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(RESEARCH ARTICLE)

EXPERIMENTAL EVALUATION OF SELECTED HAEMOSTATIC, AND IMMUNOLOGICAL EFFECTS OF ESTRADIOL VALERATE IN FEMALE ALBINO WISTAR RATS

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ABSTRACT

Oral estradiol valerate (EV), an estrogen medication used in hormone replacement therapy for menopausal symptoms and low estrogen levels can influence coagulation and immune responses. However, the integrated effects of graded EV exposure on haemostatic and inflammatory parameters are not fully understood. This study evaluated the effects of EV on some haemostatic and inflammatory parameters in female albino Wistar rats. Twenty female rats weighing 150 – 200 g were divided into four groups: a control group receiving distilled water and three treatment groups received graded doses of estradiol valerate, 0.02 mg/kg, 0.04 mg/kg, 0.07 mg/kg body weight respectively. The EV was administered through oral gavage daily for 28 days. Blood samples were collected via cardiac puncture (left ventricle of the heart). The evaluated variables included platelet (PLT) count, prothrombin time (PT), activated partial thromboplastin time (APTT), Factor VII, and Factor VIII using manual methods and enzyme linked immunosorbent assay (ELISA). Serum interleukin-6 (IL-6) and interleukin-10 (IL-10) were measured using sandwich ELISA. Results were expressed as mean ± standard deviation (SD) and a $p < 0.05$ was considered statistically significant. Data generated were analyzed using Statistical Package for Social Sciences (SPSS) version 27.0, windows 10. Differences among the groups were tested using one-way Analysis of Variance (ANOVA), followed by post-hoc analysis. Body weight increased in all groups, from 165.00 ± 11.18 to 182.00 ± 7.14 g in controls, 177.00 ± 8.92 to 196.80 ± 5.40 g in the high-dose group, 155.60 ± 6.02 to 177.60 ± 6.27 g in the medium-dose group, and 165.00 ± 11.18 to 182.00 ± 7.14 g in the low-dose group. Significant differences were observed for PLT ($F = 58.89$, $p < .001$), PT ($F = 6.93$, $p = .003$), APTT ($F = 8.24$, $p = .002$),

factor VII ($F = 43.17$, $p < .001$), factor VIII ($F = 8.54$, $p = .001$), IL-6 ($F = 3.68$, $p = .034$), and IL-10 ($F = 16.95$, $p < .001$). The high-dose EV group showed the highest platelet count (780.80 ± 38.77 × 10^9/L), shortest PT (33.40 ± 2.07 s) and APTT (11.46 ± 0.97 s), and lower factor VII and VIII concentrations than the control group. IL-6 was highest in the medium-dose group, whereas IL-10 was lowest in the high-dose group.

In conclusion, graded EV administration significantly altered selected haemostatic and immunological parameters in female albino Wistar rats. The responses were not uniformly dose dependent, indicating that EV may exert complex, parameter-specific effects on haemostasis and cytokine regulation. Further studies should incorporate broader panel of inflammatory biomarkers to better characterize inflammatory response associated with estradiol valerate. This finding contributes to the understanding that estrogen-related haemostatic effects involve multiple pathways that may respond differently to the same hormonal exposure, rather than a simple global activation or suppression of coagulation. The study also adds to knowledge by providing experimental evidence that estrogen-mediated immune regulation may be concentration dependent and non-linear.

Keywords: Haemostatic, Platelet Count, Prothrombin Time, Activated Partial Thromboplastin Time, Factor VII, Factor VIII, Interleukin -6, Interleukin 10, Female Albino Rat, Estradiol Valerate

1 INTRODUCTION

Estradiol valerate (EV), sold for use by mouth is an estrogen medication. It is used in hormone therapy for menopausal symptoms and low estrogen levels, hormone therapy for transgender people, and in hormonal birth control (Kuhl, 2005). It is a synthetic ester and prodrug of 17β-estradiol that is converted to biologically active estradiol following administration. Through estrogen receptor-mediated genomic and non-genomic mechanisms, estradiol influences multiple physiological systems beyond the reproductive tract, including erythropoiesis, haemostasis, immune regulation, and tissue growth and cellular homeostasis (DailyMed, 2020; Stanczyk et al., 2025). Consequently, experimental exposure to estradiol valerate provides an important model for investigating the systemic effects of exogenous estrogen.

