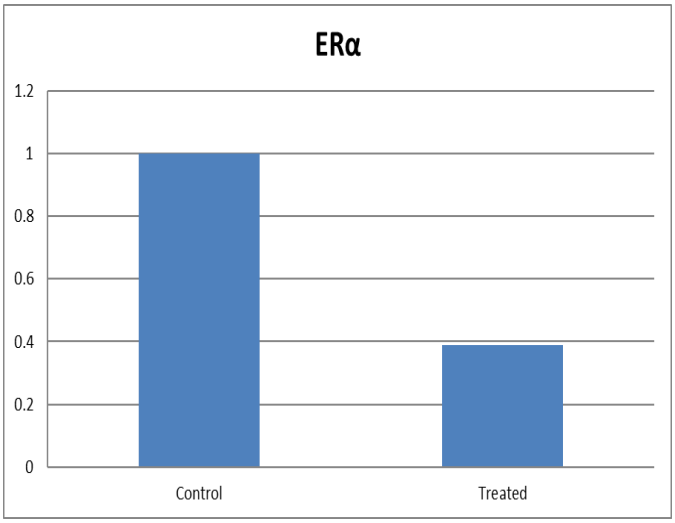

Figure 5: fold change comparison between the group expressed ERα gene . and show a significant decrease in the VCD group as compared with the control group. induced by 80 mg/kg/daily/2 weeks Im injected of VCD

Estrogen stabilizes ERα protein and enhances its transcription. Hypoestrogenism reduces ERα mRNA and protein levels, as seen in VCD-treated rodents (40–50% decrease in hypothalamic ERα) (25).

VCD accelerates ovarian follicle atresia, leading to hypoestrogenism—a hallmark of its toxicity. Estrogen is a key regulator of ERα expression; its absence disrupts the positive feedback loop that maintains receptor levels. In the brain, estrogen binding to ERα stabilizes the receptor and enhances Esr1 transcription via estrogen response elements (EREs) in the promoter region. Rodent studies demonstrate that VCD-induced ovarian failure reduces circulating estradiol (E_2) by 60–70%, correlating with a 40–50% decline in ERα mRNA and protein levels in the hypothalamus and hippocampus (26). This hypoestrogenic state mimics postmenopausal conditions, where ERα downregulation exacerbates cognitive decline and mood disorders (27).

VCD can generate reactive oxygen species (ROS) in the brain by depleting glutathione (GSH) and impairing mitochondrial function. Oxidative stress activates redox-sensitive transcription factors like NF-κB and AP-1, which compete with estrogen-activated ERα for binding to coactivators such as steroid receptor coactivator-1 (SRC-1). In murine models, VCD exposure increases hippocampal lipid peroxidation (measured by MDA levels) by 2-fold, while concurrently reducing ERα expression by 30%. Antioxidant treatment with N-acetylcysteine (NAC) partially restores ERα levels, confirming oxidative stress as a mediator(28). ROS also oxidize ERα's DNA-binding domain, impairing its ability to regulate target genes [29].

Furthermore VCD has been linked to the induction of oxidative stress, inflammation, and mitochondrial dysfunction in the brain—all of which are thought to be important mechanisms driving neurodegeneration (30).

VCD triggers microglial activation and pro-inflammatory cytokine release (e.g., IL-1β, TNF-α) in the brain, particularly in regions with high ERα density. IL-1β suppresses Esr1 transcription by activating NF-κB, which recruits histone deacetylases (HDACs) to the ERα promoter, inducing chromatin condensation and silencing gene expression (31).

VCD promotes DNA hypermethylation and histone modification at the Esr1 promoter. In rodent hippocampal neurons, VCD exposure increases DNA methyltransferase (DNMT) activity, adding methyl groups to CpG islands in the ERα promoter region—a process linked to gene silencing Concurrently, histone H3 lysine 27 trimethylation (H3K27me3), a repressive chromatin mark, is enriched at Esr1 loci, as shown by ChIP-seq analysis. Demethylating agents like 5-azacytidine restore ERα expression in VCD-treated models, underscoring epigenetic dysregulation as a key mechanism (32).

ERα in the hypothalamus regulates gonadotropin-releasing hormone (GnRH) neurons, which coordinate reproductive hormone release. VCD-induced ERα loss disrupts this feedback, leading to elevated FSH and LH levels due to diminished estrogen-negative feedback. Paradoxically, GnRH neurons themselves exhibit reduced ERα expression, impairing pulsatile GnRH secretion and further destabilizing the HPG axis. This creates a vicious cycle: hormonal imbalances exacerbate ERα suppression, while ERα deficiency amplifies neuroendocrine dysfunction (33).

ERα in the prefrontal cortex modulates serotonin and dopamine signaling. VCD-induced ERα suppression reduces tryptophan hydroxylase (TPH2) expression, lowering serotonin synthesis and increasing depressive-like behaviors in rodent forced-swim tests (34). Similarly, ERα knockdown in the amygdala elevates anxiety-like behaviors, linked to dysregulated corticotropin-releasing hormone (CRH) signaling (35).

Hypothalamic ERα regulates energy homeostasis and autonomic function. VCD-treated mice exhibit leptin resistance, hyperphagia, and weight gain due to ERα loss in pro-opiomelanocortin (POMC) neurons. Autonomic dysfunction, including elevated blood pressure and heart rate variability, is also observed (36).

