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Contraceptive Activity of *Vitex doniana* Stem Bark Methanol Extract in Female Albino Rats

Angela Nnenna Ukwuani-Kwaja[1], Ibrahim Sani[1] and Abdulhamid Zubairu[1*]

[1]Department of Biochemistry, Faculty of Life Science, Kebbi State University of Science and Technology Aliero, Kebbi State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author ANU-K designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author IS performed the statistical analysis, and managed the analyses of the study and author AZ carryout the bench work and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The acceptability and accessibility of modern contraceptive drugs are limited especially in northern Nigeria. These contraceptives also have numerous side effects hence there is need to search for safe natural alternatives from medicinal plants. This research was aimed at evaluating the contraceptive effect of stem bark methanol extract of *Vitex doniana* in female albino rats.
Place and Duration of Study: Department of Biochemistry, Faculty of Life Science, Kebbi State University of Science and Technology Aliero, Kebbi State, Nigeria. Between August 2019 to July 2020.
Methodology: *Vitex doniana* stem bark was extracted with methanol and the extract was subjected to qualitative phytochemical screening. Acute toxicity (LD50) of *V. doniana* stem bark extract was determined using up and down method and anti-fertility effect was evaluated via (anti-ovulation, anti-implantation and serum hormonal assay).
Results: The results for phytochemical screening showed the presence of alkaloids, flavonoids, tannins, steroids, glycoside, balsam and volatile oil. The LD50 of the extract was estimated to be greater than 5000 mg/kg as no mortality or any sign of toxicity are recorded within 14 days. The anti-

**Corresponding author: E-mail: mrconfirmre@gmail.com;*

fertility studies, methanol stem bark extract of *Vitex doniana* showed anti-ovulation activity through alteration of estrous cycle, changes in the histology of ovarian corpus luteum and decreasing number of follicles of extract treatment groups compared to control. Serum hormonal assay showed significant decrease ($P<0.05$) in oestrogen and progesterone level respectively in the extract treated groups compared to control group. Also, anti-implantation effect was observed in drug treated group (levenogesterel) and group treated with 400 mg/kg of *V. doniana* stem bark as there was no evidence of conception.

Conclusion: The present study revealed that methanol stem bark extract of *Vitex doniana* is relatively nontoxic at acute dose and possess a moderate amount of antifertility agent.

Keywords: Contraceptive; antiovulation; antiimplantation; hormonal assay; vitex doniana.

1. INTRODUCTION

In the developing world an increase in the use of modern fertility regulating methods (contraceptives) vigorously improves the reproductive health of women [1]. These antifertility agents (contraceptives) are substance that prevents fertilization, ovulation, implantation, and destroys the zygote or causes abortion in females, Globally approximately 137 million women have unmet need for contraception [2], and this unmet need is particularly high in Africa where there is low contraceptive patronage, generally due to lack of access or lack of knowledge to contraceptive options in particular [3]. In Nigeria, The Multiple Indicator Cluster Survey conducted in 2011 revealed that contraceptive prevalence rate is 17.5% and unmet need for contraception is 19.4% [4].

Herbal medicines and their derivatives have been incorporated into traditional medicine virtually since the beginning of recorded history. But it is only in recent times that the broader use of medicinal plants is beginning to garner acceptance in the more expansive international domain [5]. There are certain challenges in the process, including lack of quality control and toxicological studies, the imperative to increase product shelf life, and compliance with international regulatory standards that need to be overcome before their full market potential can be realized [5].

However, even with the previously mentioned drawbacks of herbal medicine, the use of plant based contraceptive agents are better because of their more cultural acceptability, more compatibility with the human body, lesser side effects compared to synthetic contraceptive agents [5]. *Vitex doniana* is one of the agents used for several medicinal purposes [6]. Parts of the plant are locally used by traditional healers for the treatment of various ailments including antifertility effect. However, scientific information on the toxicity and antifertility effect of *Vitex doniana* in man and animal are lacking. The present study investigated the acute and chronic toxicity as well as antifertility effect of methanol stem bark extract of *Vitex doniana* in rats.