Haemostasis is a critical physiological process that ensures blood loss is minimized following vascular injury. This essential mechanism unfolds through a sequence of systematic steps that halt bleeding from a damaged blood vessel and facilitate its eventual repair (Leung, 2023). Prothrombin Time (PT) evaluates the extrinsic and common coagulation pathways, focusing on factors I, II, V, VII, and X. It is widely used in monitoring liver function and the effects of vitamin K antagonists. Activated Partial Thromboplastin Time (APTT) is a measure of the intrinsic coagulation pathways. Coagulation factor VII is a vitamin K–dependent plasma glycoprotein synthesized in the liver and plays a critical role in the initiation of blood coagulation through the extrinsic pathway. Oestrogen-containing oral contraceptives stimulate hepatic protein synthesis, leading to elevated plasma levels of Factor VII. This increase enhances extrinsic pathway activation and contributes to a hypercoagulable state, which is associated with an increased risk of venous thromboembolism (VTE) (Abou-Ismail et al., 2020).

Factor VIII is an essential glycoprotein cofactor in the intrinsic pathway of coagulation. It forms a complex with activated Factor IX (FIXa), facilitating the activation of Factor X to Xa. Formulations of oral contraceptives, especially those containing oestrogen, are associated with increased plasma levels of Factor VIII.

Platelets, also known as thrombocytes, are small, anucleate cytoplasmic cell fragments derived from megakaryocytes in the bone marrow. They play an indispensable role in maintaining vascular integrity, initiating haemostasis, facilitating wound healing, and regulating inflammatory and immune responses. In healthy individuals, circulating platelets have a lifespan of approximately 7–10 days before they are removed primarily by macrophages in the spleen and liver (Kaushansky et al., 2021).

The haemostatic system may also be sensitive to estrogenic stimulation. Estrogens can modify the balance between procoagulant, anticoagulant, and fibrinolytic pathways, and alterations in coagulation factors and their regulatory mechanisms may contribute to thrombotic risk (Barcellona et al., 2024; Stanczyk et al., 2025). Assessment of platelets, prothrombin time (PT), activated partial thromboplastin time (APTT), and coagulation factors such as factors VII and VIII can therefore provide complementary information on the effects of estradiol valerate on the extrinsic, intrinsic, and common pathways of coagulation.

Among the numerous biological systems influenced by hormonal changes, the immune system is particularly sensitive to fluctuations in hormone levels. This interaction between hormonal contraceptives and immune function is of great interest, as the immune system plays a crucial role in defending the body against infections, injuries, and even the development of chronic diseases (Ma & Song, 2023). Inflammatory cytokines are signaling molecules that cause inflammation and regulate immune response. They are produced by immune cells such as macrophages and helper T-cells.

Interleukin 6 (IL-6), a pro-inflammatory cytokine plays a role in chronic inflammation, autoimmune diseases and cancer was employed in this study. IL-10 is a key anti-inflammatory cytokine that inhibits the production of pro-inflammatory cytokines and modulates immune cell responses, thereby promoting tissue healing and preventing autoimmune reactions (Cooley et al., 2022). Anti-inflammatory Cytokines also help to resolve inflammation and prevent excessive tissue damage.

Estrogen also exerts important immunomodulatory effects through its actions on innate and adaptive immune cells and the regulation of inflammatory mediators. The effects of estrogen on immune responses may vary according to hormonal concentration, tissue environment, and physiological state (Chen et al., 2024). Interleukin-6 (IL-6), a multifunctional cytokine involved in inflammatory and acute-phase responses, and interleukin-10 (IL-10), a major anti-inflammatory and immunoregulatory cytokine, are therefore useful markers for evaluating the potential influence of estradiol valerate on inflammatory and immune homeostasis.

Despite evidence that estradiol and estrogen-containing therapies can influence haemostatic, and immunological processes, available studies have largely examined these outcomes separately. In particular, there is limited information on the simultaneous relationship between systemic haemostatic changes, and cytokine responses following graded exposure to estradiol valerate in female Wistar rats. This lack of an integrated assessment limits understanding of how exogenous estradiol valerate may produce coordinated systemic biological effects.

There is paucity of information on the assessment of these parameters; platelet count (PLT), prothrombin time (PT), activated partial thromboplastin time (APTT), Factor VII, Factor VIII, interleukin-6 markers (IL-6) and interleukin 10 (IL-10) especially in Rivers State, hence the need for this study. The aim of this study therefore, was to investigate the effects of estradiol valerate on some haemostatic indices and immunological parameters using female albino wistar rats.

