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Contraceptive Effect of Methanol Leaves Extract of *Dalbergia saxatilis* on Female Albino Rats

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Abstract

Contraception is the deliberate use of artificial methods or other techniques to prevent pregnancy as a consequence of sexual intercourse. This research was aimed at studying the contraceptive effect of methanol leaves extract of *Dalbergia saxatilis* on female albino rats. Standard laboratory methods were used to test for the presence of the following phytochemicals; alkaloids, anthraquinones, anthraquinone glycosides, cardiac glycosides, flavonoids, phenols, saponins, tannins, and terpenoids. The LD50 of *Dalbergia saxatilis* was determined by the up and down method and fifteen rats were used. The contraceptive effect was evaluated via anti ovulation, anti-implantation, and hormonal assay respectively in female albino rats. Female albino rats were treated with varying concentrations of methanol leaf extract of *Dalbergia saxatilis*. *Dalbergia saxatilis* revealed the presence of alkaloids, flavonoids, saponin, saponin glycoside, glycoside, cardiac glycoside, tannins and steroids, balsams, anthraquinone and terpenoid were not detected. The results showed that *Dalbergia saxatilis* is relatively safe as LD50 is >3000 mg/kg. Subchronic (21 days) administration of methanol leaves extract of *Dalbergia saxatilis* exhibited a reduction in serum progesterone and estrogen levels significantly ($P<0.05$) at 300 mg/kg BW and 600 mg/kg BW when compared to the normal control. The histology of the anti-implantation effect of the extract on the uterus showed that the endometrial thickness reduced in a dose-dependent manner while the anti-ovulation effect also showed a reduction in the number of follicles in a dose-dependent manner. Therefore, the use of *Dalbergia saxatilis* as an antifertility agent in traditional medicine has pharmacological basis and this research validates the traditional claim.

Keywords: Herbal medicine, Contraceptive agents, Hormones, Ovulation, *Dalbergia saxatilis*

Introduction

Contraception is the deliberate use of artificial methods or other techniques to prevent pregnancy as a consequence of sexual intercourse (1). The worldwide contraceptive prevalence by any method for women between the ages 15 - 49 has been reported to be 59.85% and 31% in Sub-Saharan Africa (2, 3). The prevalence of contraceptive use in Nigeria is 15.98% (3) which is well below the world and regional averages. Sub-Saharan Africa has been reported to have the highest maternal mortality rates in the world, sometimes as high as 1 maternal death per 100 births in some areas within the sub-region (4). Unintended pregnancies, unsafe abortions, and death resulting from it are high owing to the low prevalence of contraceptive use (2).

Various forms of contraceptive are available ranging from hormonal, barrier, chemical and herbal. Several studies on the use of contraceptives in Sun-Saharan Africa have listed misinformation, misperceptions, and fear of health side effects as barriers to regular contraceptive (5-7). Authors have listed the fear of side effects, partner objection, and religious beliefs as some of the reasons for the low prevalence of contraceptives in Nigeria (8-10). Low prevalence of conventional contraceptive specifically in northern Nigeria is majorly attributed to its non-accessibility to the rural population and cultural unacceptability (11). As such, there is high reliance on herbal contraceptives because traditional medicine enjoys a wider acceptability due to the fact it blends readily into the socio-cultural life of the people in whose culture it is deeply rooted and also due to its accessibility and cost (10, 12). In 2017, the Nigerian government committed to achieving a modern contraceptive prevalence rate of 27% among all women aged 15–49, regardless of marital status, by 2020 (13). This has however not been achieved, largely due to poor counseling and knowledge on the proper use of contraceptives. In Nigeria, abortion has not been legalized (14), as such; proper knowledge on the use of contraceptives is paramount to address unwanted pregnancies which could prompt illegal abortions, thereby placing women at risky. The use of contraceptives is on the increase worldwide (6); this is however not without challenges like limited access to contraception, the cost of contraceptives, fear of side-effects, cultural or religious disapproval, and gender-based barriers. In developing countries, reliance on herbal medicine in health management is on the increase too (3). *Dalbergia saxatilis* has been used as a decoction in traditional medicine for ailments, such as cough, smallpox, skin lesions, bronchial ailments and toothache (15). The traditional acquaintance and availability of this plant could mitigate the challenges associated with modern contraceptives. Therefore, the present study is designed to evaluate the contraceptive effect of *Dalbergia saxatilis.*

Materials and Methods

Plant Collection and Identification

The leaves of *Dalbergia saxatilis* were collected in August 2019 from Zuru, Zuru Local Government Area of Kebbi State, Nigeria, and were taken to the herbarium unit in of Plant Science and Biotechnology Department, Kebbi State

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University of Science and Technology, Aliero where it was identified and authenticated by a botanist. A Voucher specimen (102A) was deposited at the Herbarium for future reference.