Many women in rural areas are not able to access information, supplies and services that could facilitate preventing unplanned pregnancies and planning the number and timing of desired pregnancies [7].

The need to tackle numerous challenges attributed to modern contraceptives has thrown many researchers into search for herbal alternatives. Since ancient times, mankind has used plants to cure diseases and relieve physical sufferings. Because of their cultural acceptability, compatibility with biological system, and lesser side effects. Therefore this research is designed to search for newer and more potent herbal contraceptive with no toxic effects, which may be accessible, generally acceptable and less expensive.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Sample

The plant sample was collected in July 2019 from Zuru town, Zuru Local Government Area of Kebbi State. It was authenticated by a Taxonomist from Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aleiro, with a voucher specimen (V.N. 307) deposited in the herbarium of the same Department.

2.2 Plant Preparation and Extraction

The stem bark of *V. doniana* were washed with clean water and allowed to dry under shade for

two weeks. It was then grinded to coarse powder using grinding machine. One thousand five hundred grams (1500 g) of the powdered stem bark was soaked in 4000 ml of methanol for 72 hrs [8]. It was then filtered using muslin cloth and the filtrate was evaporated using an oven set at 45°C.The dried extract was stored in an air tight container and kept in refrigerator at 4°C. The percentage yield of the extract was calculated using the formula.

Percentage yield = weight of extract / weight of plant sample × 100

2.3 Phytochemical Screening of *Vitex doniana*

The Phytochemical screening for the presence of saponins, tannins, alkaloids, flavonoids, tannins, steroids, saponins, glycosides, cardiac glycosides, saponin glycosides, balsams, anthraguines, and volatile oil were carried out according to the methods described by [9,10,11].

2.4 Experimental Animals

The albino (Wistar) rats used in this study were purchased from Animal House, Usmanu Danfodiyo University, Sokoto in August, 2019. They were transported in plastic ventilated cages to Animal House, Faculty of Science, Kebbi State University of Science and Technology, Aleiro. The rats were allowed to acclimatize for two (2) weeks before the commencement of the experiment. The rats were fed with standard rodent pellets and were allowed access to water *ad libitum*.

2.5 Acute Oral Toxicity Studies (LD50)

The acute oral toxicity study was conducted according to Organization for Economic and Cultural Development for testing of chemicals (OECD, 2001) guideline and up and down method was used for the study. A total of Fifteen (15) 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 group 1, 2, 3, 4 and 5 respectively. After the administration, food was withheld for a further 3-4 hrs. The animals were observed individually at least once during the first 30 min after dosing, periodically at 8, 14, 24, and 48 hrs intervals. The animals were observed for signs of drowsiness, hair loss, loss of appetite, salivation, tremors, convulsion and bulging of the eyes. The animals were thereafter observed for a period of 14 days for any signs of delayed toxicity and mortality.

2.6 Anti-ovulatory Activity

Female albino rats are divided into four groups, of three animals each, fasted overnight and allowed free access to water ad libitum. Vaginal smear from each rat are examined daily for 8 days before administration of the extract and those rats that exhibited normal regular cycles are used. Extract and vehicle were administered orally in the estrous cycle, the vaginal smear is also observed daily for 8 days. The treatments were as follows:

Group I Received distilled water (1 ml/ 100 g) and served as control (negative control)

Group II Received levenogesterol at 1 mg/kg.

Group III Received methanol extract of *Vitex doniana* at the dose of 250 mg/kg

Group IV Received methanol extract of *Vitex doniana* at the dose of 500 mg/kg

Extract was administered for 15 days to cover four regular estrous cycles. The vaginal smear and body weight of each animal are observed every morning between 9 and 10 am on the 16th day, 24 hrs after the last dose was administered the rats from each group were anesthetized and sacrificed. Ovaries are dissected out, suspended in normal saline placed on microscopic slide with cover slip to count number of follicles and *corpuse luteum* [12]. The blood samples were also collected in plan bottles and subjected to hormonal assay.

