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Effects of *Laurus nobilis* on pregnancy and fetal growth using Sprague-Dawley rats

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Abstract

Background: Pregnancy-related nutritional choices significantly affect maternal reproductive health and fetal health. *Laurus nobilis* is widely used worldwide as a food-medicinal herb, even among pregnant women; however, its effects on the health of pregnant women and fetuses are underexplored. This study investigated the effects of *L. nobilis* on pregnancy and fetal growth in Sprague-Dawley rats.

Methods: Twenty-four pregnant rats were randomly assigned to four groups: control, low dose (50 mg/kg), medium dose (100 mg/kg), and high dose (200 mg/kg) of the herb. Animals were sacrificed after 19 days after oral administration of *L. nobilis*. Body weight, number of litters, and weights of the uterus, ovary, and litter were measured. Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and progesterone were determined using ELISA. Histological analyses of the ovaries and uteri were performed.

Results: The increase in body weight was highest in the control group (+38.0%) and significantly lower in the treatment group. Ovary weights did not differ significantly across groups, but uterus weight was markedly lower in the high-dose group compared to the control. The number of litters and litter weights decreased with increasing *L. nobilis* doses. Serum FSH, LH, and progesterone levels were significantly reduced in the treatment groups. Superoxide dismutase and catalase expression levels revealed a dose-dependent decrease, whereas malondialdehyde levels increased. Histological examination revealed dose-dependent alterations in the ovaries and uteri.

Conclusion: These findings suggest that *L. nobilis* consumption during pregnancy may adversely affect maternal health, fetal growth, and reproductive hormone levels in a dose-dependent manner. The mechanisms underlying these effects need to be elucidated, and the safety of L. nobilis consumption during pregnancy in humans needs to be determined.

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Highlights

What is current knowledge?

- Adequate nutrition rich in macronutrients throughout pregnancy is crucial for optimal birth outcomes, maternal health, and child development.
- Bay leaf (*Laurus nobilis*) is an aromatic herb with various biological and pharmacological properties, including antioxidant and wound-healing potency.
- Oxidative stress has been implicated in female reproductive complications, such as follicular abnormalities, faulty meiosis, and reduced fertilization rates.

What is new here?

- Increased *L. nobilis* consumption results in significantly lower uterine weight and a decrease in the number and weight of litters.
- *L. nobilis* significantly decreases the serum levels of FSH, LH, and progesterone.
- *L. nobilis* consumption distorts the expression levels of antioxidant enzymes, resulting in increased oxidative stress in pregnancy.
- Histological examination revealed dose-dependent degenerative changes in the ovaries and uteri, which may adversely affect maternal reproductive and fetal health.

Introduction

The female reproductive system is a complex network of internal and external organs (1). It produces gametes and certain sex hormones (2,3) and maintains the zygote throughout pregnancy until the mature fetus is ready for delivery (4). Pregnancy involves the gestation period from ovum fertilization to embryo implantation in the female body, ending with either spontaneous or voluntary abortions or delivery (5). Throughout pregnancy, the mother's body undergoes significant anatomical and physiological changes (6) to support the growing baby and prepare her for labor and delivery (7). Pregnancy also leads to hormonal, immunological, and metabolic changes in the woman's body, significantly affecting maternal and fetal health (8). Alterations in hormone levels affect the complex physiological adjustments required for fetal growth (9).

Nutrition, through diet choice, has immense short- and long-term effects on pregnancy health (10,11). An adequate nutritional regimen rich in macronutrients throughout pregnancy is essential for achieving the best possible birth outcomes, maternal health, and child development (11). Several maternal dietary factors have been explored with adverse pregnancy and child health and development outcomes (12).

Human nutrition relies heavily on nutrients derived from vegetables and herbs (13). Bay Leaf (*Laurus nobilis*), also known as bay laurel or Roman laurel (14), is an important herb used in meals, pharmaceuticals, and cosmetics (15). Grown in the southern Mediterranean and other parts of the world (16), bay leaf is a very aromatic and fragrant herb used as a condiment in soups, fish, meat products, stews, puddings, and beverages (17,18). *L. nobilis*' biological and pharmacologic properties include nematocidal, insecticidal, antibacterial, antifungal, and antioxidant properties (18). *L. nobilis* also boosts the immune system due to its antidiarrheal, anti-inflammatory, and anti-diabetic potentials (19). This medicinal herb has wound healing, anticonvulsant, antioxidant, antimicrobial, antiviral, and anticholinergic properties (18,20). It also has the potential to reduce the concentrations of uric acid and blood cholesterol (21).