Plant Preparation and Extraction

The leaves of *D. saxatilis* leaves were dried at room temperature for two weeks and were cut into smaller pieces. Two hundred and fifty grams of the plant material was macerated with two liters of methanol left in an air-tight aspirator for seventy-two hours. The mixture was then filtered using a sterile muslin cloth. The filtrate was evaporated using a rotary evaporator at 45°C and subsequently dried in a drying cabinet at 45°C and labeled methanol extract. The weight of the dried extract was recorded for the calculation of yield and stored in an air-tight bottle in a refrigerator until required for analysis.

Phytochemical Analyses

Qualitative screening using standard methods was used to test for the presence of the following phytochemicals; alkaloids, anthraquinones, anthraquinone glycosides, cardiac glycosides, flavonoids, phenols, saponins, tannins, and terpenoids (16-19).

Acute Toxicity of Dalbergia saxatilis

The acute oral toxicity study was conducted according to the Organization for Economic and Cultural Development for testing of chemicals (20) guideline and up and down method was used for the study. A total of Fifteen animals were randomly divided into five (5) groups of three (3) animals each and used for the experiment. A single oral limit test dose of 1000, 2000, 3000, 4000, and 5000 mg/kg body weight was administered to groups 1, 2, 3, 4, and 5 respectively (21).

Determination of Contraceptive Activity

Estrus Cycle Monitoring

Three weeks prior to the treatment, vaginal smears were collected and observed each morning (8-10 a.m.) to determine estrus cyclicity of each animal. This involved sampling the cells of the vaginal canal with sterile saline using a glass slide and automatic pipette. The recovered smears containing cells were placed on microscope slides, dried and examined microscopically. Cell descriptions were used to classify rats based on the stages of the estrus cycle (proestrus, estrus, metestrus and diestrus).

Study design

Fifteen female albino rats were divided into five groups. The vaginal smear from each rat was examined daily for ten days before administration of the extract and those rats that exhibited normal regular cycles were used. The treatments were as follows: Group I (Control) received distilled water (1 ml/100g BW); Group II received cypoterone acetate (1 ml/kg BW); Group III received Methanol leave extract of *Dalbergia saxatilis* (MLEDS) (150 mg/kg); Group IV received methanol leave extract of *Dalbergia saxatilis* (300 mg/kg); Group V received methanol leave extract of *Dalbergia saxatilis* (600 mg/kg).

Anti-ovulation Activity

Twenty-four hours after the last dose, the rats from each group were anesthetized and sacrificed. The ovaries were dissected out, placed formalin, and prepared histologically to count the number of follicles and corpus luteum (22).

Anti-Implantation Activity

Female and male albino rats were used for the study. The rats were paired overnight in the evening of the proesterus phase with sexually active males in the ratio of 3:1. Successful mating was confirmed by the presence of vaginal plug and or sperm cells in the vaginal smear the following morning between 9.00 and 10.00am. The day sperm cells are found in the vaginal smear was considered as day 1 of pregnancy. Thereafter, female rats were randomly divided into five groups of four rats each and were treated as follows: Group I received distilled water (1 ml/100g BW); Group II received cypoterone acetate (1 ml/kg BW); Group III received methanol leave extract of *Dalbergia saxatilis* (150 mg/kg); Group IV received methanol leave extract of *Dalbergia saxatilis* (300 mg/kg); Group V received methanol leave extract of *Dalbergia saxatilis* (600 mg/kg).

On day 21 of gestation, each rat was sacrificed, the uterus extracted and examined for signs of implantation (22).