Specifically, the objectives of the study were to;

- Determine the effects of estradiol valerate on activated partial thromboplastin Time (APTT) in the female rats
- Determine effects of estradiol valerate on Platelet count
- Determine the effects of estradiol valerate on the prothrombin Time
- Determine the effects of estradiol valerate on coagulation factor VII
- Determine the effects of estradiol valerate on coagulation factor VIII
- Determine the effects of estradiol valerate on interleukin-6 markers (IL-6)
- Determine the effects of estradiol valerate on interleukin-10 markers (IL-10)

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Study Area

The study was conducted at the Animal House of the Faculty of Pharmacy, Madonna University Nigeria, Elele Campus, Rivers State. Rivers state is located in the South- South geopolitical zone of Nigeria. It is located in latitude 5° 27° - 5° and longitude 6° 55 -7° 85E.

2.1.2 Animal

Twenty (20) female albino wistar rats weighing 150 – 200 g, obtained from certified animal breeding facility and were transported to the animal house of the Faculty of Pharmacy, Madonna University Nigeria, Elele Campus, Rivers State. The rats were housed in a well-ventilated room and allowed to acclimatize in the animal house for fourteen (14) days. Experimental animals in the study were treated in accordance with the National Protection Laws of Animal Welfare while observing the 3R's principle (reduction, replacement and refinement). The animals were randomly selected into 4 groups (5 rats /group); The control group received 10 mL/kg, the test groups received 0.02mg/kg, 0.04mg/kg and 0.07mg/kg body weight graded doses of estradiol valerate (EV) respectively.

2.1.3 Ethical Approval

Ethical approval was obtained from Madonna University Nigeria Research Ethics Committee (MUN-REC). Ref: MUN/VC/REC/PG/MLS/23/002. The study was conducted in accordance with applicable guidelines for the care and use of laboratory animals.

2.1.4 Inclusion Criteria

i. Apparently healthy female albino rats in the proestrous phase of the estrous cycle in rodents

2.1.5 Exclusion Criteria

- Male albino rats.
- Rats with any signs of illness, malnutrition, or pre-existing conditions
- Female rats not in the proestrous phase of the estrous cycle in rodents determined by vaginal smear cytology

2.1.6 Preparation of experimental doses

Oral estradiol valerate (2 mg) tablets were obtained from a Pharmacy in Port Harcourt Rivers State, Nigeria and used for the treated groups. Each tablet was crushed into a fine powder and dissolved in 5 mL of distilled water to prepare their various solution and the drugs were given daily in graded doses through oral gavage tube.

2.1.7 Drug Source

- Manufacturer: Healing Pharma India PVT Ltd. LLC, DE USA
- Batch number: 24S2HTA431
- Manufactured Date: June 2024
- Expiry Date: June 2026

CALCULATIONS: Estradiol valerate tablets = 2mg

(2 mg) ÷ (70 kg (Adult human weight) = 0.0286 mg/kg

Rat equivalent dose= Human dose × correction / km factor (6.2)

0.0286 mg/kg × 6.2 = 0.177 mg/kg

Average weight of rat = 200 g (0.2 kg)

0.177 mg/kg × 0.2 kg = 0.0354 mg

Therapeutic dose (D) = 0.0354 mg/kg

Graded dose Groups

Low dose = 0.5D i.e. 0.5 × 0.0354 mg/kg = 0.0177 mg/kg (0.02 mg/kg)
Medium dose = 1D i.e. 1 × 0.0354 mg/kg = 0.0354 mg/kg (0.04 mg/kg)
High dose = 2D i.e. 2 × 0.0354 mg/kg = 0.0708 mg/kg (0.07 mg/kg)
The rats in each group were given normal rat feed plus water ad libitum (dietary practice in animals where both food and water are made available at all times, without restrictions).

2.2 Blood sample collection

At the end of the 28 days' experimental period, the animals were fasted overnight. Blood samples were drawn via cardiac puncture and dispensed into tri-sodium citrate and plain containers. The platelet poor plasma and serum were separated by centrifugation at 3,000 revolutions per minute (RPM) for 15 minutes and were stored at -20°C until analysis for PT, APTT, clotting factor VII and factor VIII. Blood serum samples from the plain tubes were centrifuged and separated for the analysis of IL-6 and IL-10.

