



Evaluation of the Biochemical Effects of Ethanolic Extract of *Cola Nitida* (Kola Nut) on Female Reproductive Hormones in Albino Rats

Arunsi Ogbonnaya Mba^{1*}, Nwahiri Jude Donatus², and Lucky Chiamaka Veronica²

¹Department of Medical Laboratory Science, Abia-State University, Uturu

²Department of Medical Laboratory Science, Madonna University, Elele

Corresponding Author: Arunsi Ogbonnaya Mba

Department of Medical Laboratory Science, Abia-State University, Uturu

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ABSTRACT

The Kola nut (*Cola nitida*) is commonly used in West Africa due to its stimulant characteristics and cultural importance; yet, its possible effects on female reproductive endocrine function are poorly understood. This study investigated the biochemical effects of ethanolic extract of *Cola nitida* on selected reproductive hormones in female albino rats. Experimental study design was utilised. Female albino rats were randomly assigned into three groups. Group I was the control and was given distilled water and normal rat meal. Group II and III were given normal rat feed and distilled water supplemented with the ethanolic *C. nitida* extract at 100 and 400 mg/kg body weight respectively for 28 days. At the end of the therapy period blood samples were taken and sera were separated for hormonal analysis. Serum concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin and progesterone were measured by enzyme linked immunosorbent assay (ELISA). Data were represented as mean $\pm$ standard deviation and analysed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test and significance was established at $p < 0.05$. The results demonstrated significant changes in the concentrations of all the studied hormones. The FSH concentration was lowered from 5.15 ± 0.13 mIU/mL in control group to 4.78 ± 0.15 mIU/mL in high dosage group ($p = 0.015$). Likewise, there were substantial reductions in prolactin and LH concentrations in all therapy groups ($p = 0.001$ and 0.004 , respectively). However, the progesterone concentration was considerably enhanced from 1.60 ± 0.08 ng/mL in the control group to 2.43 ± 0.15 ng/mL in the high-dose group ($p < 0.001$). These results demonstrate that the ethanolic *C. nitida* extract altered the reproductive hormonal profile of female albino rats, characterised by decrease in FSH, LH and prolactin and increase in progesterone. The modifications observed, particularly at higher doses, indicate that long-term use of *C. nitida* may impact on the hypothalamic-pituitary-ovarian axis and subsequently alter the female reproductive endocrine function.

Keywords: *Cola nitida*; kola nut; reproductive hormones; follicle-stimulating hormone; luteinizing hormone; progesterone; prolactin; albino rats.

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INTRODUCTION

Women's reproductive health is an integral component of overall health and well-being, especially during the reproductive years. Normal reproductive function requires the precise coordination of the hypothalamic-pituitary-ovarian (HPO) axis and regulates the synthesis, secretion and physiological actions of hormones involved in follicular development, ovulation, endometrial preparation, implantation and maintenance of pregnancy [1]. Disruption of this endocrine axis may adversely affect reproductive function, contributing to monthly irregularities, anovulation, decreased fertility, problems with pregnancy, and other reproductive illnesses. Therefore, it is vital to identify nutritional, environmental, pharmacological and plant derived chemicals that may alter reproductive hormone concentrations so as to

comprehend aspects that may influence female reproductive health [2]. The main hormones involved in the regulation of female reproductive processes are follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone and prolactin. FSH and LH are gonadotropins synthesised and produced by the anterior pituitary gland in response to gonadotropin-releasing hormone (GnRH) from the hypothalamus [3]. FSH is involved in the recruitment and maturation of ovarian follicles, and stimulates granulosa cells to produce oestrogen. LH participates in follicle maturation and after the LH surge in the middle of the cycle, LH produces ovulation and stimulates the formation and functional activity of the corpus luteum. Progesterone, largely secreted by the corpus luteum after ovulation, is necessary for conversion and maintenance of the endometrium to prepare it for implantation and to

support early pregnancy. Another hormone of the anterior pituitary is prolactin, which is mostly involved with development of the mammary glands and breastfeeding, but also interacts with the reproductive endocrine system and may affect ovarian function and the secretion or action of other reproductive hormones [4].