Hormonal Assay

Blood samples (serum) were collected on the last day of anti-ovulation study (16^{th} day) via cardiac puncture. The samples were centrifuged for 15 min at 3000 rpm using a bench top centrifuge. Enzyme-linked-immunoasorbent serologic assay (ELISA) techniques are used to assay for progesterone and oestrogen level using standard laboratory methods.

Data Analysis

The data was analysed using Statistical Package for Social Sciences (SPSS) windows program version 20.0 while graphs were drawn with GraphPad Prism, Version 5.0. The data was presented as means ± standard error of mean (SEM) analysed by one way analysis of variance (ANOVA) followed by Duncan's Post Hoc Comparison test. $P<0.05$ was considered to be significant.

Results

Results of Phytochemical Screening

Table 1: Phytochemical Constituents of *Dalbergia saxatilis* leaves extract

Phytochemicals	Results
Tannins	+
Steroids	+
Balsams	ND
Anthraquinone	ND
Alkaloids	+
Flavonoids	+
Saponin	+
Saponin glycoside	+
Glycoside	+
Cardiac glycoside	+
Terpenoid	ND

KEY: + = Present, ND = Not detected

Result of LD_{50}

The animals are dosed with methanol leaves extract of *Dalbergia saxatilis* within a time interval of 48hrs with an initial dose of 1000 mg/kg, all the animals survived and the dose was increased to the subsequent doses 2000 mg/kg, 3000 mg/kg, 4000 mg/kg and 5000 mg/kg respectively. The testing was terminated when the upper limit (5000 mg/kg) was reached without mortality and the LD_{50} was assumed to be greater than 5000 mg/kg BW.

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Effect of MLEDS on Implantation

The endometrium section shows product of conception and placental tissue which is another possible evidence evidence that implantation occurred (Figure 1). In the group administered with the standard drug, the endometrial thickness is about 5µm. The stroma cells which depict a viable endometrium are dense and some have change to decidua (Figure 2). The group administered with the lowest dose of the extract (150 mg/kg) showed a thick endometrium measuring about 11.567µm. The thickness of the endometrium at the lowest dose is an indication that the extract could not effectively inhibit implantation. The decidualisation of the endometrium shows that implantation might have possible taken place (Figure 3). In the group administered with 300 mg/kg of the extract, the endometrium became thin and measured about 6.547µm (Figure 4). When the endometrium is thin, conception is unlikely to occur because decidualisation of the stroma is not possible when the endometrium is thin. In the group treated with the highest dose of the extract (600 mg/kg), the endometrial glands were viewed to be thin (3µm). This does not possibly favour implantation and as such, implantation might not have possibly taken place. The absence of decidualisation is an indication of the potency of the extract. The reduction in the endometrial thickness from 11.567µm to about 6.547µm and subsequently to about 3µm is an indication that the effect of the extract could possibly be dose dependent.

The anti-implantation results on the histology of the uterus is presented in Table 2. The endometrial glands in the control group and the group treated with the lowest dose of the extract were regular and showed decidua changes with evidence of conception. The group treated with the standard drug (cypoterone acetate), 300 mg/kg and 600 mg/kg showed secretory changes with thin endometrium. The endometrial thickness of the control group was significantly higher than those of the other groups while there was no significant difference between the group treated with the standard drug and the group treated with 300 mg/kg. There was evidence of product of conception in the control group and the group that received the lowest dose of the extract (150 mg/kg).

Anti-ovulation Activity

The rats of the control group showed mature follicles, corpus luteum and regular ovarian stroma. This is evidence that ovulation had occurred (Figure 6). The group administered with the standard drug showed less mature follicles and regular ovarian stroma. Also, no corpus luteum seen indicating that either ovulation did not occur, or it was irregular (Pate 5). The group administered with 150 mg/kg and 300 mg/kg of the extract showed matured follicles and regular ovarian stroma similar to that of the control group indicating that ovulation had taken place. The group administered with 600 mg/kg of extract showed premature follicles and regular ovarian stroma indicating that the process of ovulation might have been altered by the administration of the extract.

The anti-ovulation activity of methanol leaves extract of *D. saxatilis* is presented is Table 3. The number of corpus luteum in the normal control group were significantly higher ($P<0.05$) than in the treated groups. Even though the group treated with the highest concentration of the extract had the least number of corpus luteum, this was however not significantly different ($P>0.05$) from the other treated groups. There was no significant difference in the number of mature follicles in the control group and the group that received the lowest dose of the extract, however, there was no significant difference between the group treated with the standard drug and the group that received the highest concentration of the extract.