Plants, herbs, and spices contain bioactive compounds that can affect oxidative stress (22). Oxidative stress has been implicated in female reproductive complications (23), such as follicular abnormalities, faulty meiosis, reduced fertilization rates, delayed embryonic development, and some diseases, such as polycystic ovarian syndrome and ovarian endometriosis cysts (24). It can also harm maternal and placental health, cause gestational diabetes mellitus, and potentially lead to fetal mortality (6). Oxidative stress accelerates ovarian and uterine aging (25,26).

L. nobilis and its essential oils are generally safe for consumption in small quantities for most people. In some parts of the world, pregnant women consume diets with high *L. nobilis* content because it is believed to strengthen pregnancy, promote fetal growth, and improve maternal and child health. However, there are no credible studies supporting the high consumption of *L. nobilis* by pregnant women (27). Hence, this study aimed to investigate the effects of *L. nobilis* on pregnancy and fetal growth using histological, hormonal, oxidative stress, and fetal parameters in a Sprague-Dawley rat model.

Methods

Experimental design and animal groups

Freshly harvested *L. nobilis* leaves were air-dried and ground into a powdery form for easy administration to rats in the treatment groups. Twenty-four (24) female pregnant Sprague-Dawley rats weighing (152 ± 29 g) were randomly assigned to four groups consisting of six (6) rats:

Group A: served as control group (Were administered with 10 ml distilled water)
Group B: low dose group (Administered with 50mg/kg *L.nobilis*)
Group C: medium dose group (Administered with 100mg/kg of ground *L.nobilis*)
Group D: High-dose group (200 mg/kg of ground *L.nobilis*).

The rats in the treatment groups were orally administered ground bay leaf mixed with water daily for 19 days (Within Sprague-Dawley rats' gestation period of 21-23 days) at the specified concentrations. All animals had ad libitum access to rat feed and clean water. The animals were housed in standard clean cages under moderately constant environmental conditions, proper aeration, and adequate lighting.

All procedures were carried out according to the standard international guidelines on animal use for research (National Research Council, 2011). The Animal Research Ethical Committee of Bowen University approved this study.

Animal sacrifice and sample collection

Pregnant Sprague-Dawley rats were fasted for 12 h before being sacrificed 20 d after oral administration. The ovaries, uteri, and fetuses were harvested for histological and biochemical analyses. Ovaries and uteri were dissected and fixed in Bouin's solution to prevent tissue degradation. Biochemical samples were homogenized and preserved on ice. Fetuses were measured for various parameters, including crown-rump length, tail length, fetal weight, umbilical cord length, and placental weight.

Biochemical analysis

Before the rats were sacrificed, 5 ml of blood was collected from their saphenous veins and stored at -20 °C for analysis. Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and progesterone were determined in all pregnant rats in each group using enzyme-linked immunosorbent assay (ELISA) kits.

Histological procedure

The sections were dewaxed in two stages of xylene, with each stage lasting for 2 minutes. They were rehydrated in a series of descending concentrations of alcohol (100%, 95%, 80%, 70%, and 50%) for 2 minutes each. The tissues were rinsed in distilled water for 3 minutes and then stained with hematoxylin for 15-20 minutes. The sections were rinsed in running tap water for 1-5 minutes until they turned blue, then they were immersed in 70% ethanol containing 0.5% hydrochloric acid for about 5 seconds to remove the excess dye. The sections were rinsed in running tap water for 10-15 minutes and stained in eosin solution for 2-5 minutes. The excess eosin dye was rinsed off in running tap water for 1-5 minutes. Then, the sections were dehydrated in increasing alcohol concentrations, cleared in xylene, and mounted in a synthetic resin medium. Four micrometer-thick paraffin sections were created for microscopic examination.

Statistical analysis

The findings of this study were expressed using mean and standard deviation in the statistical analysis. Normality was determined using the SPSS software version 20. The mean difference between each group was evaluated using a one-way analysis of variance (ANOVA) compared to the control group at a significance level of 0.05.