Reproductive hormones concentrations are affected by complicated feedback processes involving the brain, pituitary gland and ovaries. Thus, changes in the secretion or activity of any of these hormones may affect ovarian function and reproductive capacity. For example, aberrant gonadotropin secretion can affect follicular development and ovulation while changes in progesterone secretion can affect endometrial receptivity and pregnancy maintenance. Likewise, deviations in the levels of prolactin may lead to disruptions in the regular reproductive function. Therefore, it is crucial to investigate compounds that can influence these hormonal pathways especially where these compounds are regularly used as foods, drinks, traditional treatments or stimulants [5].

In many African communities especially in Nigeria and other West African countries, plant derived products are used widely because of its nutritional, cultural, medical and social significance. Natural products are thought to be safe, yet regular or large doses of natural products can cause significant physiological consequences, due to the presence of biologically active ingredients. Alkaloids, flavonoids, tannins, saponins, phenolic chemicals and other secondary metabolites that can affect metabolic and endocrine systems are found in several medicinal and food plants [6]. Increased usage of plant-based stimulants hence necessitates exploration of their possible effects on physiological systems in addition to their intended or traditional uses.

One plant product of enormous cultural and economic value in West Africa is the Kola nut, which is obtained mainly from *Cola nitida* and *Cola acuminata*. The nut is typically used in ceremonies, social gatherings and cultural customs and is generally chewed due of its stimulating effects. It is also used to improve alertness, minimise the feeling of weariness and maintain awake during prolonged physical or mental activities [7]). The pharmacological activities of kola nut are mainly due to the presence of a wide range of phytochemical elements such as methylxanthine alkaloids like caffeine and theobromine, tannins, flavonoids, saponins and other bioactive compounds. Among these chemicals, caffeine is one of the major contributions to the stimulant effect of kola nut [8].

Caffeine and other methylxanthines may act through many methods including regulation of adenosine receptors and intracellular cyclic adenosine monophosphate (cAMP)-dependent pathways. These systems can affect neurotransmitter release, sympathetic

nerve activity, metabolic activities, and endocrine responses. The hypothalamus and pituitary gland are sensitive to neuroendocrine signals and drugs that may alter central nervous system activity may have the ability to influence reproductive hormone release. Previous studies have suggested that consumption of caffeine may alter circulating levels of some reproductive hormones including prolactin and gonadotropins, but the direction and magnitude of these effects appear to be dependent on variables such as dose, duration of exposure, physiological status and the specific preparation [10].

Because the biological activity is not due just to caffeine, the possible reproductive effects of kola nut are of special interest. Other phytochemicals in the nut, including as tannins, alkaloids, flavonoids and related substances may interact with endocrine and metabolic processes. Experimental data has raised concerns that prolonged or excessive exposure to various plant-derived stimulants may influence reproductive processes however evidence regarding *C. nitida* and female reproductive hormones is scarce and conflicting. Differences in plant species, extraction procedures, dosages, duration of exposure, animal models, and hormonal indicators examined may partly explain the heterogeneity of published findings [11].

Little attention has been paid to the effects of kola nut on the female reproductive endocrine system, despite its wide usage in West Africa. There is in particular dearth of evidence on the effects of ethanolic *C. nitida* extract on circulating FSH, LH, progesterone and prolactin concentrations. The importance of determining if exposure to different doses of kola nut extract affects these hormones lies in the fact that these changes could serve as biochemical indications of its possible influence on the HPO axis. This is particularly the case in populations where kola nut is regularly ingested and in certain instances in large amounts.

Therefore, this study was aimed at determining the biochemical effects of ethanolic extract of *Cola nitida* (kola nut) on several female reproductive hormones (FSH, LH, progesterone and prolactin) in albino rats after 28 days of exposure to various doses of the extract. The results are intended to help to the knowledge of the possible reproductive endocrine effects of *C. nitida* and provide new scientific information on the safety implications of the frequent ingestion of *C. nitida*.