Effect of extract on oestrogen and progesterone

The hormonal assay results shows a significant decrease ($P<0.05$) in oestrogen and progesterone in all the treated groups respectively when compared to the control The relationship between hormonal level of control group, standard drug (Cypoterone acetate) group and groups treated with the extract revealed a significant reduction ($P<0.05$) in serum oestrogen and progesterone of drug treatment group when compared to the normal control. Also there was significant reduction ($P<0.05$) in serum oestrogen and progesterone of groups treated with 150 mg/kg 300 mg/kg and 600 mg/kg when compared to control (Figure 11 and Figure 12). However serum oestrogen of group treated with 300 mg/kg and 600 mg/kg of extract showed significant reduced ($P<0.05$) when compared to standard drug (cypoterone acetate) group. While Serum progesterone level of the normal control and the group treated with 150 mg/kg of extract and standard drug (cypoterone acetate) were not significantly deferent ($P>0.05$).

Table 2: Anti-implantation effect of methanol extract of *d. saxatilis* on histology of uterus in female albino rats

Treatment	Endometrial Gland	Endometrial Stroma	Endometrial Thickness (µm)	Evidence of Conception
Control	Regular Glands (Deciduous endometrium)	Decidual changes	11.68 ± 0.83^{d}	Product of conception
Cypoterone Acetate	Secretory changes (Thin endometrium)	Regular stroma	5.88 ± 0.13^{b}	-
D. saxatilis (150 mg/kg)	Regular glands (Thick endometrium)	Decidual changes	9.00 ± 0.41^{c}	Product of conception
D. saxatilis (300 mg/kg)	Secretory Changes (Thin endometrium)	Regular stroma	5.83 ± 0.39^{b}	-
D. saxatilis (600 mg/kg)	Secretory Changes (Thin endometrium)	Regular stroma	2.88 ± 0.051^{a}	-

Mean values having common superscript letters in a column are not significantly different.

Table 3: Anti-ovulation activity methanol leaves extract of *d. saxatilis* on female albino rats

Treatment	Corpus Luteum	Mature Follicles
Control (normal saline)	8.67 ± 0.88^{b}	7.00 ± 1.15^{c}
Cyproterone Acetate	2.00 ± 1.00^{a}	4.33 ± 0.33^{a}
MLEDS (150 mg/kg)	3.33 ± 0.88^{a}	$5.33\pm0.33b^{c}$
MLEDS (300 mg/kg)	2.33 ± 0.33^{a}	3.33 ± 0.33^{a}
MLEDS (600 mg/kg)	1.67 ± 0.33^{a}	2.67 ± 0.33^{a}

Values are expressed as mean ± standard error of mean. Mean values having common superscript letters in a column are not significantly different.

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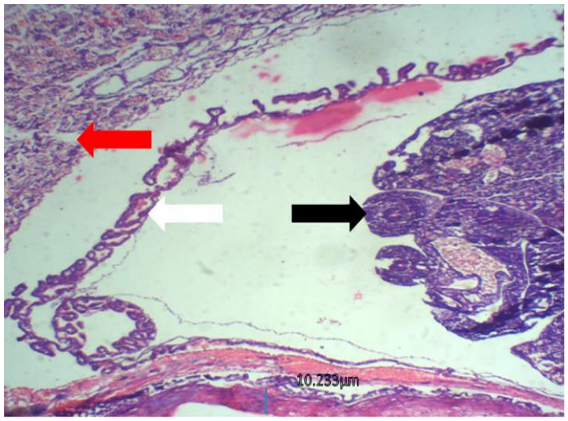

Figure 1. Photomicrograph of rat's uterus obtained from group 1 (H and E stain, x 100 magnification). Endometrium section shows placental tissue (white arrow), product of conception (black arrow) and deciduous endometrial tissue (red arrow)