Results

Body weight

As shown in Table 1, there was a high percentage increase in the weight of Group A rats (+38.0%) and a significant ($p < 0.05$) increase in Group B (+6.7%) and Group C (+9.4%) when compared to Group D (+2.7%)

Table 1. Initial and final body weights of experimental models

Group	Initial body weight (g)	Final body weight (g)	Percentage difference in body weight (%)
Group A	140.7 ± 9.20	194.7 ± 24.70	+ 38.0
Group B	134.3 ± 3.21	143.3 ± 4.93	+ 6.7
Group C	148.7 ± 3.22	162.7 ± 5.13	+ 9.4
Group D	171.7 ± 11.37	176.3 ± 9.01	+ 2.7

Number of litters and weight of the uterus, ovary, and litter

The weights of the ovaries did not differ significantly ($p < 0.05$) across the different groups. However, the weight of the uterus was markedly lower in Group B (Low-dose group) than in the other groups (Table 2). Additionally, litter weight was significantly decreased in the treatment groups (Groups B, C, and D) compared to the control group.

Table 2. Number of litters and weights of uterus, ovary, and litter of pregnant Sprague Dawley rats after the oral administration of *L. nobilis*

Group	Uterus weight (g)	Ovary weight (g)	Litter weight (g)	Number of litters
Group A	2.97 ± 0.420	0.13 ± 0.210	2.87 ± 0.180	23
Group B	1.97 ± 0.088	0.12 ± 0.008	0.98 ± 0.063	18
Group C	2.73 ± 0.210	0.14 ± 0.012	1.39 ± 0.043	16
Group D	2.92 ± 0.230	0.13 ± 0.008	1.20 ± 0.052	17

Fetal parameters

As shown in Table 3, there was a significant ($p < 0.05$) decrease in the fetal parameters between the control group (Group A) and the treatment groups (Groups B, C, and D). The reduction of crown-rump length and umbilical cord length was dose-dependent. The tail length and placenta weight showed a dose-dependent increase, with the high-dose group (Group D) having the highest measurements significantly lower than the control group (Group A).

Table 3. Measurements of fetal parameters of Sprague Dawley rats after oral administration of *L. nobilis*

Group	Crown-Rump Length (cm)	Tail Length (cm)	Placenta Weight (g)	Umbilical Cord Length (cm)
Group A	3.87 ± 0.15	1.24 ± 0.057	0.59 ± 0.023	3.00 ± 0.078
Group B	3.31 ± 0.13	0.72 ± 0.036	0.33 ± 0.036	2.22 ± 0.051
Group C	3.47 ± 0.05	0.91 ± 0.032	0.47 ± 0.025	1.73 ± 0.080
Group D	2.57 ± 0.12	0.70 ± 0.030	0.50 ± 0.023	1.70 ± 0.069

Hormonal assay

The analysis of FSH, LH, and progesterone revealed a significant ($p<0.05$) decrease in the serum levels of these hormones in the treatment groups compared with the control group. It was observed that the hormonal (FSH, LH, and progesterone) levels of the treatment groups decreased with increased dosage of the grounded bay leaves administered (Table 4).

Table 4. Mean and standard error of FSH, LH, and progesterone serum levels in the different groups after administration of *L. nobilis*.

Group	FSH (mIU/ml)	LH (mIU/ml)	Progesterone (mIU/ml)
Group A	2.533 ± 0.052	15.54 ± 0.380	65.53 ± 0.430
Group B	2.107 ± 0.030	13.82 ± 0.043	60.45 ± 0.018
Group C	1.867 ± 0.009	10.98 ± 0.047	53.95 ± 0.910
Group D	1.370 ± 0.031	7.767 ± 0.150	36.30 ± 0.670

Biochemical assay

Ovary: Biochemical analysis (Table 5) revealed significantly lower levels of superoxide dismutase and catalase in the treatment groups than in the control group, indicating higher levels of oxidative stress markers in the ovary ($p < 0.05$). Conversely, malondialdehyde expression levels were significantly ($p < 0.05$) higher in the treatment groups than in the control group. The decrease in relative expression levels of superoxide dismutase and catalase in the treatment groups was dose-dependent. Similarly, the increase in the expression levels of malondialdehyde in the treatment groups was dose-dependent.

Uterus: Biochemical analysis of the uteri for oxidative stress markers revealed a similar pattern to that observed in the ovary, as indicated in Table 5. The relative expression levels of these markers in the uteri indicated that superoxide dismutase and catalase were significantly lower in the treatment groups than in the control group. In contrast, malondialdehyde levels were significantly higher in the treatment groups than in the control group. Significant ($p < 0.05$) differences in the expression levels of these markers were all dose-dependent.

Table 5. Biochemical assay of malondialdehyde, superoxide dismutase, and catalase in ovarian and uterine serum after administration of *L. nobilis*.