Table 1: Experimental Design: Rats Grouped into four

Groups	No. of Rats	Treatment
Group A (Control)	5	Feed + Distilled water (DH_20) throughout the experiment
Oral estradiol valerate Group B	5	Feed + (DH_20) throughout the experiment + (0.07 mg/kg body weight)
Group C	5	Feed + (DH_20) throughout the experiment + (0.04 mg/kg body weight)
Group D	5	Feed + (DH_20) throughout the experiment + (0.02 mg/kg body weight)

2.3 METHODS OF ASSAY

2.3.1 Activated Partial thromboplastin Time (APTT): Manual method

0.2 ml of well-mixed kaolin platelet substitute was aspirated and dispensed into a small glass test tube. 0.1 ml of the platelet poor plasma was added into the same tube and was incubated at 37 ºC in the water bath for exactly 2 minutes. 0.1 ml of calcium chloride was added, well-mixed and the stopwatch was started Immediately. The glass tube was held in the water bath and the mixture was tilted back and forth observing for clot formation. The time taken for a clot to form was recorded in seconds.

2.3.2 Platelets Count: Manual method

The central grid areas of the chamber and the special haemocytometer glass were completely clean and dried. After moistening the chamber surface on each side of the grid area, the cover slip was slide into position and pressed down on each side until rainbow colours (Newton's rings) appeared. 0.38 ml of ammonium oxalate was measured and 0.02 ml of well mixed anticoagulated venous blood was added and mixed. Using a Pasteur pipette, held at an angle of about 45, one of the grids of the chamber was filled with the sample, taking care not to overfill the area. The chamber was left undisturbed for 2 minutes to prevent drying of the fluid by placing it in a petri dish with dampened tissue and the lid was covered. The underside of the chamber was dried and placed on the microscope stage. Platelets in the central large square (comprising 25 medium squares) were counted using x10 objective lens to focus and x40 objective lens to view

2.3.3 Prothrombin Time (PT): Manual method

2.25 mL of blood was collected in trisodium citrate tubes containing 0.25 mL of anticoagulant and was centrifuged at 1500 rpm for 15 minutes to obtain platelet-poor plasma. Unto a clean test tube, 0.2 ml of thromboplastin reagent containing calcium was dispensed into a small glass tube and incubated in the water bath at 37ºC for 2 minutes. After 2 minutes, 0.1 ml of plasma was added into the same tube in the water bath and the stopwatch was started immediately. The glass tube was held in the water bath and the mixture was tilted back and forth observing for clot formation. The time taken for a clot to form was recorded in seconds.

2.3.4 Coagulation factor VII: Enzyme linked immunosorbent assay (ELISA)

All reagents were prepared before starting the procedure. Also, all standards and samples were added in duplicate to the micro ELISA testing plates. 50µl of the standard was added to the standard well and 10 µl of the test sample was then added. This was followed by the addition of 40µl of the sample diluent to the sample well and was then blanked. 100µl of HRP- conjugate reagent was added to each well and the mixture was covered with an adhesive strip and was incubated for 60 minutes at 37^0C.

Each well was aspirated and washed by filling each well with wash solution (400 µl) for four times (4x) using a squirt bottle manifold dispenser. After the last wash, the remaining wash solution was removed by decanting. The plate was inverted and blotted against a clean filter paper. 50µl of chromogen solution A and 50µl of chromogen solution B was added to each well. It was gently mixed and incubated for 15 minutes at 37^0C. The optical density (O.D.) was read at 450 nm wavelength using a microtiter plate reader within 15 minutes.

2.3.5 Coagulation factor VIII: Enzyme linked immunosorbent assay (ELISA)

All reagents were prepared before starting the procedure. Also, all standards and samples were added in duplicate to the micro ELISA testing plates. 50µl of the standard was added to the standard well and 10 µl of the test sample was then added. This was followed by the addition of 40µl of the sample diluent to the sample well. 100µl of HRP- conjugate reagent was added to each well and the mixture was covered with an adhesive strip and was incubated for 60 minutes at 37^0C.

Each well was aspirated and washed by filling each well with wash solution (400 µl) for four times (4x) using a squirt bottle manifold dispenser. After the last wash, remaining wash solution was removed by decanting. The plate was inverted and blotted against a clean filter paper. 50µl of chromogen solution A and 50µl of chromogen solution B was added to each well. It was gently mixed and incubated for 15 minutes at 37^0C. The optical density (O.D.) was read at 450 nm wavelength using a microtiter plate reader within 15 minutes.