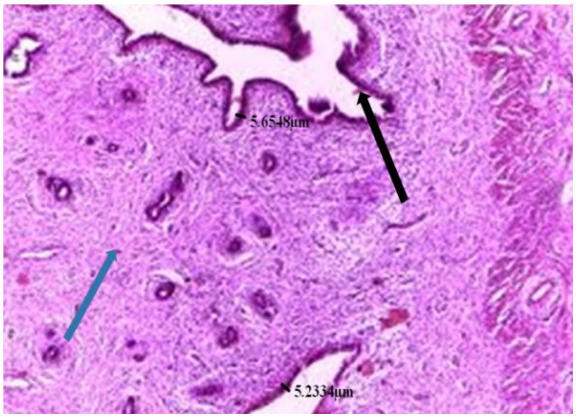

Figure 2. Photomicrograph of rat's uterus obtained from group administered with standard drug (cypoteroine acetate, 1 mg/kg) (H and E stain, x 100 magnification). Showing thin endometrial gland (black arrow) and dense stroma (Blue Arrow)

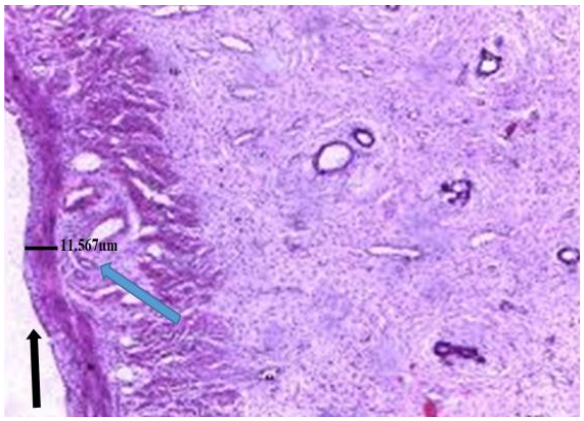

Figure 3. Photomicrograph of rat's uterus obtained from group administered with 150 mg/kg of methanol leaves extract of *D. saxatilis* (H and E stain, x 100 magnification). Showing placental tissue ie possible evidence of conception (blue Arrow) and a thick endometrial cavity (black Arrow)

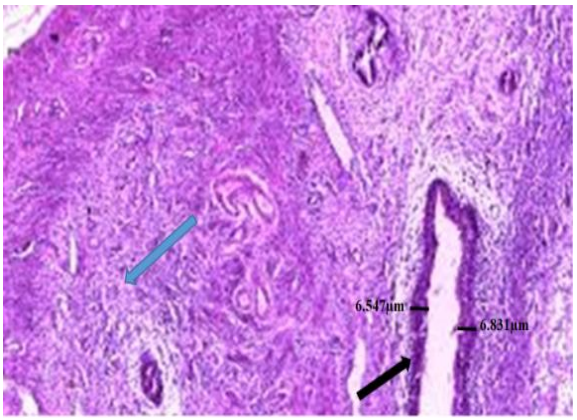

Figure 4. Photomicrograph of rat's uterus administered with 300 mg/kg of methanol leaves extract of *D. saxatilis* (H and E stain, x 100 magnification). Showing compact stroma (Blue arrow) and thin endometria (black arrow)

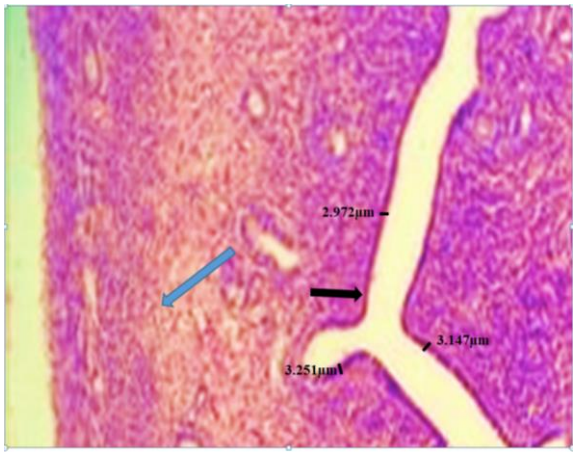

Figure 5. Photomicrograph of rat's uterus administered with 600 mg/of methanol leaves extract of *D. saxatilis* (H and E stain, x 100 magnification). Showing thin endometrial glands (Black arrow) and compact stroma (Blue arrow)

