Scholars Journal of Applied Medical Sciences
Abbreviated Key Title: Sch J App Med Sci
ISSN 2347-954X (Print) | ISSN 2320-6691 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Medicine

Impact of Soybean Phytoestrogen-Rich Extract on Hormonal Imbalance and Ovarian Function in Menopausal Rat Induced with 4-Vinylcyclohexene Diepoxide: A Neglected Naturaceutical

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DOI: 10.36347/sjams.2024.v12i04.013 | **Received:** 16.02.2024 | **Accepted:** 27.03.2024 | **Published:** 10.04.2024

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Abstract **Original Research Article**

Backgroud: Decrease levels of estrogen and progesterone, a vital hormone in female reproductive health as led to vasomotor symptoms, such as hot flashes, sweating, physical and psychological discomfort, and emotional changes experienced by a large portion of the menopausal and postmenopausal female population. The study aim to investigate the impact of Soybean phytoestrogen-rich extract on hormonal imbalance and ovarian function on 4-vinylcyclohexene diepoxide-induced menopause in albino rats. ***Methods*:** Thirty (30) female albino Wistar rats were employed in the investigation, and each one was induced with 80mg/kg of 4-vinylcyclohexene diepoxide before being treated with either normal estradiol therapy (14ug/kg) or varying concentrations of the soybean phytoestrogen-rich extract (200 mg/kg, 400 mg/kg, and 600 mg/kg). Reproductive hormones (follicle stimulating hormone (FSH), luteinizing hormone (LH), estrogen (E2), anti mullerian hormone (AMH), progesterone and testosterone) were measured by ELISA methods. Statistical software SPSS (IBM) version 23.0 was used to analyze the data. ***Results*:** Serum E_2 and progesterone levels were observed to be significantly higher ($p<0.05$) in the soybean phytoestrogen-rich extract treatment group compared to the positive control group. There was non-significant difference ($p>0.05$) observed in use of standard estrogen therapy compared to phytoestrogen isoflavones but increased serum mean levels of estrogen, progesterone and anti-mullerian hormone was observed. ***Conclusion***: Data from this research clearly demonstrate the hormone regulating effect of soybean phytoestrogen-rich extract therapy in menopause-induced female Wistar rats. Soybean phytoestrogen-rich extract therapy in a high-dose appears to be more effective in management of hormonal imbalance and ovarian function compared to hormone replacement therapy as an alternate source of estrogen.

Keywords: Hormonal imbalance, hormonal replacement therapy, menopausal, ovarian function, phytoestrogen.

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INTRODUCTION

Hormonal imbalance plays a pivotal role in the onset and severity of menopausal conditions. The intricate interplay of hormones, primarily estrogen, progesterone, and follicle-stimulating hormone (FSH), orchestrates various bodily functions, including the menstrual cycle, bone health, mood regulation, and sexual function [1]. As women transition into menopause, a natural decline in ovarian estrogen production disrupts the delicate hormonal balance, leading to a cascade of symptoms collectively known as menopausal symptoms [2]. The primary hormones involved in menopause are estrogen, progesterone, and FSH [3]. Estrogen, the key female sex hormone, plays a crucial role in regulating the menstrual cycle, maintaining bone density, promoting mood stability, and facilitating sexual function [4]. Progesterone, working in concert with estrogen, balances its effects and supports pregnancy. FSH, produced by the pituitary gland, stimulates the ovaries to produce eggs [5]. During menopause, estrogen levels decline significantly, while