2.7 Anti-Implantation

Twenty white albino rats of both sexes weighing between 150 to 190 g were used for the studies. The animals are kept under room temperature, and are allowed to acclimatize for two weeks. The female 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 10.00 am. The day sperm cells are found in the vaginal smear was considered as day 1 of pregnancy. Thereafter, three female rats and one (1) male rat are randomly divided into five (5) groups of four (4) rats each and were treated as follows:

Group I Treated with distilled water (1 ml/ 100 g) and serve as (Negative control)

Group II Treated with levenogesterel at 1 mg/kg (Positive control)

Group III Treated with methanol extract at 100 mg/kg body weight

Group IV Treated with methanol extract at 200 mg/kg bodyweight

Group V Treated with methanol extract at 400 mg/kg bodyweight

All the treatment was by oral administration for 19 consecutive days. On day 20 of gestation, each rat is laparatomised under high ether anaesthesia. The uterine horns are exteriorized and incised at the greater curvature of the horns. The latter are examined for sites of implantation and resorption. Numbers of corpora lutea of pregnancy, number of live foetuses and placentae were also determined as stated by [13,14].

2.8 Hormonal Assay

Blood samples (serum) collected on the last day of anti-ovulation study (16th day) via cardiac puncture where subjected to enzyme-linked-immunoasorbent serologic assay (ELISA) techniques used to assay for progesterone and oestrogen level using standard laboratory methods as described by Osunuga et al. [15].

2.9 Data Analysis

The data generated from the study are presented as Mean ± Standard deviation and subjected to one-way analysis of variance (ANOVA) and statistical difference between means were subjected to Duncan multiple comparison test using statistical package for social science (SPSS) version 20. Values are considered statistically significant at $P<0.05$. Graphs are plotted using Microsoft excel and Prism software, micrographs and diagrams were presented where necessary using digital camera.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Results of phytochemical screening

The qualitative phytochemical screening of the methanol stem bark extract of *V. doniana* is presented in Table 1.

3.1.2 Acute oral toxicity (LD_{50})

The testing was terminated when (5000 mg/kg) was reached without mortality, the animals were further observed for fourteen (14) days for any apparent sign of delayed toxicity, no mortality and no sign of toxicity and the LD_{50} was considered to be greater than 5000 mg/kg b.w.

3.1.3 Anti-ovulation effect of the extract in female albino rats

All the animals used in this study present regular estrous cycle (Proestrus, Estrous, Metestrus and Diestrus) for eight days (two cycles) before treatment with methanol stem bark extract of *V. doniana* (Table 2). However upon treatment with extract and standard drug (levenorgestrel), estrous cycle of all the animals in the standard drug (levenorgestrel) group became irregular during two successive cycles. Two rats each from groups treated with 250 and 500 mg/kg b.w of methanol stem bark extract of *V. doniana* showed irregular estrous cycle (Table 2).

The histology of the ovaries of groups treated with MSBEVD revealed a dose dependent decrease in follicles and curpus luteum respectively when compare to control, also standard drug (levenorgestrel) treated group showed lesser number of both curpus luteum and follicle respectively when compare to control (Plates 1-4).

3.1.4 Anti-implantation effect of the extract in female albino rats

The animals treated with 400 mg/kg of methanol stem bark extract of *V. doniana* showed no evidence of conception, however groups treated with 100 and 200 mg/kg revealed placenta and primitive feutal tissues respectively indicating implantation has taking place while standard drug (levenorgestrel) treated group I showed no evidence of conception (Plates 5-9).