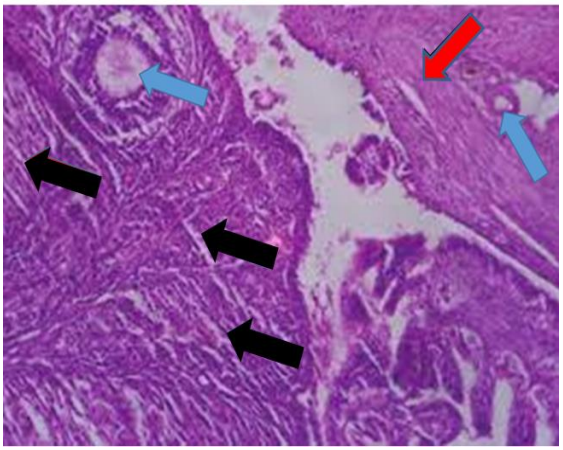

Figure 6. Photomicrograph of rat's ovary obtained from control Section shows matured follicles (blue arrow), corpus luteum (black arrow) and regular ovarian stroma (red arrow)

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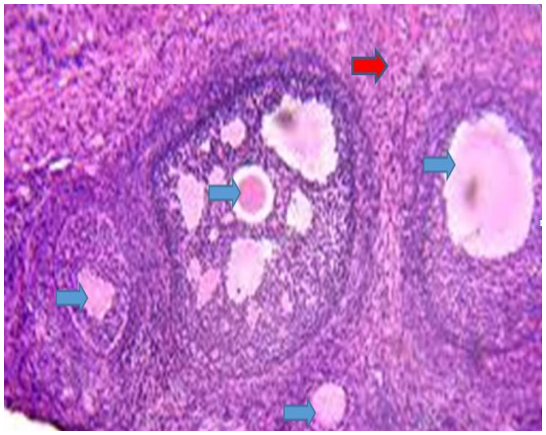

Figure 7. Photomicrograph of rat's ovary administered with standard drug (cypoterone acetate, 1mg/kg) shows mature follicles (blue arrow), and regular ovarian stroma (red arrow)

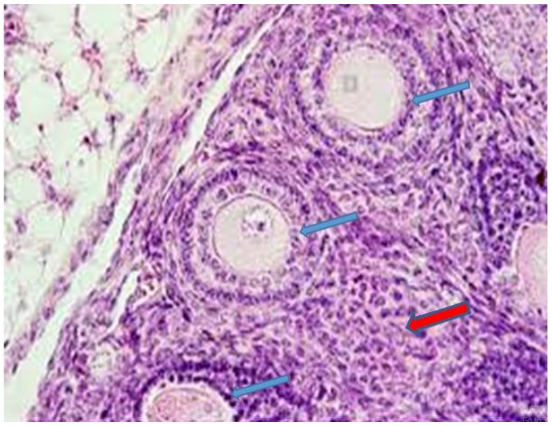

Figure 8. Photomicrograph of rat's ovary administered with 150mg/kg of methanol leaves extract of *D. saxatilis* section shows matured follicles (Blue arrow), and regular ovarian stroma (red arrow)

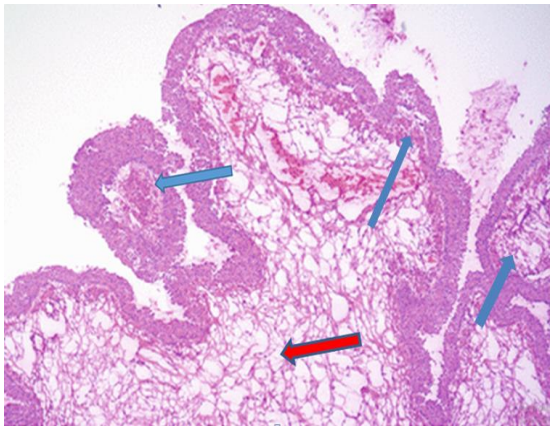

Figure 9. Photomicrograph of rat's ovary administered with 300 mg/kg of methanol leaves extract of *D. saxatilis*. Section shows matured follicles (blue arrow), and regular ovarian stroma (red arrow)