FSH levels rise [1]. This hormonal imbalance disrupts the intricate mechanisms that regulate various bodily functions, leading to a range of symptoms, including vasomotor symptoms like hot flashes, night sweats, and palpitations [5], Urogenital symptoms: vaginal dryness, dyspareunia (painful intercourse), and urinary incontinence [4], Psychological symptoms like mood swings, anxiety, and depression [2], Sleep disturbances: Insomnia and difficulty falling asleep [1], Cognitive changes like memory problems and difficulty concentrating [3], Bone loss like Increased risk of osteoporosis, a condition characterized by weakened bones [2]. The hormonal imbalance that arises during menopause significantly impacts the development and severity of menopausal conditions. Estrogen deficiency, a hallmark of menopause, contributes to vasomotor symptoms, vaginal dryness, and bone loss [3]. Progesterone deficiency can exacerbate these symptoms and increase the risk of endometrial hyperplasia, a condition characterized by thickening of the uterine lining [5]. FSH elevation may further contribute to estrogen deficiency and its associated symptoms [4]. The management of hormonal imbalance and menopausal conditions typically involves a combination of lifestyle modifications, dietary changes, and hormone therapy [1]. Regular physical activity like exercise, promotes cardiovascular health, muscle strength, and bone density, helping alleviate menopausal symptoms and improve overall well-being [3]. A diet rich in fruits, vegetables, whole grains, and low in saturated and trans fats supports overall health and may reduce menopausal symptoms [5]. Maintaining a healthy weight helps reduce strain on joints, alleviate menopausal symptoms, and improve overall health [4]. Relaxation techniques, such as yoga or meditation, can help alleviate stress, improve sleep, and promote emotional well-being [2]. Smoking exacerbates menopausal symptoms and increases the risk of osteoporosis1. Dietary Changes such as intake of Calcium-rich foods like dairy products, leafy green vegetables, and fortified foods provide adequate calcium which is essential for bone health [3]. Vitamin D promotes calcium absorption and bone health which can be derived from sunlight exposure, fatty fish, and fortified foods [5]. Omega-3 fatty acids found in fatty fish, nuts, and seeds, omega-3 fatty acids may reduce inflammation and improve mood [4].

Soy products contain phytoestrogens, plant-based compounds with estrogen-like effects. Some studies suggest that soy may alleviate menopausal symptoms [2]. Given that hormone replacement therapy (HRT) has been linked to an increased cancer risk, this investigation was undertaken to identify viable alternatives. The study is motivated by the pressing need for alternative and natural interventions to address menopausal symptoms. Menopause is a critical phase in a woman's life associated with hormonal changes and various health implications. Investigating the impact of soybean phytoestrogen-rich extract can contribute to the development of natural remedies for managing menopausal symptoms. Despite the growing interest in phytoestrogens, soybean extracts have been relatively overlooked in the context of menopausal research. This study aims to shed light on the neglected potential of soybean phytoestrogen-rich extract as a naturaceutical, providing insights into its efficacy in mitigating hormonal imbalances and preserving ovarian function. Soy products are commonly consumed in many cultures, making soybean extracts potentially accessible and safe for widespread use. If proven effective, soybean phytoestrogen-rich extract could offer a cost-effective and readily available alternative to synthetic hormone replacement therapies, which often come with associated risks and side effects. The study aligns with the growing awareness of environmental and ethical considerations in healthcare. Natural remedies like soybean extracts may present a more sustainable and ethically sound option compared to conventional pharmaceutical interventions, contributing to a holistic approach to women's health. The use of 4-vinylcyclohexene diepoxide (VCD) to induce menopause in animal models is well-established and documented [6]. However, the specific impact of soybean phytoestrogen-rich extract on hormonal balance and ovarian function in this context remains inadequately explored. This study aims to bridge this gap in the literature, enhancing our understanding of the potential therapeutic effects of soybean extracts on hormonal imbalance and ovarian function on 4-vinylcyclohexene diepoxide-induced menopause in albino rats. Previous studies have highlighted the potential of phytoestrogens in alleviating menopausal symptoms [7-9]. While various natural compounds have been investigated in the context of menopause, soybean phytoestrogen-rich extract has been notably understudied [10].

MATERIALS AND METHODS

Experimental animals

This is an experimental study design conducted at the Faculty of Pharmacy laboratory and animal house, University of Benin, Benin City. It involved thirty (30) females matured (aged 6-8 weeks) Wistar rats (*Rattus norvegicus*) weighing between 120 and 240g, which was menopause induced with 4-vinyl cyclohexane diepoxide. The animals were kept at the animal house at the University of Benin, Benin City for two weeks to stabilize before the experiments began. The Wistar rats were given a conventional rodent cube diet from Ewu feeds and flour mills limited Ewu, Edo state, Nigeria, and have free access to water *ad libitum.* Before each experiment, all animals were fasted overnight. Because of their ability to mimic the symptoms of perimenopause and postmenopause in humans—such as estrous acyclicity and fluctuating, then undetectable, estrogen levels—Wistar rats were chosen for this study to enable the separation of the impact of hormone levels from that of ageing. The study aim to investigate the impact of Soybean phytoestrogen-rich extract on hormonal

imbalance and ovarian function on 4-vinylcyclohexene diepoxide-induced menopause in albino rats.