MATERIALS AND METHOD

Study Area

This study was carried out in Madonna University Teaching Hospital Elele, Rivers State, Nigeria. Elele town is located in the South Eastern part of Nigeria. It is located in latitude 5° 27' - 5° and longitude 6° 55' - 7° 85'E. The climate of the area is tropical with the mean daily temperature of 29°C for most of the year. The annual rainfall in this region is between 217 and 240cm. There are other towns and village that surround Elele

town, they include Isiokpo town, Omagwa, Ahoda, Omoku, Owerri town and others.

Research Design

This study employed an experimental research design using female albino rats to evaluate the comparative biochemical effects of Ethanolic extract of kola nut on female reproductive hormones of albino rats. Rats were divided into experimental groups and administered plant juices orally for 28 days, after which serum hormonal levels were analysed.

Experimental Animals

A total of 12 female albino rats, aged 8–10 weeks and weighing 150–200 g, were used for the study. Animals were procured from an accredited animal house and were allowed to acclimatize for two weeks before the experiment.

Inclusion and Exclusion Criteria

Inclusion:

1. Healthy female albino rats
2. Body weight within 150–200 g
3. No prior exposure to chemicals or medications

Exclusion:

1. Sick or weak animals
2. Albino rats showing signs of distress
3. Albino rats below or above the weight range

Ethical Considerations

Ethical approval was obtained from the Research Ethics Committee (REC) of Madonna University. All procedures conformed to international guidelines for the care and use of laboratory animals. Humane handling, minimal stress exposure, and proper euthanasia methods were strictly adhered to.

Preparation of Ethanolic Kola Nut Extract

Collection and identification: Fresh kola nuts (*Cola nitida*) were sourced from a local market and the kola nuts were carefully inspected to ensure there was no from physical damage or rot and were washed thoroughly with clean water before use.

Preparation of crude Ethanolic extract: The kola nuts were washed, air-dried, and the seed coat removed. The nuts were oven-dried at 40–50°C until a constant weight is obtained, then pulverized into a fine powder using an electric grinder. A measured quantity (e.g., 200 g) of the powdered *Cola nitida* was soaked in 70% ethanol (1:10 w/v) at room temperature for 48–72 hours with intermittent stirring. The mixture was filtered using muslin cloth followed by Whatman No. 1 filter paper to remove particulate matter. The filtrate was concentrated under reduced pressure using a rotary evaporator to remove the ethanol and obtain the crude ethanolic extract. The concentrated extract was further dried in a water bath at low temperature (or desiccator) to obtain a solid extract. The dried extract was stored in an airtight container and kept in a refrigerator at 4°C until use.

Dose preparation: The dried extract was reconstituted in distilled water to prepare dosing solutions immediately prior to administration. Dose volumes were adjusted according to the most recent body weight of each rat and administered by oral gavage.

Reagent

The reagents that were used in this work were of analytical grade. They were purchased commercially and the manufacturer's standard operating procedure (SOP) was strictly followed.

- Follicle stimulating Hormone (FSH) AccuBind® ELISA Test with product number: 425-300
- Luteinizing Hormone (LH) AccuBind® ELISA Test with product number: 625-300
- Prolactin Hormone AccuBind® ELISA Test with product number: 725-300
- Progesterone Hormone AccuBind® ELISA Test with product number: 525-300

Experimental Grouping and Administration

A randomized controlled experimental design was used. Eighteen (12) female albino rats were randomly assigned into three groups (n = 4 per group):

1. Group I (Control): received normal rat feed and distilled water (vehicle) orally.
2. Group II (Low dose): received distilled water, normal rat feed and Ethanolic kola nut extract at 100 mg/kg body weight orally.
3. Group III (High dose): received distilled water, normal rat feed and Ethanolic kola nut extract at 400 mg/kg body weight orally.

Animal Feeding and Housing

Rats were housed in well-ventilated cages under standard laboratory conditions (12-hour light/12-hour dark cycle, temperature 25 ± 2°C). They were fed standard rat pellets and given water ad libitum. Bedding was changed regularly to maintain hygiene.

Sample Collection

At the end of the treatment period (day 28), animals were fasted overnight with access to water. Under light anaesthesia, blood samples were collected by ocular puncture into plain sample tubes.