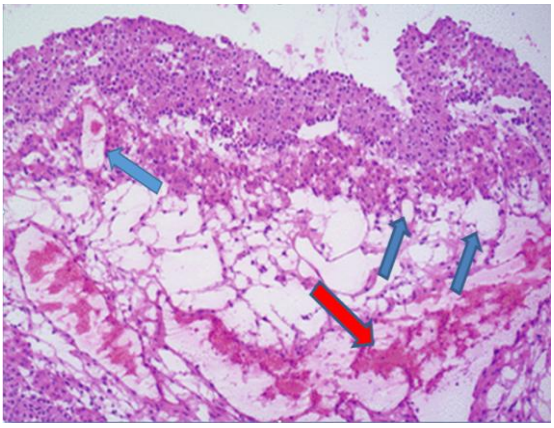

Figure 10. Photomicrograph of rat's ovary administered with 600 mg/kg of methanol leaves extract of *D. saxatilis*. Section shows premature follicles (blue arrow), and regular ovarian stroma (red arrow)

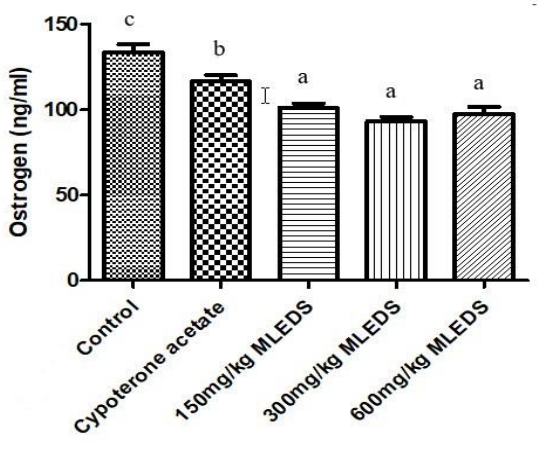

Figure 11. Effect of different concentration of methanol leaves extract of D. saxatilis on oestrogen concentration in female albino rats

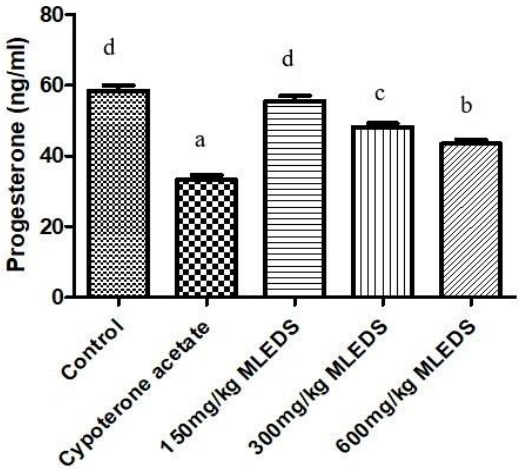

Figure 12. Effect of different concentration of methanol leaves extract of D. saxatilis on progesterone concentration in female albino rats

Discussion

Phytochemicals are substances produced mainly by plants, and these substances have biological active constituent used in the pharmaceutical industry, in medicine and pharmacologically plants represent the main source to obtain various active ingredients (23, 24). In the present study, the phytochemical screening indicated the presence of compounds such as steroids, tannins, and alkaloids. Hiremath and Rao, (25) reported that these compounds exhibit anti-fertility activity, this suggests that the anti-fertility effect observed in the present study may be due to the presence of one or more of these compounds. Saha *et al*. (26) have also reported the presence of an abundant amount of steroids, saponins, and flavonoids in *Dalbergia saxatili* leaves. This also agrees with the present finding which showed the presence of steroids, saponins, and flavonoids. Acute toxicity studies LD50 are usually carried out to determine the dose that will cause death or serious toxic effect when administered within a short period of time. It also shows the dose which kills 50% of the animal population within short period of time and this serves as a bases to establish doses that should be used in subsequent studies (27). The oral median lethal dose value for the methanol stem leaves extract of *D. saxatilis* obtained in rats was found to be above 5000 mg/kg in the present study.