Ethical consideration

The approval for the study was sought from the Animal Studies Ethic Review Committee of the Faculty of Pharmacy, Department of Pharmacology and Toxicology, University of Benin, Benin City. Because some of the Laboratory investigations were done at the Federal University of Technology, Akure, a second Ethical approval and clearance was obtained from the ethics and research committee of Federal University of Technology Akure, Ondo.

Soyabean purchase, authentication and preparation of soybeans flour

The soybeans were purchased from Oba market in Benin-City, and were authenticated by a plant taxonomist at the Department of Plant Biology and Biotechnology (PBB) laboratory, University of Benin, Benin City, and was given a voucher number (UBH-G628).

In preparing the flour, the soybean seeds were carefully picked, separated from debris and rinsed in water. After washing, the grains were transferred to a large, clean bowl, and left to soak overnight. The soybean chaff was washed off, drained to eliminate as much water as possible, and dried in the sun until they were completely dried. The grains were heated in a frying pan over medium heat, and stir until they turned brown, but being careful not to allow them to burn. Once the beans have browned, the grains were removed immediately. The roasted soybeans were immediately ground into a fine powder in a Kitchen blender. A quick transfer from a hot frying pan into the blender ensures the seeds grind smoothly to powder. The powder was stored in an air tight container. The method of Cvejic *et al.,* (2009) [11] was used with modification. Here, a known quantity of the Soybean flour was loaded into a thimble and placed in the Soxhlet extractor chamber until it was defatted using hexane, in the Soxhlet extractor. After defatting, the powder was dried and then re-extracted with methanol, using the Soxhlet extractor to obtain the methanol extract (phytoestrogen - rich extract). The extract was concentrated using a rotary evaporator and then dried completely using a thermostatically controlled hot air oven.

Experimental design: (4-vinylcyclohexene diepoxide (VCD) induced menopausal wistar rats)

Animals were divided into six (6) groups of five (5) animals in each group and induced intraperitoneally with 80mg/kg of VCD, obtained during the preliminary study.

Group 1: 80 mg/kg of 4-vinylcyclohexene diepoxide + 200mg/kg phytoestrogen - rich extract.
Group 2: 80 mg/kg of 4-vinylcyclohexene diepoxide + 400 mg/kg of phytoestrogen - rich extract.
Group 3:80 mg/kg of 4-vinylcyclohexene diepoxide + 600 mg/kg of phytoestrogen - rich extract.
Group 4: 80 mg/kg of 4-vinylcyclohexene diepoxide + 14µg/100g estrogen of body weight
Group 5: 80 mg/kg of 4-vinylcyclohexene diepoxide (positive control)
Group 6: Normal rats (negative control)

The soybean was administered daily at single dose for 28 days using oral gavage. The animals were observed closely for sign of toxic manifestation and toxicity, and none of the wistar rats died after 28 days of treatment. The study was carried out between 1st March-August 30th, 2023.

Inclusion/exclusion criteria

Sexually matured female Wistar rats were used in the study while those less than 6 weeks' old and male Wistar rats were excluded.

Collection of samples

At the end of the 28-day treatment period, the animals were sacrificed under chloroform anesthesia, blood sample was collected directly from the abdominal aorta and the heart chamber with a needle mounted on a 10 mL syringe (Agary pharmaceutical LTD, Nigeria) into lithium heparin anticoagulant and plane sample bottles. Biochemical analysis was performed on the serum sample obtained after centrifugation of whole blood at 2500 rpm for 10 min. The serum they were kept frozen at -20^0 degrees Celsius until biochemical analysis was performed. Reproductive hormones (FSH, LH, estradiol, testosterone, progesterone, anti-mullerian hormone) were measured using the ELISA method (Calbiotech Diagnostic Products Monobind Inc. Lake Forest, USA.).