The collected blood was allowed to clot at room temperature for 30 minutes and centrifuged at 3000 rpm for 10 minutes to obtain serum. Serum was aliquoted into labelled microcentrifuge tubes and stored at -80°C until hormonal assays were performed.

Biochemical Analysis

Luteinizing Hormone (LH)

Test: Quantitative measurement of serum LH using a LH Sandwich Enzyme Linked Immunoassay (ELISA) kit.

Principle: The assay employed a sandwich enzyme-linked immunosorbent assay (ELISA). Anti-LH antibodies were precoated onto microplate wells. Serum

LH bound to the capture antibody; after washing, a biotinylated detection antibody specific for LH was added, followed by streptavidin–horseradish peroxidase (HRP). Addition of tetramethylbenzidine (TMB) substrate produced a colorimetric reaction proportional to the amount of LH in the sample. The reaction was stopped with an acidic stop solution, and absorbance was measured spectrophotometrically at 450 nm. Concentrations were determined by interpolation from a standard curve.

Procedure: The microplate was equilibrated to room temperature. Standards, controls, and serum samples (in duplicate) were added to designated wells. The plate was incubated for the specified time at room temperature. Wells were washed (usually 3–5 times) with the supplied wash buffer to remove unbound substances. Biotinylated antibody was added to each well and incubated for the specified time. After washing, streptavidin–HRP conjugate was added and incubated. Following the final washes, TMB substrate was added and incubated until adequate color development occurred. The enzymatic reaction was stopped with stop solution, and the optical density was read at 450 nm using a microplate reader within 15 minutes. A standard curve was plotted (absorbance versus concentration), and sample LH concentrations were calculated. Results were expressed in mIU/mL or ng/mL depending on the kit used.

Follicle-Stimulating Hormone (FSH)

Test: Quantitative measurement of serum FSH using a FSH Sandwich Enzyme Linked Immunoassay (ELISA) kit.

Principle: The assay used a sandwich enzyme-linked immunosorbent assay (ELISA) format similar to the LH assay. Capture antibodies specific for FSH were precoated onto the microplate wells and bound FSH present in the samples. A biotin-labeled detection antibody and streptavidin–horseradish peroxidase (HRP) were subsequently added to form an antibody–antigen–antibody complex. Addition of tetramethylbenzidine (TMB) substrate produced a measurable color change proportional to the concentration of FSH in the sample.

Procedure: Standards, controls, and serum samples (in duplicate) were added to the precoated wells. The plate was incubated for the time specified by the kit at room temperature. The wells were washed thoroughly according to the manufacturer's instructions. Biotinylated anti-FSH antibody was added to each well and incubated. After washing, streptavidin–HRP conjugate was added and incubated. The wells were washed, TMB substrate was added, and the plate was incubated for color development before the addition of stop solution. Absorbance was read at 450 nm using a microplate reader, and FSH concentrations were calculated from a standard curve. Results were reported in ng/mL as specified by the kit manufacturer.

Progesterone

Test: Quantitative measurement of serum progesterone using a Competitive Progesterone Enzyme Linked Immunoassay (ELISA) kit.

Principle: Most progesterone kits for small molecules used a competitive enzyme-linked immunosorbent assay (ELISA) format. Progesterone present in the sample competed with enzyme-labelled progesterone for binding to a limited number of anti-progesterone antibody binding sites. The intensity of the signal was inversely proportional to the concentration of progesterone in the sample. Following incubation and washing, tetramethylbenzidine (TMB) substrate was added, and colour development was measured spectrophotometrically at 450 nm.

Procedure: Standards, controls, and appropriately diluted serum samples (in duplicate) were added to the wells containing a fixed amount of antibody. Enzyme-labelled progesterone conjugate was added (as required by the kit), and the mixture was incubated to allow competition between labelled and unlabelled progesterone molecules. After the incubation period, the plate was washed to remove unbound materials. TMB substrate was added and allowed to react for the prescribed period. The reaction was stopped, and the absorbance was read at 450 nm using a microplate reader. Progesterone concentrations were calculated by comparing sample absorbance values with the standard curve, taking into account the inverse relationship between signal intensity and progesterone concentration. Results were reported in ng/mL or nmol/L as specified by the kit manufacturer.