Ovary			
Group	**Malondialdehyde (µmol/ml)**	**Superoxide dismutase (µmol/ml)**	**Catalase (µmol/ml)**
Group A	306.4 ± 2.86	430.4 ± 4.35	388.1 ± 0.98
Group B	329.2 ± 2.32	405.0 ± 0.88	321.6 ± 4.09
Group C	374.7 ± 3.49	322.5 ± 1.76	283.9 ± 1.80
Group D	426.0 ± 1.61	284.4 ± 3.17	225.7 ± 7.36
Uterus			
Group	**Malondialdehyde (µmol/ml)**	**Superoxide dismutase (µmol/ml)**	**Catalase (µmol/ml)**
Group A	290.8 ± 3.93	401.4 ± 0.60	392.9 ± 1.72
Group B	303.1 ± 1.38	298.1 ± 1.32	300.7 ± 1.36
Group C	328.8 ± 1.86	244.5 ± 0.53	265.5 ± 2.37
Group D	394.6 ± 1.99	204.5 ± 0.67	217.2 ± 2.89

Histological Examination

Photomicrographs of sections of the ovary

The ovary photomicrographs of the control group (Figure 1) and different concentrations of *L. nobilis* (Figures 2, 3, and 4) are shown in Figures 1-4.

Control group

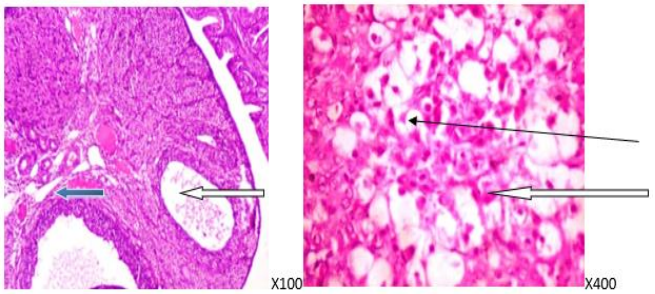

Figure 1. Normal antral follicles (White arrow) with normal theca cells (Blue arrow) within the ovarian cortex and degenerated corpus luteum (Slender arrow) in the control group.

Low-dose treatment group

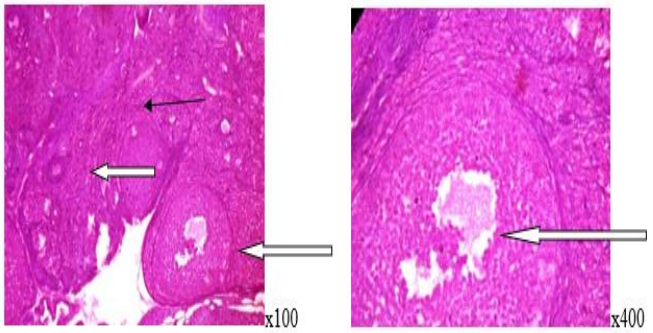

Figure 2. Normal antral and Graafian follicles (White arrow) with normal luteinized stromal cells and normal connective tissue (Slender arrow) in the ovaries of the low-dose treatment group.

Medium-dose treatment group

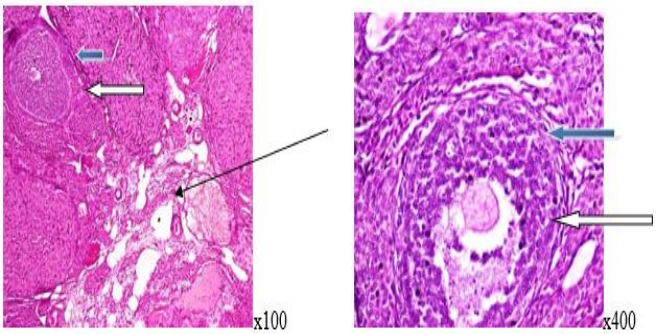

Figure 3. Normal antral follicles (White arrow) with normal theca cells (Blue arrow) within the ovarian cortex, luteinized stromal cells, and mild vascularization (Slender arrow) in the ovary of the medium-dose treatment group.