This suggests that the plant extract is non-toxic as no death was recorded. The Organization for Economic Cooperation and Development (20), recommended chemical labeling and classification of acute systemic toxicity based on oral median lethal dose values as very toxic if less than or equal to 5 mg/kg, toxic if greater than 5 mg/kg but less than or equal 50 mg/kg, harmful if greater than 50 mg/kg but less than or equal 500 mg/kg, and non-toxic or not harmful if greater than 500 mg/kg or less than or equal 2000 mg/kg (28). Based on this classification, the oral median lethal dose obtained for rats found to be above 5000 mg/kg, is relatively safe orally. This study agreed with Idris et al. (29) findings whom also reported that the LD50 of *Dalbergia saxatilis* leaves to be above 5000 mg/kg. Estrous cyclicity is controlled by a cascade of neuroendocrine events, involving the activation of the hypothalamic-pituitary-gonadal axis (30). Two modes of gonadotropin-releasing hormone (GnRH) are well established to regulate the estrous cycle one is a tonic or pulse mode of secretion which is responsible for the stimulation of follicular development and steroidogenesis; the other is a surge mode, which is solely responsible for the induction of luteinizing hormone (LH) surges, eventually leading to ovulation (30). Estrogenic chemicals (phytosteroids) are known to cause infertility by shortening the time of the transport of egg, disrupting the estrous cycle, lowering the plasmic progesterone, and decreasing pregnanediol (31). Steroids might be the contributory factor responsible for the disruption of the estrous cycle in the present study this agrees with the findings of Shibeshil et al. (30) who also suggest steroids might be the reason behind the disruption of the estrous cycle in their study. The ovary consists of an aggregation of three endocrine tissues, the stroma, the follicle, and the corpus luteum. The corpus luteum is a direct continuation of follicle development, it forms after ovulation from the hemorrhagic tissues of the ruptured follicle and it becomes the major source of progesterone circulating in plasma (32, 33). In the present study, there is a decrease in the number of corpora lutea and the number of follicles, this may also be due to the inhibitory effect of *Dalbergia saxatilis* on preovulatory follicles. The present study agrees with Beim et al. (34) finding who also found a reduction in the number of developing follicles and corpora lutea in their experiment.

Endometrium plays an important role in the implantation process and also involves vital hormonal regulations (35). These hormonal related changes in uterine glands diameter cause the thickening of the endometrium (environment favorable for embryonic implantation) or cause the thinning of endometrium (environment unsuitable for embryonic implantation) (35). In the present study, *Dalbergia saxatilis* showed an uterotrophic activity as decreasing endometrium thickness was observed in the treatment groups. Hernandez-Nieto et al. (36) also reported the effect of a decrease in endometrium thickness associated with embryonic implantation which agrees with the present study. Hormonal activity depends mainly on the levels of progesterone, estrogen, and their receptors, however also on the rates of progesterone and estrogen metabolism (e.g. up-regulation of enzymes that convert estradiol (E2) to estrone or estrone sulfate or remove sulfate from E2 and estrone) (37). Although progesterone and estrogen are the key modulators of endometrial maturation, their roles in this process are to regulate the expression of numerous endometrial proteins (38). In the present study, there was a reduction in serum concentration of both progesterone and estrogen suggesting that the extract has a hormonal effect. The research of (38) supports the present by mentioning that at low concentration, estrogen and progesterone inhibits gonadotropins, but a high concentration of estrogen and progesterone stimulates them. Cervix and Cervical mucus vary in response to both natural and artificial hormonal changes, it is commonly believed that cervical mucus thinning is associated with normal fertility and that progestogen-induced thickening is an essential contraceptive mechanism this thickening affect fertility by interfering with sperm transport across the cervix (39). In the present study, the histology of the cervix was not affected suggesting that the extract does not have an effect on the cervix and likewise sperm transport.

Conclusion

Dalbergia saxatilis is relatively safe and can be used in clinical formulations based on its high LD50. The administration of methanol leaves extract of *Dalbergia saxatilis* led to a reduction in serum progesterone and estrogen levels and the thinning of the endometrium. These show that *Dalbergia saxatilis* is a potential antifertility agent. Therefore, the use of *Dalbergia saxatilis* as an antifertility agent in traditional medicine has a pharmacological basis and this research authenticates the claim in traditional.

Ethical issue

Authors are aware of and comply with, best practices in publication ethics specifically with regard to authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests, and compliance with policies on research ethics. Authors adhered to publication requirements that submitted work is original and has not been published elsewhere in any language.

Competing Interests

The authors declare that there is no conflict of interest.

Authors' contribution

All authors of this research contributed to data collection, data analyses, and manuscript writing.

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