Prolactin

Test: Quantitative measurement of serum prolactin using a Rat Prolactin Sandwich Enzyme Linked Immunoassay (ELISA) kit.

Principle: A sandwich enzyme-linked immunosorbent assay (ELISA) format was used. Microplate wells were coated with anti-prolactin capture antibodies, and prolactin present in the samples bound to the capture antibodies. Following washing, a biotinylated detection antibody and streptavidin–horseradish peroxidase (HRP) were added. Tetramethylbenzidine (TMB) substrate produced a colorimetric reaction proportional to the prolactin concentration in the samples, which was measured spectrophotometrically at 450 nm.

Procedure: Kit reagents were equilibrated to room temperature, and standards, controls, and serum samples were added to the wells in duplicate. The plate was incubated as directed by the manufacturer, and the wells were washed to remove unbound proteins. Biotinylated anti-prolactin antibody was added to each well and incubated, followed by washing. Streptavidin–HRP conjugate was added and incubated, after which the wells were washed. TMB substrate was added and incubated until adequate color development occurred. The reaction was then stopped with stop solution, and absorbance was read at 450 nm using a microplate reader. A standard curve was generated, and prolactin concentrations were

calculated and expressed in ng/mL or ng/dL, as appropriate for the assay kit used.

Statistical Analysis

Data were recorded as mean $\pm$ standard deviation (SD). Statistical evaluation was performed using SPSS, Graph Pad Prism, or similar software.

Differences between groups were analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Values of $p < 0.05$ were considered statistically significant.

RESULT

Table 1: Showing the mean $\pm$ SD of FSH, Prolactin, LH and Progesterone among different study groups.

Groups	FSH (ng/mL)	Prolactin(ng/mL)	LH(mIU/ml)	Progesterone(ng/mL)
Group 1	5.15 $\pm$ 0.13	17.25 $\pm$ 0.96	7.38 $\pm$ 0.43	1.60 $\pm$ 0.08
Group 2	5.08 $\pm$ 0.17	16.50 $\pm$ 1.29	7.03 $\pm$ 0.43	2.05 $\pm$ 0.13
Group 3	4.78 $\pm$ 0.15	12.75 $\pm$ 1.26	5.93 $\pm$ 0.53	2.43 $\pm$ 0.15
F-value	6.915	16.740	10.610	44.673
p-value	0.015	0.001	0.004	0.000

Significant at $p < 0.05$.

Table 1 shows the levels of FSH, prolactin, LH and progesterone among the three study groups. Group 1 recorded mean values of 5.15 $\pm$ 0.13, 17.25 $\pm$ 0.96, 7.38 $\pm$ 0.43 and 1.60 $\pm$ 0.08, respectively. Group 2 recorded 5.08 $\pm$ 0.17, 16.50 $\pm$ 1.29, 7.03 $\pm$ 0.43 and 2.05 $\pm$ 0.13, while

Group 3 recorded 4.78 $\pm$ 0.15, 12.75 $\pm$ 1.26, 5.93 $\pm$ 0.53 and 2.43 $\pm$ 0.15. ANOVA showed significant differences among the groups for FSH (F=6.915, $p=0.015$), prolactin (F=16.740, $p=0.001$), LH (F=10.610, $p=0.004$), and progesterone (F=44.673, $p<0.001$).

Table .2: Showing the Post hoc comparison (Tukey HSD),of the mean value of FSH, LH, PROGESTERONE and PROLACTIN among different groups fed with kola nut (*cola nitida*)

Comparison	FSH (ng/ml)	Prolactin(ng/ml)	LH (mIU/ml)	Progesterone(ng/ml)
Group 1 vs Group 2	0.768	0.654	0.557	0.002*
Group 1 vs Group 3	0.016*	0.001*	0.004*	0.000*
Group 2 vs Group 3	0.049*	0.004*	0.021*	0.005*

Significant at $p < 0.05$.