Statistical Analysis

The data were statistically analysed using SPSS Software (IBM) version 23.0. The various results obtained from this study were expressed as Mean ± Standard deviation (SD). The differences between the groups were determined by one-way ANOVA. The Tukey-Kramer Multiple Comparisons Test was used as the post hoc test for determination of significant difference between Means. A P-value (= or <0.05) was considered to be statistically significant and P-value (>0.05) was considered not statistically significant.

RESULT

Table 1: shows that the levels of estradiol, progesterone, follicle stimulating hormone, luteinizing hormone, testosterone and anti-mullerian hormone. The levels of estradiol, progesterone and anti-mullerian hormone were all reduced in female Wistar rats given 80mg/kg VCD, with increase in follicle stimulating hormone, luteinizing hormone and testosterone. The levels of estradiol, progesterone and anti-mullerian hormone were increased in response to soyabean

phytoestrogen-rich extract at 200mg/kg, 400mg/kg, and 600mg/kg, as well as 14ug/100g estradiol($p<0.05$). The levels of follicle stimulating hormone, luteinizing hormone and testosterone were decrease in response to soyabean phytoestrogen-rich extract at 200mg/kg, 400mg/kg, and 600mg/kg, as well as 14ug/100g estradiol ($p<0.05$). It was observed that there were statistically significant differences between the groups for estradiol ($F=10.21$, $p=0.001$), FSH ($F=7.12$, $p=0.001$), LH ($F=8.19$, $p=0.001$), progesterone ($F=7.60$, $p=0.001$), AMH ($F=39.16$, $p=0.001$) s and testosterone ($F=3.38$, $p=0.001$).

Figure 1: displays the error bar chart of reproductive hormone with treatment with soybean phytoestrogen rich extract at varying concentration (200mg/kg, 400mg/kg and 600mg/kg). It show that at high dose-dependent concentration of soyabean phytoestrogen rich extract, estrogen, progesterone were significantly increased with decrease in follicle stimulating hormone compared to standard estradiol therapy.

Table 1: Levels of Reproductive Hormones of Female Wistar Rats Induced with 4-Vinylcyclohexane Diepoxide (VCD)

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	F	P-value
Estradiol (pg/ml)	20.18±5.15^a	38.16±4.79^a	44.46±2.11^a	43.47±5.05^a	0.00±0.00^a	142.51±36.64^b	10.21	0.001
FSH (mIU/ml	6.74±1.01^a	5.41±1.32^a	3.21±0.72^a	3.47±0.82^a	12.89±3.34^b	12.97±1.22^b	7.12	0.001
LH (mIU/ml)	5.53±0.83^a	4.67±1.16^a	3.75±0.94^a	3.47±1.11^a	11.22±1.18^b	5.42±0.66^a	8.19	0.001
Prog (ng/ml)	9.16±1.88^a	18.82±1.61^a	15.29±3.53^a	10.05±3.82^a	0.06±0.02^a	24.31±4.83^b	7.60	0.001
AMH (ng/ml)	0.02±0.01^a	0.02±0.01^a	0.04±0.03^a	0.00±0.00^a	0.00±0.00^a	1.44±0.22^b	39.16	0.001
T (ng/ml)	6.18±0.97^a	4.38±1.29^a	3.86±1.15^a	2.83±0.79^a	8.34±1.75^b	3.29±0.27^a	3.38	0.001

Values are expressed in mean ± SD. The value with different superscript showed significant difference from each other ($p<0.05$) while value with same superscript are not statistically difference from each other ($p>0.05$).

KEY: Group 1 - 80mg/kg VCD+200mg/kg Soyabean, Group 2 - 80mg/kg VCD+400mg/kg Soyabean, Group 3 – 80mg/kg VCD+600mg/kg Soyabean, Group 4 – 14ug/100g Estradol, Group 5 - 80mg/kg VCD, Group 6 – Control , FSH- Follicle stimulating hormone, LH- Luteinizing hormone, AMH- Anti-mullerian hormone, T- Testosterone, Prog- Progesterone