3.1.5 Effect of the extract on hormones (Oetrogen and Progesterone)

The relationship between hormonal level of control group, standard drug (levenorgestrel) group and groups treated with methanol stem bark extract of *V. doniana* revealed a significant reduction ($P<0.05$) in serum oestrogen and progesterone of drug treatment group when compared control. Also there was significant

reduction (P<0.05) in serum oestrogen and progesterone of groups treated with 250 and 500 mg/kg when compared to control (Table 3). However serum oestrogen of group treated with MSBEVD 250 and 500 mg/kg significant reduced (P<0.05) when compared to standard drug (levenorgestrel) group. While Serum progesterone level of group treated with MSBEVD at 500 mg/kg and standard drug (levenorgestrel) were not significantly different (P>0.05).

Table 1. Qualitative phytochemical constituents of methanol stem bark extract of *V. doniana*

Phytochemicals	Results
Alkaloids	+
Flavonoids	+
Tannins	+
Steroids	+
Saponin	-
Glycoside	+
Cardiac glycoside	-
Saponin glycoside	-
Balsam	+
Anthraguinone	-
Volatile oil	+

Key: + = Present, - = Not detected

Table 2. Effect of methanol stem bark extract of *V. doniana* on Estrous Cycle

	Normal Control	Levenogesterel (1.074 mg/Kg)	*V. doniana* (250 mg/kg)	*V. doniana* (500 mg/kg)
Corpus luteum	4	0	5	4
Follicle	12	2	8	5

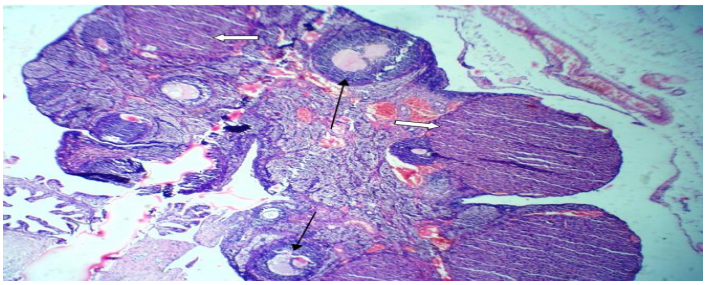

Plate 1. Photomicrograph of rat's ovary obtained from control

Section shows matured follicles (long arrow), corpus luteum (white arrow) and regular ovarian stroma.

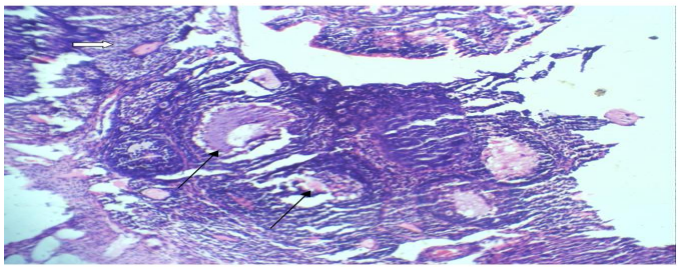

Plate 2. Photomicrograph of rat's ovary administered with (Levenogesterol)

Section shows matured follicles (long arrow), corpus luteum (white arrow) and regular ovarian stroma.

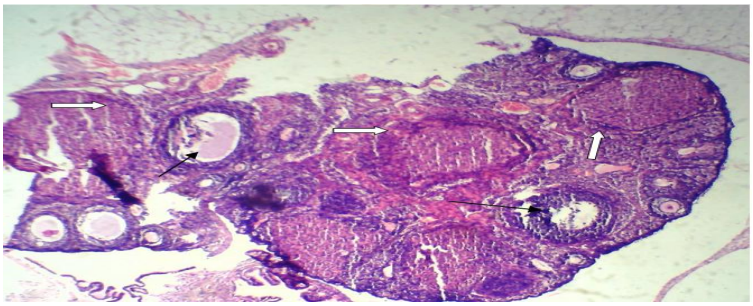

Plate 3. Photomicrograph of rat's ovary administered with 250 mg/kg

Section shows matured follicles (long arrow), corpus luteum (white arrow) and regular ovarian stroma.

