GPH-International Journal of Biological & Medicine Science
(Online-ISSN 3050-9610)
Volume 09 | Issue 02 | February 2026
Published by: Global Publication House
DOI: https://doi.org/10.5281/zenodo.19203236
Available online at www.gphjournal.org
Journal homepage: https://gphjournal.org/index.php/bs/index

Phytosterol Promotes Ovulatory Functions in Wistar Rats by Shortening the Estrous Cycle

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Abstract

Menstrual cycle interruption is one of the most predominant reproductive endocrinopathies globally. The present study investigated the ovulatory functions of phytosterol using female Wistar rat as experimental models. Fifteen (15) female Wistar rats were randomly divided into three (3) groups, five (5) rats each. Group I served as control, and rats in this group had free access to normal rat chow and clean drinking water ad libitum. Groups II and III served as treatment groups and received 1000 and 2000mg/kg body weight of phytosterol respectively. Vaginal smears of each experimental rat were collected morning of every day of the study between 7 - 9 a.m. for four weeks (2 weeks before treatment and 2 weeks during treatment) for the determination of estrous cycle. Our findings show a significant and dose dependent reduction in the values of proestrus, estrus, metestrus and diestrus amongst Groups 2 and 3 rats administered graded doses (1000mg/kg and 2000mg/kg body weight) of phytosterol compared to Group 1 (control) rats ($p<0.05$); Suggesting a possible ovulatory modulatory effect of phytosterol. Phytosterol offers a promising alternative in the management of menstrual dysfunction.

Keywords:

Phytosterol, proestrus, estrus, diestrus, and metestrus.

INTRODUCTION

Menstrual cycle interruption is one of the most predominant reproductive endocrinopathies, affecting approximately 30–40% of reproductive-age women globally, with 50% of them experiencing infertility [1,2]. Infertility is defined as the inability to conceive after a year of

consistent unprotected sexual intercourse [3]. Infertility in most women is attributed to abnormalities linked to ovarian folliculogenesis. Premature maturation and differentiation of granulosa cell has been proven to be responsible for preantral follicular growth, cyst development cum anovulation [4]. Infertility is commonly managed with medications like gonadotropins, clomiphene citrate and metformin. These interventions were aimed at improving ovulatory activity, reducing menstrual irregularity and improving estrous cycle [5].

Treatments for menstrual irregularity and anovulatory functions may reduce symptoms, but they are commonly associated with reasonable adverse effects. [6] Furthermore, some interventions like gonadotropins cum surgical procedures are quite expensive, time-consuming, and their utilization require close monitoring, hence the need to develop a novel bio-active substance that is more efficient, better tolerated and less expensive than currently available pharmacological interventions and surgical procedures. Literature has shown that plants including Tribulus terrestris [7], A*llium fistulosum* [8], C*raterispermum schweinfurthi* [9], Turmeric [10, 11] and Cucumber [11, 12] are effective in restoring hormonal profiles, follicular indices, ovarian functions and oxidative stress in rats. These findings imply that plants with fertilizing qualities can ameliorate menstrual abnormalities and improve estrous cycle.

Interest in plant-derived compounds, such as phytosterols, for the management of female infertility is growing worldwide, partly due to their potential effects on hormonal balance and antioxidant activity [1, 2]. Products from medicinal plants with antioxidant and reproductive benefits are particularly prominent in Africa [13]. Several herbal remedies have demonstrated notable positive effects on reproduction [14, 15].

Phytosterols are structurally like cholesterol and play a key role in maintaining the integrity of plant cell membranes [16]. Their primary natural sources are vegetable oils and related products [16]. Local studies suggest that phytosterols may exert modulatory effects on the female endocrine system [17]. Many plant-derived compounds influence fertility through various molecular mechanisms, including direct interactions with hormone receptors, regulation of gene expression, and modulation of enzymatic pathways [18, 19]. Phytosterols may mimic or modulate the synthesis and release of key reproductive hormones [20]. These actions can either enhance or disrupt intracellular hormonal signalling, potentially alleviating certain hormonal imbalance [20]. Inspite of the above documented properties, its menstrual and estrous related functions have not yet been explored in animal models. The effects of phytosterol on the estrous cycle was studied in this regard.

MATERIALS AND METHODS

Source of Phytosterol

Phytosterol was purchased from Wakunaga of America Co., LTD. Mission Viejo, CA92691 U.S.A. Capsules were dissolved in tween 80 and constituted into 1000mg/kg and 2000mg/kg body weight respectively.

Sourcing and Handling of Experimental Rats

A total of 15 adult female Wistar rats weighing between 100-250g were procured from PAMO University of Medical Sciences animal house, Port Harcourt, Nigeria. Procured rats were placed in plastic cages, one for each study group, after two (2) weeks of acclimatization, experimental rats were subsequently divided into study groups and used for the study and cared for under standard laboratory conditions [21].

Ethical Consideration

The protocol guiding the research, experimental design and methods, were reviewed and given approval by the PAMO University of Medical Sciences Research, Grants and Ethics Committee vide a communication referenced PUMS/REC/2025030.