Table 2: The table in 4.2 shows the multiple comparisons of the mean values of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Prolactin and Progesterone among the different experimental groups treated with different experimental groups treated with ethanolic extract of *Cola nitida*. Values were considered significant at $P < 0.05$

DISCUSSION

The present study assessed the effect of ethanolic extract of *Cola nitida* on selected female reproductive hormones namely follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin and progesterone in albino rats. Statistically significant variations were seen in the concentrations of all hormones investigated across the three study groups. Total FSH, LH and prolactin concentrations dropped with increasing exposure to the extract although progesterone concentration increased. The results indicate that the ethanolic extract of *C. nitida* may potentially influence the control of the endocrine functions of the female reproductive system, especially at the higher dose.

The mean FSH concentration was gradually decreased from 5.15 $\pm$ 0.13 mIU/mL in Group I to 4.78 $\pm$ 0.15 mIU/mL in Group III. One-way analysis of variance revealed that there was a significant difference between the groups (F = 6.915, $p = 0.015$). Post hoc

analysis of Tukey revealed that the difference was statistically significant between Group I and Group III and Group II and Group III but was not statistically significant between Group I and Group II. FSH is a gonadotropin released from the anterior pituitary gland and has a significant function in ovarian follicular recruitment, follicular maturation and regulation of ovarian steroidogenesis [12]. The decrease in FSH especially in group III may reflect a change in the secretion of pituitary gonadotropins or disturbance of normal hypothalamic –pituitary-ovarian (HPO) axis. However, because the hypothalamus gonadotropin releasing hormone (GnRH) and ovarian feedback were not directly examined in the present investigation, the exact mechanism responsible for the observed decline cannot be definitely determined. abnormalities in reproductive physiology have been observed to be associated with abnormalities in circulating FSH concentrations [13]. It was emphasised that perturbations in hypothalamic–pituitary control can affect gonadotropin secretion. Therefore, the current finding indicates that the administration of higher dose (200 mg/kg body weight) of *C. nitida* extract may impair the normal gonadotropic regulation [14].

Prolactin concentration was also substantially different among the study groups (F=16.740, $p=0.001$). The mean concentration of maximum was found in group I (17.25 $\pm$ 0.96) and minimum in group III (12.75 $\pm$ 1.26).

The post hoc test of Tukey showed significant difference between Group III and the other two groups (Group I and Group II). Prolactin is involved in mammary gland development and lactation but also regulates female reproductive function through interactions with the hypothalamic–pituitary–ovarian system. The present finding of a gradual decrease in prolactin may suggest an alteration in pituitary endocrine function or in the hypothalamus processes controlling prolactin release. The major inhibitor of prolactin release is hypothalamic dopamine and hence alterations in hypothalamic-pituitary signalling can significantly impact circulating prolactin levels. Alterations in pituitary and hypothalamus function have been shown to have a considerable effect on circulating prolactin concentrations [15]. Similarly, changes in hypothalamic regulation mechanisms have been shown to affect prolactin secretion. Although dopaminergic activity was not assessed in the present investigation, the observed reduction in prolactin implies *C. nitida* extract may alter one or more pathways regulating this hormone [16].

Luteinizing hormone also demonstrated significant variation among research groups ($F = 10.610$, $p = 0.004$). Group I had the greatest mean LH levels (7.38 ± 0.43) while Group III had the lowest value (5.93 ± 0.53). Pairwise comparison showed substantial differences between group III and each of the other groups. LH is a key reproductive hormone crucial in follicular maturation, ovulation, and the growth and maintenance of the corpus luteum. The drop in LH in Group III may reflect a change in gonadotropin secretion or hypothalamic-pituitary control. Changes in GnRH production are known to affect pituitary LH secretion, and adjustments in the reproductive endocrine status may be related to changes in circulating LH concentrations [17]. However, GnRH secretion was not directly measured and altered hypothalamic regulation remains a conceivable explanation rather than a validated mechanism. However, the marked reduction in serum LH after the exposure to higher dose of *C. nitida* extract indicates that the extract may interfere with the normal gonadotropic regulation.