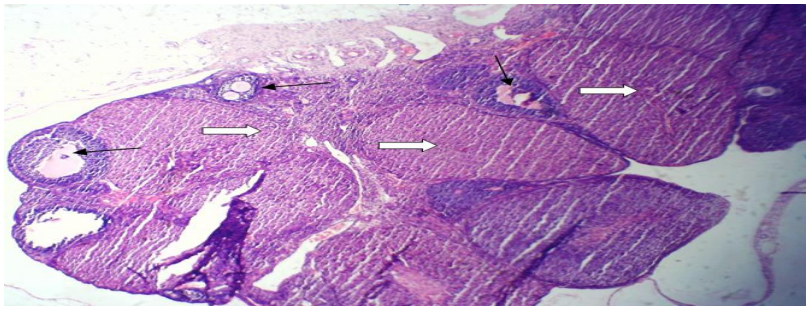

Plate 4. Photomicrograph of rat's ovary administered with 500 mg/kg

Section shows matured follicles (long arrow), corpus luteum (white arrow) and regular ovarian stroma.

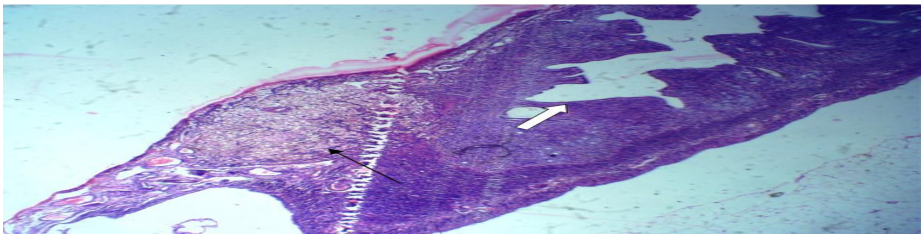

Plate 5. Photomicrograph of rat's uterus obtained from control

Endometrium section showing placental tissues (arrow) and endometrial cavity (white arrow)

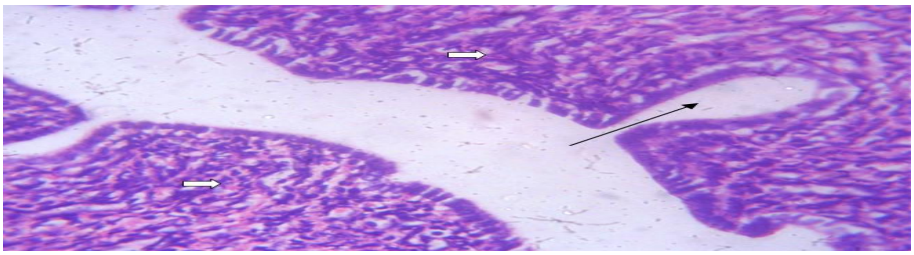

Plate 6. Photomicrograph of rat's uterus obtained from group administered with (Levenogesterol)

Section showing tubular endometrial glands (Arrow head) and compact stroma (white arrow).

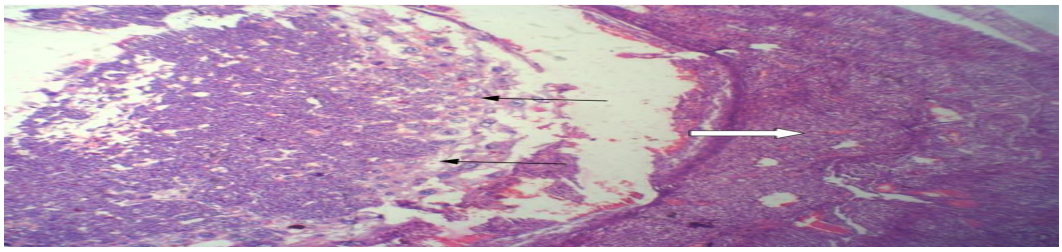

Plate 7. Photomicrograph of rat's uterus obtained from group administered with 100 mg/kg of methanol stem bark extract of *V. doniana*

Endometrium Section show placental tissues (arrows) and deciduous endometrial tissues (white arrow).