Acute Toxicity

Acute toxicity level of phytosterol was found to be >3000mg/kg body weight as previously reported by Carlos and Ma'rcia-regina, (2017) [22] and further validated by Saronee *et al.*, (2024) [9]

Study Design

A total of Fifteen (15) female Wistar rats were used for the study and were divided randomly into three (3) groups of five (5) rats each. Group I served as control, and rats in this group had free access to only normal rat chow and distilled water ad libitum. Groups II and III served as treatment groups and received 1000 and 2000mg/kg body weight of phytosterol respectively. Experimental rats were granted unhindered access to standard rat chow and clean drinking water.

Determination of Estrus Cycle

1. Vaginal smears of each experimental rat were determined as previously described by Mclean et al., (2012) [23].
2. Smears were collected morning of every day of the study between 7 - 9 a.m. for four weeks (2 weeks before treatment and 2 weeks during treatment).
3. A 1ml rubber pipette, beakers, distilled water, glass slide, and a light microscope were used. And animals were held in a supine position while 0.5ml of distilled water was flushed into the vaginal opening twice with the rubber pipette.
4. Vaginal fluid was carefully collected and deposited on a glass plate for microscopic examination. [24]
5. Rubber pipettes used for the study were carefully washed to do away with any remaining cells before being used on the next animal.
6. vaginal smears staining protocol was as reported by Mclean *et al.*, (2012] [23, 24]. In 100 ml of double distilled water (ddH2O), 0.1 g of crystal violet powder was added and well blended. The crystal violet stain (0.1 percent) was kept at room temperature in a securely sealed container until needed. Crystal violet dye (0.1 percent) was applied to one side of the cover slip with an eye dropper.

7. Excess fluid was drained from the borders of the slide using filter paper until the stain was uniformly dispersed throughout the surface.
8. The proportion of nucleated epithelial cells, cornified epithelial cells, and leukocytes was determined immediately under a standard light microscope.
9. Animals whose vaginal smears contained mainly leukocytes (60 percent) were categorized as diestrus using the Tropp and Markus [25] method. Proestrus smears had a high percentage of nucleated epithelial cells (60%) and a low percentage of leukocytes (10%). Estrus was defined as smears with a high percentage of cornified cells (90%). Metestrus was defined as smears that comprised predominantly cornified cells (60%) with a substantial number of leukocytes (20%) and nucleated epithelial cells (20%).
10. After cytological assessment, stained smears were preserved, and photomicrographs were made.

Statistical Analysis

Statistical analysis was performed using SPSS to determine mean values and standard error of mean (SEM). An ANOVA test to determine the mean differences among all treatment groups was performed, and a post hoc test using LSD. Data were expressed as mean ± standard error of mean and a p-value <0.05 was considered significant.

RSULTS

Table 1 shows significant and dose dependent reduction in the values of proestrus, estrus, metestrus and diestrus (estrus cycle) amongst Groups 2 and 3 rats administered graded doses (1000mg/kg and 2000mg/kg body weight) of phytosterol relative to Group 1 (control) rats ($p<0.05$). These findings suggest that phytosterol can stimulate ovulation and manage menstrual dysfunction by shortening the estrous cycle.

Table 1: Values of Phytosterol Administration on Estrus Cycle

Groups	Treatment	Proestrus	Estrus	Metestrus	Diestrus
GROUP 1	Control 1.17±0.01	3.37±0.04	2.76±0.02	5.11±0.06	
GROUP 2	Low Dose Phytosterol Group (1000mg/kg)	0.75±0.03	2.09±0.17*	0.72±0.01*	3.42±0.03*
GROUP 3	High Dose Phytosterol Group (2000mg/kg)	0.36±0.04*	1.09±0.04*	1.75±0.05*	1.81±0.02*

Values expressed as Mean ± SEM. n=5. * = significant compared to Group 1(Control) ($p<0.05$)

DISCUSSION

Endocrine hormones play a significant role on the overall activity of the female reproductive system. [26] The hypothalamus controls the cyclic changes in the ovaries and uterus via Gonadotropin-releasing hormone (GnRH) [27, 28]. This hormone is carried in the bloodstream and causes the pituitary gland to release the gonadotropins: follicle stimulating hormone (FSH) and the luteinizing hormone (LH) [29]. The pituitary gland synthesizes the follicle-stimulating hormone (FSH) during the initial phases of the menstrual cycle. This is needed to produce follicles in the ovaries [30]. In the present study, oral treatment of phytosterol reduced the rats' reproductive cycle compared to control rats. However, an extended estrous cycle has been reported previously following lead acetate treatment probably due to hormonal disruption or inhibition of the hypothalamo-pituitary-ovarian-axis Kolawole *et al.,* (2014) [31].

A cycle with a diestrus phase of about 4 days and/or an estrous length of a minimum of 3 days was regarded anomalous [24, 28]. We observed significant differences in the length of the phases of estrous cycle in the phytosterol treated groups relative to control. A remarkable shortening of the proestrus, estrus, metestrus, and diestrus phases, was observed in the 1000mg/kg and 2000mg/kg treated phytosterol groups. A shorter reproductive cycle suggests an early ovulation onset which is indicative of a possible fertility potential [32, 33, 34].

Finally, findings from this study reveal that phytosterol had a positive effect on the estrous cycle in female rats, suggesting that phytosterols are beneficial to the rats' reproductive health. However, further study is recommended to determine long-term consumption safety and precise mechanisms of action.

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