High-dose treatment group

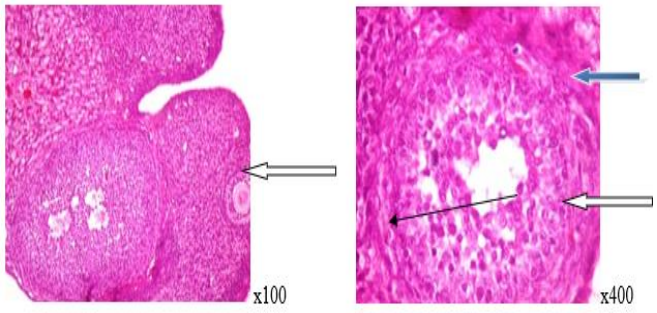

Figure 4. Normal antral follicles (White arrow) with normal theca cells (Blue arrow) within the ovarian cortex and mild vascular congestion of the ovarian stroma (Slender arrow) in the ovarian section of the high-dose treatment group.

Photomicrographs of sections of the uterus

The uterus photomicrographs of the control group (Figure 5) and different concentrations of *L. nobilis* (Figures 6, 7, and 8) are shown in Figures 5-8.

Control group

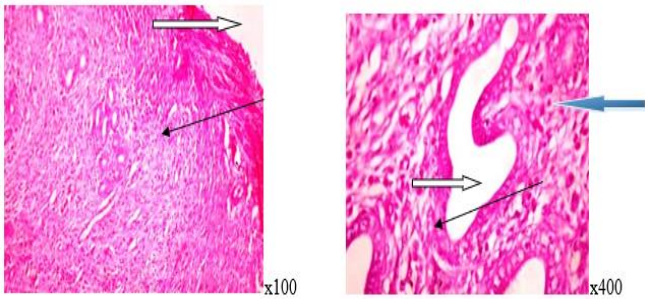

Figure 5. Normal endometrial epithelial layer (White arrow), normal endometrial gland (Blue arrow), and moderate infiltration of endometrial stroma by inflammatory cells (Slender arrow) in the uterine section of the control group.

Low-dose treatment group

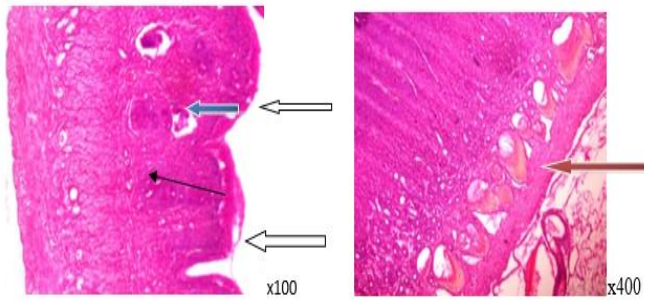

Figure 6. Normal endometrial epithelial layer (White arrow), normal endometrial gland (Blue arrow) with severe infiltration of the endometrial stroma by inflammatory cells (Slender arrow), and mild vascular congestion of the myometrium (Red arrow) in the uterine section of the low-dose group.

Medium-dose treatment group

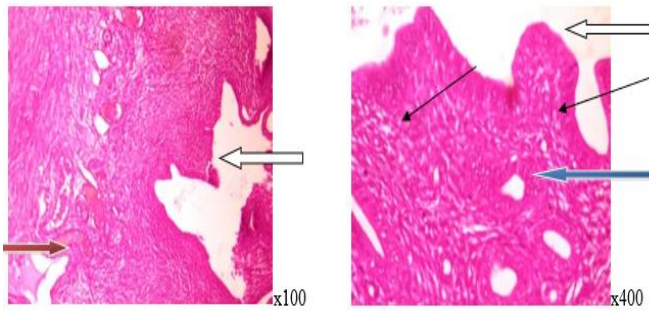

Figure 7. Normal endometrial epithelial layer (White arrow), normal endometrial gland (Blue arrow) with moderate infiltration of inflammatory cells (Slender arrow), and mild vascular congestion (Red arrow) in the uterine section of the medium-dose group.

High-dose treatment group

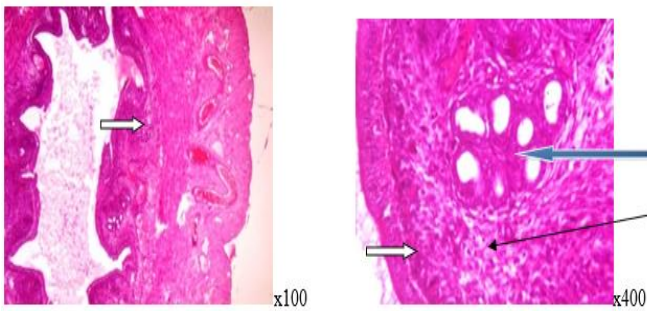

Figure 8. Normal endometrial epithelial layer (White arrow), moderate proliferation of endometrial gland (Blue arrow) with epithelial hyperplasia, and severe infiltration of the endometrial stroma by inflammatory cells (Slender arrow) in the uterine section of the high-dose group.