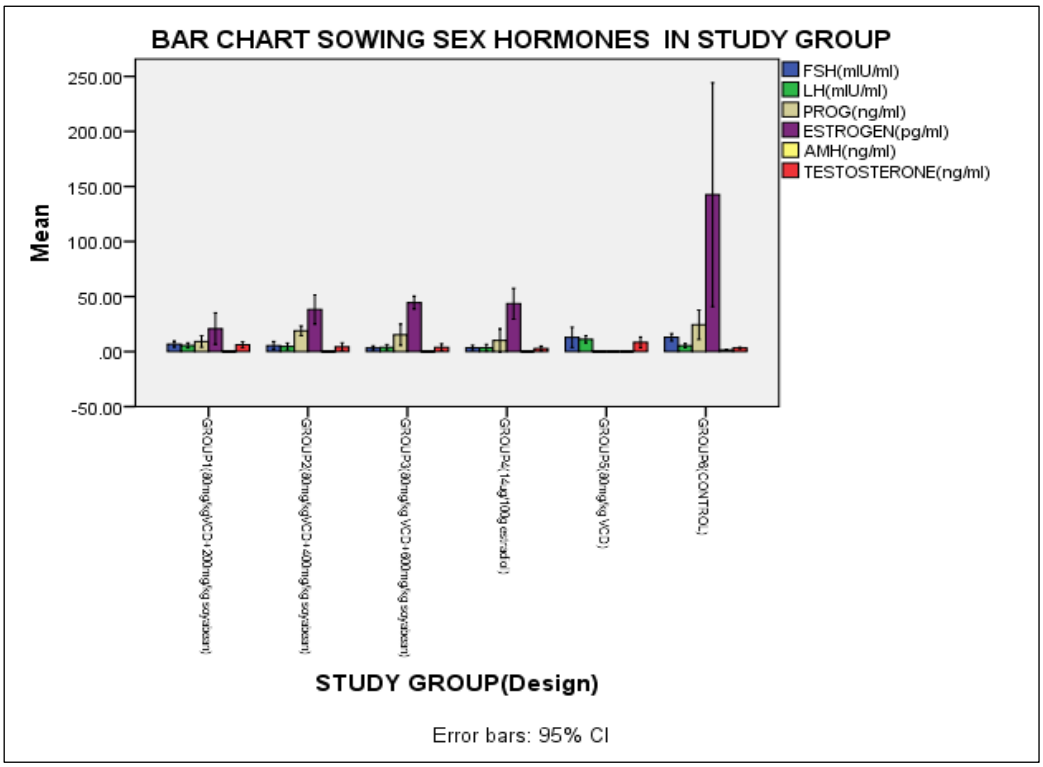

Figure-1: displays the error bar chart of reproductive hormone with treatment with soybean phytoestrogen rich extract at varying concentration(200mg/kg, 400mg/kg and 600mg/kg). It show that at high dose-dependent concentration of soyabean phytoestrogen rich extract, estrogen, progesterone were significantly increased with decrease in follicle stimulating hormone compared to standard estradiol therapy

DISCUSSION

Phytoestrogens, plant-derived compounds with estrogenic activity, have been investigated for their potential in alleviating menopausal symptoms. Soybean phytoestrogen-rich extract, containing compounds such as genistein and daidzein, has shown affinity for estrogen receptors, suggesting its capacity to modulate hormonal imbalances associated with menopause [8, 12].

Menopause is characterized by a decline in ovarian function, leading to hormonal fluctuations. The impact of soybean phytoestrogen-rich extract on ovarian function in the context of 4-vinylcyclohexene diepoxide (VCD)-induced menopause is crucial. Previous research indicates that VCD disrupts ovarian function in animal models, making it a suitable tool for studying menopausal changes [6]. The neglected status of soybean extracts as a naturaceutical prompts an exploration of the potential mechanisms underlying their impact on hormonal balance and ovarian function. Studies suggest that phytoestrogens may act through estrogen receptor modulation, antioxidant effects, and anti-inflammatory pathways [10, 13]. The study contributes to a comparative analysis of natural interventions for menopausal symptoms. Comparisons with existing treatments, such as hormone replacement therapy (HRT), can provide insights into the safety, efficacy, and potential advantages of soybean phytoestrogen-rich extract [9, 14]. Previous research has demonstrated the estrogenic activity of phytoestrogens, suggesting their potential in addressing menopausal symptoms [12]. Johnson *et al*., (2018) [10] proposed that the effects of soybean phytoestrogen-rich extract on hormonal balance may involve modulation of estrogen receptors and antioxidant pathways.