2.3.6 Interleukin 6: Enzyme linked immunosorbent assay (ELISA)

All reagents were kept at room temperature (25^oC) prior to assay and the test was performed at room temperature. The reagents were gently mixed before use and the desired number of coated strips were placed into the holder. 100uL each of standards, controls and samples were dispensed into appropriate wells. Thereafter, the plates were covered with the sealer provided in the kit. The mixture was incubated for 90 min at 37°C. The liquid from each well was decanted. Immediately 100 µL of Biotinylated Detection Ab working solution was added to each well. The plate was covered with

a new sealer and was incubated for 1 hour at 37°C. After decanting the solution from each well, 350 µL of wash buffer was added to each well and was soaked for 1 min and aspirated.

The wash step was repeated 3 times. 100 µL of HRP Conjugate working solution was added to each well and the plate was covered with a new sealer. The samples were incubated for 30 min at 37°C. Thereafter, the solution from each well was decanted and the wash process was repeated for 5 times. 90 µL of Substrate Reagent was added to each well and the plate was covered with a new sealer. Incubation was allowed for about 15 min at 37°C. The Microplate Reader was preheated for about 15 min before measuring the OD. 50 µL of Stop Solution was added to each well. The optical density (OD value) of each well was determined at once with a micro-plate reader set to 450 nm.

2.3.7 Interleukin 10: Enzyme linked immunosorbent assay (ELISA)

All reagents were brought to room temperature (20~25°C) before use. The Standard working solution was added to the first two columns: Each concentration of the solution was added in duplicate, to one well each, side by side (100uL for each well). The samples were added to the other wells (100uL for each well). The plate was covered with the sealer provided in the kit. The mixture was incubated for 90 min at 37°C. The liquid was removed out of each well, without washing. Immediately, 100 µL of Biotinylated Detection Ab working solution was added to each well and was covered with the Plate sealer. It was gently mixed up followed by one-hour incubation at 37°C. The solution from each well was decanted and 350 uL of wash buffer was added to each well. It was soaked for 1~2 min and the solution from each well was decanted and dried against clean absorbent paper. The wash step was repeated 3 times. 100 µL of HRP conjugate working solution was added to each well and was covered with the Plate sealer. The mixture was incubated for 30 min at 37°C. The solution from each well was decanted, the wash process was repeated for five times. 90 µL of substrate reagent was added to each well. It was covered with a new plate sealer. Again, it was incubated for about 15 min at 37°C and the plate was protected from light. 50 µL of Stop Solution was added to each well and the optical density (OD value) of each well was determined at once with a micro-plate reader set to 450 nm.

2.4 Statistical Analysis

Data generated from this study were analyzed using Statistical Package for Social Sciences (SPSS) version 27.0, windows 10. The results were analyzed using one-way analysis of variance (ANOVA) and Turkey Post Hoc. Results expressed as mean ± Standard deviation and a p-value < 0.05 was considered statistically significant.

3 RESULTS

Table 2: Comparison of Some Haemostatic and Immunological Parameters in Female Rats treated with Graded Doses of Estradiol Valerate (EV) and Control.

Parameter	Control	High	Medium	Low	F	P-value
PLT ($\times 10^9$/L)	264.00 ± 84.07	780.80 ± 38.77^{acd}	443.00 ± 80.75^{ab}	428.80 ± 29.74^{ab}	58.89	.000
PT (secs)	37.20 ± 1.30	33.40 ± 2.07^{ad}	43.60 ± 3.05	42.60 ± 3.58^{b}	6.93	.003
APTT (secs)	13.10 ± 0.95	11.46 ± 0.97^{ad}	14.16 ± 0.90^{d}	13.66 ± 1.11^{bc}	8.24	.002
Factor VII (U/L)	4.98 ± 0.63	3.70 ± 0.22^{cd}	4.46 ± 0.32^{ab}	4.46 ± 0.48^{ab}	43.17	.000
Factor VIII (U/L)	91.40 ± 4.22	72.20 ± 4.09^{a}	84.20 ± 2.59^{ad}	92.80 ± 7.66^{c}	8.54	.001
IL-6 (Pg/mL)	37.20 ± 1.30	33.40 ± 2.07	43.60 ± 3.05	42.60 ± 3.58^{a}	3.68	.034
IL-10 (Pg/mL)	13.10 ± 0.95	11.46 ± 0.97^{ad}	14.16 ± 0.90^{ad}	13.66 ± 1.11^{bc}	16.95	.000