Discussion

Food and supplements consumed during pregnancy impact the health of expectant mothers and fetuses (28). Pregnancy outcomes depend on comprehensive prenatal care that promotes healthy nutritional habits (29). Pregnant women must understand the risks and advantages of various dietary choices (10). Considering the use of *L. nobilis* in a wide variety of dishes, there is a need to study the effects of bay leaves on female reproductive organs and fetal parameters.

Laurus nobilis treatment significantly affects fetal parameters, manifested as decreased litters, litter weight, and placental weight. Our study was in concordance with Sathasivam et al. (30) 's study, which reported a positive correlation between placental weight and birth weight. These may result from a significant decrease in the transfer of nutritional substances from the mother to the fetus. Although our study's sample size was small, our results indicate the nutritional value of *L. nobilis*.

Hormonal assays revealed a dose-dependent decrease in FSH and LH levels. However, there was a dose-dependent decrease in progesterone levels in the treatment groups compared with the control group. As reported by Bosch et al. (31), these hormonal deficits result from decreased gonadotropin production. This suggests that *L. nobilis* influences gonadotropin activity, which can impair implantation and embryo development in the early stages of pregnancy. This may also affect the mother's immune response, potentially leading to embryo rejection (32).

The treatment groups showed a decrease in SOD concentrations in the ovaries and uteri, similar to previous studies showing reduced activity with increased sucrose intake (33), while curcumin and capsaicin have been found to increase ovarian SOD levels (34). Lu et al. (35) proposed that suppressing SODs may hinder its protective effects against oxidative stress and oxygen species-mediated ailments.

Catalase expression levels showed a dose-dependent decrease in the ovary and uterus of the treatment groups compared to that in the control group. This contrasts with Kaygusuzoglu et al.'s study (36), which reported that increased catalase activity with zingerone treatment reduced oxidative DNA damage indicators and inhibited apoptosis. Repression of catalase concentrations may result in increased ovarian damage, exacerbated by apoptotic ovarian follicular cells, which decrease fecundity due to catalase suppression (37).

Administration of *L. nobilis* increased malondialdehyde levels in the ovaries and uteri, a finding consistent with that of Sadowska et al. (33). In contrast to Laurus nobilis, *Chlorella vulgaris* supplementation reduced malondialdehyde levels and could have protective effects against lipid-peroxidation-induced cell damage, as reported by Sikiru et al. (38). In our study, *L. nobilis* administration increased malondialdehyde levels, potentially damaging cell membranes.

The vasculature of the ovary plays a crucial role in tissue oxygenation, hormone transport, nutrient intake, and waste elimination (39). Low doses of *L. nobilis* do not cause adverse effects on ovarian tissue; however, increased dosages can cause vascular congestion in the ovarian stroma. Increased ovarian stromal vascularization has been linked to PCOS pathogenesis of polycystic ovary syndrome (40). High dosages of *L. nobilis* can cause endometrial gland proliferation and epithelial hyperplasia, which can affect uterine function and pregnancy, leading to pathological diseases such as endometrial hyperplasia and reduced female fertility (41). *L. nobilis* use during pregnancy causes uterine vascular congestion, which, according to Elagwany (42), is associated with pregnancy and uterine complications.

Conclusion

Extensive consumption of *L. nobilis* during pregnancy may negatively affect oxidative stress, hormonal levels, and the histological structure of the ovary and uterus. The mechanisms underlying these effects need to be elucidated, and the safety of *L. nobilis* consumption during pregnancy in humans must be determined. Hence, more clinical studies may be required to better comprehend the impact of *L. nobilis* on maternal and fetal health.

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Ethical statement

This study was approved by the Bowen University Institutional Research Ethics Committee on June 1, 2023 (Reference number BUI/COHES/ANA/01013). The experimental animals were cared for according to the committee's guidelines.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

OA: Contributed to research conceptualization and design, editing the initial draft of the article, and providing funding. JO: Performed the histological examination of ovaries and uteri and provided funding. NB: Collected data, wrote the initial and final draft of the article, and provided funding. PA: Performed data analysis and provided logistic support and funding. AA: Performed animal care and oral administration of *L. nobilis*, collected data, and provided funding. All authors critically reviewed and approved the final manuscript.

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