In the current study there were a signefecant ($p < 0.05$) increase in ERβ gene exprtion in the VCD group compared with the control group as shown in fig(6 and 7)

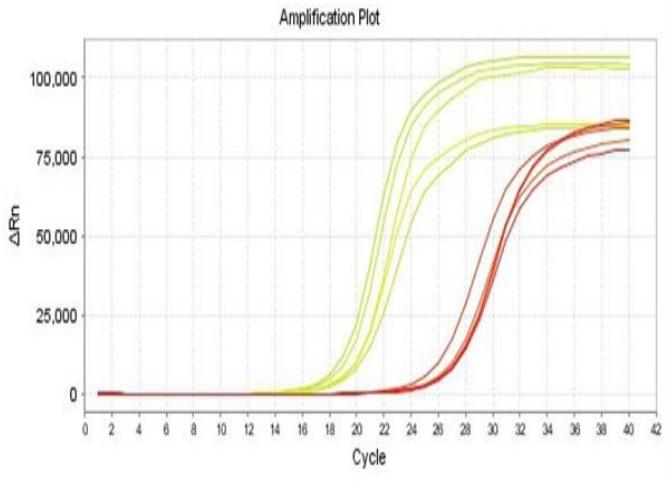

Figure 6: amplification curve of the tested samples represented the ERβ gene. this indicate a successful RNA extraction and cDNA synthesis

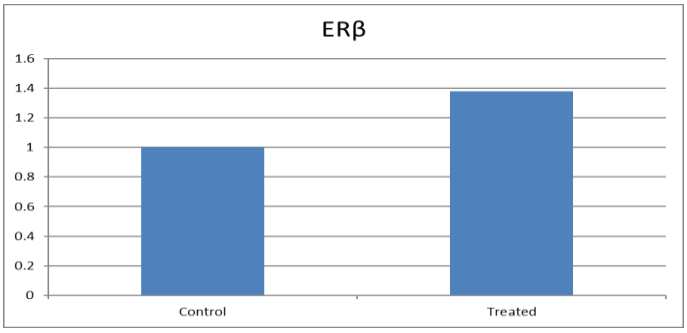

Figure 7: fold change comparison between the group expressing ERβ gene . And show a significant increase in the VCD group as compared with the control group.

VCD generates reactive oxygen species (ROS) in the brain by depleting glutathione (GSH) and impairing mitochondrial electron transport chains. ERβ is uniquely equipped to counteract oxidative damage due to its ability to activate antioxidant enzymes like superoxide dismutase (SOD) and catalase via Nrf2 signaling. In murine studies, VCD exposure increases hippocampal ERβ mRNA by 50–60%, correlating with elevated SOD activity and reduced lipid peroxidation (MDA levels) (37). ERβ knockout models exhibit exacerbated oxidative damage under VCD treatment, confirming its protective role (38).

VCD induces epigenetic modifications that favor ERβ expression. In the rodent prefrontal cortex, VCD reduces DNA methylation at CpG islands in the Esr2 promoter by inhibiting DNA methyltransferases (DNMTs), as shown by whole-genome bisulfite sequencing (39). Concurrently, histone acetylation (H3K27ac) increases at ERβ-associated enhancers, enhancing transcriptional accessibility. These changes are reversed by histone deacetylase (HDAC) inhibitors, confirming epigenetic regulation (40).

ERβ can be activated by non-estrogenic ligands, including phytochemicals and ROS-modified proteins. VCD-generated lipid peroxidation products, such as 4-hydroxynonenal (4-HNE), act as ERβ agonists, binding to the receptor's ligand-binding domain and promoting its nuclear translocation. In vitro studies on cortical neurons show that 4-HNE increases ERβ transcriptional activity by 70%, even in estrogen-free conditions (41).

VCD selectively destroys primordial and primary ovarian follicles, leading to a menopause-like state in rodents. This results in reduced estrogen production, disrupting the hypothalamic-pituitary-gonadal (HPG) axis. The loss of estrogen feedback increases gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels, mimicking human menopause (42).

ERα mediates estrogen signaling in reproductive tissues (uterus, mammary gland) and regulates negative feedback on the HPG axis. ERα deficiency impairs these functions, exacerbating LH/FSH surges and reproductive tissue atrophy (43).

The observed decrease in estrogen alpha (ERα) gene expression and increase in estrogen beta (ERβ) gene expression in the brain during low estrogen states aligns with the distinct roles and regulatory dynamics of these receptors. ERα, which is highly active in reproductive and stress-related pathways may downregulate in response to diminished estrogen levels, as its expression is often estrogen-dependent. This reduction could reflect diminished demand for reproductive signaling in low-estrogen conditions. Conversely, the compensatory upregulation of ERβ—a receptor strongly tied to neuroprotection, anti-inflammatory responses, and cognitive resilience—suggests an adaptive mechanism to preserve brain function when estrogen is scarce. ERβ's increase might mitigate neuronal damage, maintain synaptic plasticity, and stabilize mood via serotonin/dopamine modulation, counteracting the loss of estrogen's protective effects. This dynamic shift could explain why postmenopausal women or individuals with neurodegenerative diseases retain some cognitive and emotional stability despite estrogen decline. The imbalance may also contribute to pathologies: reduced ERα could impair stress adaptation or reproductive behaviors, while heightened ERβ signaling might not fully compensate for estrogen loss in all contexts. The findings highlight the potential for ERβ-targeted therapies to address estrogen-deficiency-related disorders, while underscoring the need to balance receptor-specific effects to avoid unintended consequences (44).

CONCLUSION

The current study show that the VCD decrease the estrogen inside the body, which led to a decrease in antioxidants and an increase in oxidizing substances, also damage to the ovaries which change in the production of both alpha and beta estrogen receptor in brain.

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