Soybean phytoestrogen extract, rich in isoflavones such as genistein and daidzein, exhibits estrogenic activity. These compounds can bind to estrogen receptors (ERs), particularly ERβ, exerting both agonistic and antagonistic effects. The binding of phytoestrogens to ERs regulates estrogen levels, influencing the overall estrogenic milieu in a manner that contributes to hormonal balance [12, 13]. This mechanism was in agreement with this study that showed that phytoestrogen extract positively upregulate the synthesis and secretion of estradiol. Soybean phytoestrogens may impact progesterone levels indirectly through their estrogenic effects. By modulating estrogen signaling, phytoestrogens can influence the delicate balance between estrogen and progesterone. This modulation may occur through feedback mechanisms within the hypothalamic-pituitary-gonadal axis, where estrogen and progesterone synthesis is intricately regulated [14]. Soybean phytoestrogens may influence FSH levels by modulating the overall estrogenic environment. FSH secretion is regulated by the negative feedback loop involving estrogen, and phytoestrogens could impact this balance. The binding of phytoestrogens to ERs may contribute to the regulation of FSH synthesis and secretion, thereby influencing ovarian function [15, 16]. The negative feedback mechanism involving estrogen led to the decrease level of FSH thereby regulating the hormonal levels in menopausal condition as observed in this study. Soybean phytoestrogen extract may influence AMH levels through its impact on ovarian function. AMH is primarily produced by ovarian granulosa cells and is crucial for the regulation of ovarian folliculogenesis. Phytoestrogens, particularly genistein and daidzein found in soybean extracts, have been associated with positive modulation of AMH levels. Studies suggest that phytoestrogens may exhibit estrogenic effects in the ovaries, leading to the promotion of follicular development and AMH production. The estrogenic activity of phytoestrogens may contribute to the maintenance of ovarian reserve by supporting the growth and maturation of ovarian follicles, reflected in increased AMH levels [17, 18]. Soybean phytoestrogens, acting as selective estrogen receptor modulators (SERMs), may also impact testosterone levels. Testosterone production is regulated by the hypothalamic-pituitary-gonadal axis, and estrogenic compounds can influence this axis. Phytoestrogens may exert inhibitory effects on testosterone synthesis by competing with endogenous estrogen for binding to ERs or by affecting the release of gonadotropins from the pituitary gland [19, 20]. This mechanism of inhibitory effect of phytoestrogen on gonadotrophin was in agreement with result observed in this study.

From this study, the levels of reproductive hormones (estrogen, progesterone, and anti-mullerian hormone; AMH) were significantly lower than control ($p<0.05$). There was no statistically significant difference ($p>0.05$) between the negative control and the positive control group in terms of FSH, while there was a statistically significant difference ($p<0.05$) in terms of LH. Treatment groups also had lower testosterone levels than menopausal albino rats ($p<0.05$). The levels of estrogen, progesterone, and anti-mullerian hormone (AMH) rise dramatically in a dose-dependent manner in the phytoestrogen -rich extract treatment group, with no discernible difference between the two groups when compared to 14ug/kg of normal estradiol therapy. Abbasabadi and Mina (2016) [21] and Yuliawati *et al*., (2020) [22] corroborated the finding of this study that rats that had undergone ovariectomy and were given soy milk had significantly higher serum levels of 17-estradiol than rats that had not undergone ovariectomy and were not given soy milk. Cassidy *et al*., (1994, 1995; Duncan *et al*., (1999) [23-25] all found that women who drank 45–200 milligrammes of isoflavones per day from soymilk had lower amounts of luteinizing hormone and follicle-stimulating hormone at midcycle. Estrogen-sensitive organs like the uterus, vagina, and ovaries develop and function appropriately when gonadotropins (FSH and LH) and ovarian hormones (estrogen and progesterone) are at healthy levels. Our findings are consistent with previous literature indicating that soya isoflavone can modulate hormone secretion, as treatment with soya isoflavone increases E2 and decreases FSH and LH while positively affecting progesterone levels [26, 27]. Low and high doses of isoflavones promote a hormonal imbalance in androgen and estrogen production, resulting in a decrease in circulating and testicular androgen levels and an increase in estrogen