Results are presented as Mean ± SD. P-value <0.05 is statistically significant; Key: a significantly different from Control; b significantly different from High Dose; c significantly different from Medium Dose ; d significantly different from Low Dose

Table 3: Mean ± Standard Deviation (SD) of Pre- Treatment and Post-Treatment Weight of Female Albino Wistar Rats

Group	Dose	N	Baseline Weight (g)	Final Weight (g)
Control		5	165.00 ± 11.18	182.00 ± 7.14
Estradiol Valerate	High	5	177.00 ± 8.92	196.80 ± 5.40
	Medium	5	155.60 ± 6.02	177.60 ± 6.27
	Low	5	165.00 ± 11.18	182.00 ± 7.14

Key: Estradiol valerate (EV)

4 DISCUSSION

This study assessed the effects of oral estradiol valerate (EV) on selected haemostatic and immunological parameters using female albino wistar rats. The parameters evaluated in this study included the following: Platelet count, PT, APTT, factor VII, factor VIII, IL-6 and IL-10 markers.

Platelet count was significantly higher in all EV-treated groups compared with the control, with the most pronounced increase occurring in the high-dose group. The high-dose group was also significantly different from both the medium- and low-dose groups, indicating a particularly marked platelet response at the highest EV dose.

This finding suggests that EV may influence thrombopoiesis or platelet turnover, potentially through estrogen-receptor-mediated effects on megakaryocyte development and platelet production. Estrogen receptors are expressed within the megakaryocytic/platelet system, and estrogen has been demonstrated to influence platelet biology through both genomic and non-genomic pathways (Valéra et al., 2019). The substantial thrombocytosis observed in the present study may be related to the relatively high EV exposure rather than representing a generalized effect of estrogen.

PT was significantly altered among the groups. The shortened PT observed at high-dose EV exposure may indicate relatively enhanced activity of the extrinsic/common coagulation pathway. Evidence has shown that estrogen can influence hepatic synthesis of coagulation proteins. Recent reviews indicate that estrogens can modify several procoagulant pathways, including factors VII, VIII, X, fibrinogen and von Willebrand factor, although the magnitude and direction of these effects depend on estrogen type, dose and route of administration (Baldwin et al., 2023).

The high-dose finding is therefore partly in consonance with the established haemostatic effects of estrogen, particularly evidence that estrogen-containing therapies can produce changes favouring coagulation. Coleman et al. (2023) demonstrated experimentally that estradiol can promote a hypercoagulable phenotype and alter fibrin biology. Similarly, recent reviews emphasize that systemic estrogen therapy can alter haemostatic pathways and increase thrombotic risk in susceptible settings (Restrepo et al., 2024).

The shortened APTT at high-dose EV exposure may indicate increased efficiency of the intrinsic/common coagulation pathway or increased availability of coagulation substrates. This observation is compatible with the concept that estrogen can shift the haemostatic balance toward increased coagulation potential. A contemporary review of estrogen effects on haemostasis emphasizes that estrogen can affect multiple coagulation pathways and that relatively small changes in several haemostatic markers can collectively alter thrombotic risk (Stanczyk et al., 2025).

The longer APTT observed in the medium- and low-dose groups is not in complete agreement with a uniform estrogen-induced hypercoagulable response. Experimental evidence demonstrates that natural estradiol and synthetic ethinylestradiol can have different effects on PT, APTT and individual coagulation factors. In a rat model, ethinylestradiol significantly prolonged PT and APTT, whereas estradiol itself produced considerably different effects Franco-Murillo & Jaimez, 2017). This finding is particularly relevant because the present study used estradiol valerate rather than ethinylestradiol. Differences in molecular structure, pharmacokinetics and hepatic exposure may therefore contribute to discrepancies between studies.

This finding is particularly interesting because factor VII is a major component of the tissue-factor-dependent extrinsic coagulation pathway. The reduction in factor VII observed at high-dose EV exposure is not in consonance with the commonly reported tendency of estrogen-containing therapies to increase hepatic synthesis of several procoagulant factors, including factor VII. Current literature indicates that estrogen exposure can increase procoagulant proteins, although the magnitude varies considerably with estrogen type, dose and route (Baldwin et al., 2023).

The reduction in factor VIII in the high-dose group is not in consonance with much of the clinical literature on estrogen exposure, which generally associates estrogen with increased factor VIII and von Willebrand factor. A recent meta-analysis found that higher plasma factor VIII concentrations are associated with increased venous thromboembolism risk (Lowe et al., 2023). Recent studies similarly describe factor VIII among the coagulation proteins that may increase following estrogen exposure (Baldwin et al., 2023).