In contrast, progesterone concentration showed gradual increase in study groups as compared to FSH, LH and prolactin. The mean concentration was the lowest in Group I (1.60 ± 0.08 ng/mL) and the highest in Group III (2.43 ± 0.15 ng/mL). One-way ANOVA showed a statistically significant difference between the groups ($F = 44.673$, $p < 0.001$). Tukey's post hoc analysis revealed statistically significant differences between all three groups.

Progesterone is a key ovarian steroid hormone, mostly secreted by the corpus luteum after ovulation, and is crucial for preparing and maintaining the endometrium in the reproductive cycle and early pregnancy. The increased progesterone levels in the treated groups may reflect changes in ovarian steroidogenesis or in luteal

activity. In the present investigation, ovarian histology, corpus luteum development, estrous-cycle stage, and other markers of steroidogenic activity were not investigated. Therefore, it would be incorrect to infer that the rise indicates better ovarian function or increased fertility. The outcome does not show that the *C. nitida* exposure caused a change in the circulating progesterone levels. The role of ovarian activity and luteal function in the determination of circulating progesterone concentrations was emphasized [18]. Therefore, the rise recorded in the present investigation could indicate a shift in the ovarian endocrine activity following exposure to the extract.

The concomitant decrease of FSH, LH and prolactin and increase of progesterone are very interesting. The result shows that ethanolic *C. nitida* extract did not have a uniform effect on the reproductive endocrine system but might have differential effects on pituitary and ovarian hormones. The drop in FSH and LH might indicate a modified gonadotropic regulation, while the increase in progesterone might be an expression of the alterations in ovarian steroidogenic activity. These changes in hormones may be connected to the bioactive ingredients of *C. nitida* which include methylxanthines such as caffeine and theobromine, tannins, flavonoids, saponins and other phytochemicals. Some of these chemicals exhibit biological activity altering cellular signalling, neurotransmission and endocrine functions. Thus, the hormonal effects seen may be due to the combined action of numerous phytochemical ingredients rather than a single substance [19].

The increased amplitude of the hormonal changes found in Group III is also relevant. The highest progesterone level and the lowest levels of FSH, LH and prolactin were frequently found in the high-dose group. This pattern shows that the endocrine effects of *C. nitida* may increase with increased exposure. However, two treatment doses are not sufficient to define a dose-response relationship. To establish whether the observed alterations are dose-dependent and show a consistent increase, more dose levels, larger sample sizes and longer observation times would be required.

The results showed that ethanolic *C. nitida* extract dramatically changes several reproductive hormone levels in female albino rats. The decrease of FSH, LH and prolactin and the elevation of progesterone suggest that the extract exposure might have an effect on both the pituitary and ovarian parts of the female reproductive endocrine system. Further studies, including monitoring of the oestrous cycle, ovarian and uterine histopathology, determination of oestrogen, assessment of GnRH, and evaluation of ovarian steroidogenic markers, are needed to elucidate the mechanisms involved in these hormonal changes and to determine whether they have practical consequences on female fertility.

CONCLUSIONS

The treatment of ethanolic extract of *Cola nitida* for 28 days considerably changed the levels of some selected reproductive hormones in female albino rats in the present investigation. The study groups showed a significant drop in FSH, LH and prolactin levels with general decreases being more pronounced in the high dose group. However, progesterone concentration grew steadily and there was statistically significant difference between the hormones examined.

The decrease in FSH and LH may reflect changes in hypothalamic-pituitary-ovarian control or pituitary gonadotrophin production. The decrease in prolactin suggests possible adjustment of pituitary endocrine function. The rise of progesterone could represent changes in ovarian steroidogenic or luteal activity. However, the processes behind these changes in hormone levels cannot be confirmed conclusively as the present investigation did not explicitly measure GnRH secretion, ovarian morphology, estrous-cycle stages or reproductive outcomes. To summarise, the combined findings provide experimental evidence that the ethanolic extract of *C. nitida* alters the female reproductive hormonal profile of albino rats and these effects are more prominent at the higher dose. These results emphasise the need for more studies on the possible reproductive effects of chronic or high-dose intake of *C. nitida*.

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