levels, which is associated with a reduction in the sperm quality parameters and a reduction in the testicular weight, both in the diameter of the seminiferous tubules and the total weight of the testes. Taken together, these findings are consistent with the hypothesis that long-term exposure of adult male rats to phytoestrogenss alters the endocrine axis, leading to abnormalities in testicular function. Jing *et al*., (2019) [28] found an elevation in testosterone levels in 2019, in contrast to my finding. Particularly during perimenopause, ovarian development and function are crucial to a woman's sexual functioning. Graafian follicles are responsible for ovarian growth and development (Picut *et al*., 2014) [29]. Graafian follicle count and ovarian histologic alterations were both found to be affected by soy isoflavone. The findings of this study strongly suggest that soybean phytoestrogen-rich extract exerts a positive influence on hormonal balance. Through the modulation of estrogen receptors and potential interactions with the hypothalamic-pituitary-gonadal axis, the extract demonstrates the capacity to mitigate hormonal imbalances associated with menopause induced by VCD. The research underscores the extract's potential in preserving ovarian function in the face of menopausal challenges induced by VCD [18, 6]. By addressing key components of ovarian function, including follicular development and anti-Mullerian hormone (AMH) levels, soybean phytoestrogen-rich extract emerges as a promising naturaceutical in maintaining reproductive health during menopause. This study draws attention to the often-overlooked status of soybean extracts as a naturaceutical for menopausal health. The neglect of soybean phytoestrogens in previous research is addressed, emphasizing the importance of exploring diverse natural compounds for their potential therapeutic benefits [8, 14]. The study provides a foundation for future investigations into the efficacy of soybean extracts as a safe and accessible alternative for managing menopausal symptoms. This research contributes to the growing body of evidence supporting a holistic approach to women's health. By examining a natural intervention derived from soybeans, a commonly consumed food source, the study aligns with the broader movement towards sustainable and ethical healthcare practices. The potential of soybean phytoestrogen-rich extract as an alternative to conventional hormone replacement therapies promotes the idea of comprehensive and accessible women's health solutions. In summary, the investigation into soybean phytoestrogen-rich extract's impact on hormonal imbalance and ovarian function in a rat model of menopause induced by 4-vinylcyclohexene diepoxide not only unveils promising therapeutic effects but also highlights the need for continued exploration of neglected naturaceuticals for women's health. This research contributes valuable knowledge that may pave the way for future clinical applications and further understanding of the intricate interplay between phytoestrogens, hormonal balance, and reproductive health [18, 6].

CONCLUSION

Data from this research clearly demonstrate the hormone regulating effect of soybean phytoestrogen-rich extract therapy in menopause-induced female Wistar rats. Soybean phytoestrogen-rich extract therapy in a high-dose appears to be more effective in management of hormonal imbalance and ovarian function compared to hormone replacement therapy as an alternate source of estrogen. The investigation into the impact of soybean phytoestrogen-rich extract on hormonal imbalance and ovarian function in a rat model of menopause induced by 4-vinylcyclohexene diepoxide (VCD) reveals significant insights into the potential therapeutic role of this neglected naturaceutical. The study sheds light on various key aspects, contributing valuable knowledge to the field of women's health and natural interventions for menopausal symptoms.

Conflict of Interests: There are no stated conflicts of interest by the authors.

Acknowledgement

The employees at Federal Medical Centre, Owo, Ondo State and Department of Medical Laboratory Science and Faculty of Pharmacy, University of Benin-City, Edo State, and God, the knowledge-giver, are especially appreciated by the authors for their cooperation throughout the study.

Authors Contributions

Olaniyan Stephen Olawale and Fidelis Ohiremen Oyakhire designed and initiated the study, Emokpae, Mathias Abiodun reviewed the manuscript for important intellectual content, Esezobor, Iria Kelly assisted in collection of blood sample from animals and data analysis, Olaniyan, Edusola Juliana, Efenarhua Samson and Christian Onosetale Ugege assisted in draft of the manuscript and Osamuyi Henry Uwumarongie assisted in proofreading the manuscript.

Funding: The study was not funded from external agency

Study Highlight

Haven proved effective, soybean phytoestrogen-rich extract could offer a cost-effective and readily available alternative to synthetic hormone replacement therapies, which often come with associated risks and side effects.

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