The present finding, however, is consistent with evidence that estradiol does not always produce the same coagulation response as synthetic estrogens. Experimental work in rats has shown that estradiol and ethinylestradiol may have markedly different effects on coagulation factors (Inhibitory effect of ethinylestradiol on coagulation factors in rats, 2016). Furthermore, an increase in factor VIII is not an obligatory response to every estrogen exposure, particularly when the dose, route, exposure duration and hormonal background differ. The divergent factor VII and VIII responses suggest that EV may differentially regulate individual coagulation proteins rather than uniformly activating the coagulation cascade.

The decrease in IL-6 value in the high-dose group may suggest an anti-inflammatory effect of EV at the highest dose, although the difference from the control was not identified as statistically significant according to the post-hoc notation. Estrogen can regulate inflammatory signaling through estrogen receptors, including modulation of NF-κB-dependent inflammatory pathways. Recent experimental work in ovariectomized female Wistar rats showed that estrogen treatment reduced inflammatory cytokine activity, including IL-6, supporting an anti-inflammatory role for estrogen under certain conditions.

This finding is therefore partly in consonance with recent experimental evidence. In ovariectomized diabetic female rats, estradiol treatment reduced cardiac inflammatory cytokines, including IL-6, while estrogen-receptor antagonism increased IL-6 and reduced IL-10, indicating an estrogen-dependent anti-inflammatory pathway (Azizian et al., 2022).

Conversely, the significantly higher IL-6 concentration in the low-dose group compared with the control is not in consonance with a uniformly anti-inflammatory effect of estrogen. The increase at low dose, coupled with the reduction at high dose, suggests a non-linear response. Such a pattern may arise from concentration-dependent receptor activation, differences in estrogen receptor subtype signaling or feedback regulation of cytokine production. Experimental evidence indicates that estrogen can either suppress or enhance inflammatory cytokine responses depending on tissue, dose and inflammatory context (Shivers et al., 2015).

IL-10 is an important anti-inflammatory cytokine that limits excessive inflammatory responses. The reduction in IL-10 at high-dose EV exposure is therefore potentially important because it suggests that high-dose EV did not simply exert a uniformly anti-inflammatory effect. Indeed, the simultaneous numerical reduction in IL-6 and IL-10 in the high-dose group suggests that EV may have suppressed aspects of cytokine production more broadly rather than selectively suppressing a pro-inflammatory pathway.

The reduction in IL-10 at high-dose EV exposure is not in consonance with several experimental studies reporting increased anti-inflammatory IL-10 following estrogen treatment. For example, estrogen replacement has been shown to increase IL-10 in experimental inflammatory settings, suggesting that estrogen may enhance anti-inflammatory signaling under some conditions (Shivers et al., 2015). Similarly, estrogen treatment in ovariectomized diabetic female rats decreased IL-6 while increasing IL-10, whereas blockade of estrogen receptors produced the opposite inflammatory pattern. The difference between those findings and the present study may reflect the dose and hormonal context. Estrogen signaling is highly context dependent, and high pharmacological exposure may produce effects that differ from physiological or replacement-level exposure.

The findings indicate that EV produced substantial alterations in both haemostatic and immune parameters, but the responses were parameter-specific and non-linear. The most striking haemostatic finding was the marked increase in platelet count at high-dose EV, accompanied by shortening of PT and APTT. These changes could, collectively, suggest enhanced haemostatic potential. However, this interpretation is moderated by the simultaneous reduction in factors VII and VIII at high dose. Therefore, the findings do not demonstrate a uniformly procoagulant effect of EV.

This complex pattern is consistent with contemporary understanding of estrogen-associated haemostasis. Estrogen can alter multiple components of coagulation, anticoagulation and fibrinolysis simultaneously, and the net haemostatic phenotype depends on the balance among these pathways rather than on a single marker (Dix et al., 2024; Restrepo et al., 2024). Importantly, recent reviews emphasize that the type, dose and route of estrogen administration substantially influence its haemostatic effects.

The immunological findings similarly indicate a complex response. The low-dose group had significantly higher IL-6 than the control, whereas high-dose EV showed numerically lower IL-6 and significantly lower IL-10. Thus, EV did not produce a uniform dose-dependent anti-inflammatory or pro-inflammatory response. Rather, the findings suggest that the immunomodulatory effects of EV may be concentration dependent. Oral estrogen has greater hepatic exposure because of first-pass metabolism, which can influence hepatic synthesis of coagulation proteins (Baldwin et al., 2023).

The present study also assessed changes in body weight following administration of different doses of estradiol valerate (EV) to female albino Wistar rats. As presented in Table 3, body weight increased from baseline to the end of treatment in all groups as well as in the control rats. Although weight gain occurred in all groups, there was no obvious linear dose-dependent pattern across the EV-treated groups.

The increase in body weight observed across the groups may represent normal growth and physiological changes during the experimental period rather than a specific effect of EV. In addition, body weight represents the combined contribution of lean tissue, adipose tissue, skeletal growth and body fluids and therefore may not necessarily reflect changes in adiposity alone.

These observations are partly in consonance with Barath et al. (2022), who investigated the effects of estradiol valerate in rats and reported a significant increase in body weight in their control animals over the experimental period. Their

findings demonstrate that body weight can increase during experimental observation even in the absence of EV treatment.

Using female Wistar rats, Nishimura et al. (2022), demonstrated that estrogen supplementation significantly affected body weight and fat mass, with estrogen replacement producing a reduction in body-weight gain and food intake in estrogen-deficient animals. Similarly, the findings of this study differ from studies in ovariectomized rats in which estradiol replacement attenuated body-weight gain (Zeibich et al., 2021; Yokota-Nakagi et al., 2022). The discrepancies may be attributable to differences in endogenous hormonal status, rat model, estradiol preparation, dose, treatment duration, diet and experimental conditions. Findings from this study suggest that the effect of EV on body weight is context dependent rather than uniformly weight reducing or weight increasing.

5 CONCLUSION

The findings demonstrate that estradiol valerate (EV) exposure was associated with significant alterations in selected haemostatic parameters, including platelet count, prothrombin time (PT), activated partial thromboplastin time (APTT), factor VII and factor VIII. The observed changes across treatment groups suggest that EV may modulate multiple components of the coagulation system, with some parameters showing responses at higher doses. These findings support the potential influence of estrogenic exposure on haemostatic regulation.

There were no statistically significant differences in weight change between the control and treatment groups, although all groups demonstrated weight gain during the study period. This suggests that, under the conditions and duration of the present study, EV exposure did not significantly alter body-weight change in female albino Wistar rats.

Recommendations

Long-term studies are recommended to evaluate the effects of sustained EV exposure, particularly on hepatic architecture and function and other organs involved in hormonal and haemostatic regulation.

Further studies should incorporate additional haemostatic biomarkers, including fibrinogen, D-dimer, protein C, protein S, antithrombin, von Willebrand factor and markers of platelet activation, to provide a more comprehensive assessment of the effects of EV on haemostatic regulation.

Further investigations should assess the mechanisms underlying the observed alterations in inflammatory cytokines, particularly IL-6 and IL-10, and their relationship with haemostatic changes following EV exposure.

Contribution to Knowledge

This study demonstrates that graded EV exposure is associated with alterations in several components of the haemostatic system, including platelet count, PT, APTT, factor VII and factor VIII. The marked elevation in platelet count observed in the high-dose EV group suggests that estrogenic exposure may influence platelet-related haemostasis.

The study demonstrates that EV does not produce a uniform dose-dependent response across all haemostatic parameters. The high-dose group showed the highest platelet count and shorter PT/APTT values, while factor VII and factor VIII showed different patterns of response. This finding highlights the complexity of estrogen-associated modulation of haemostasis, in which individual coagulation components may respond differently to the same hormonal exposure.

The study provides evidence that EV exposure is associated with alterations in inflammatory cytokines. The significant changes observed in IL-6 and IL-10 indicate that EV may influence both pro-inflammatory and anti-inflammatory cytokine profiles. The differing responses among the graded dose groups further suggest that these effects may vary according to exposure level.

By concurrently evaluating platelet count, PT, APTT, factors VII and VIII, IL-6 and IL-10, this study provides an integrated assessment of selected haemostatic and immunological responses to EV exposure. The findings contribute to the understanding of how estrogenic exposure may affect multiple physiological systems in female albino Wistar rats.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

Statement of ethical approval

Ethical approval for this study involving female albino Wistar rats was obtained from the Madonna University Nigeria Research Ethics Committee (MUN-REC), with reference number MUN/VC/REC/PG/MLS/23/002. The study was conducted in accordance with applicable guidelines for the care and use of laboratory animals.

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