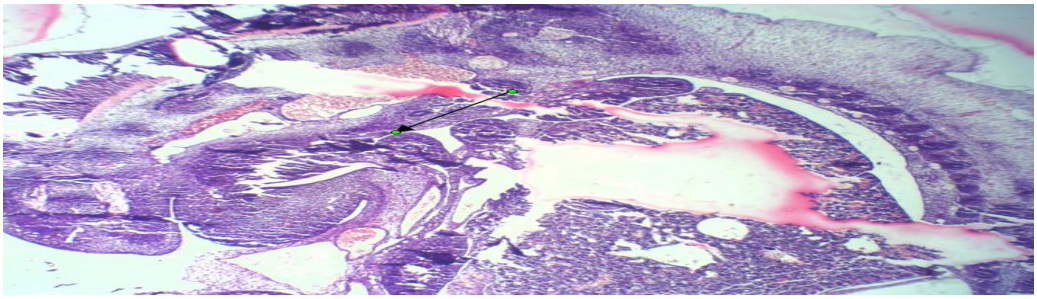

Plate 8. Photomicrograph of rat's uterus obtained from group administered with 200 mg/kg of methanol stem bark extract of *V. doniana*

Endometrium section showing product of conception (primitive fetal tissues)

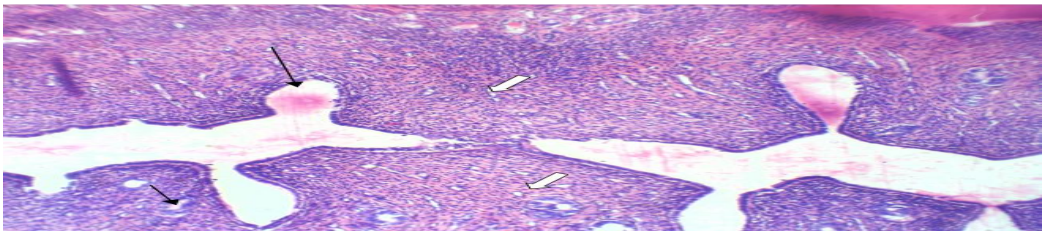

Plate 9. Photomicrograph of rat's uterus obtained from group administered with 400 mg/kg of methanol stem bark extract of *V. doniana*

Endometrium section showing tubular endometrial glands (Arrow head) and compact stroma (white arrow).

Table 3. Effect of methanol stem bark extract of *V. doniana* on hormones (Oestrogen and Progesterone)

Treatment	Serum Oestrogen (ng/ml)	Serum Progesterone (ng/ml)
Normal Control	138.53±8.72^{c}	44.90±2.85^{c}
Levenogesterel (1.074 mg/Kg)	107.60±5.97^{b}	24.27±1.79^{a}
V. doniana (250 mg/kg)	87.67±4.36^{a}	36.40±2.53^{b}
V. doniana (500 mg/kg)	85.70±2.84^{a}	21.63±1.08^{a}

Values are presented as mean ± SD (n = 3) value having same superscript are not significantly different at (P>0.05) using One-Way ANOVA, followed by Duncan multiple comparison test.

3.2 Discussion

Phytochemicals are relevant in medicine, food and the dye industry. Some of them have biological activities [16]. Others have pharmacological effects for example, flavones and tannin form important ingredients of several laxative medicines and in dyes [17]. The present study showed the presence of flavonoids, alkaloids, tannins, volatile oils, steroids, glycosides and balsams. The presence of tannin, flavonoids and steroids has been reported to be a contributing factor in disruption of estrous cycle in female rats [18]. Previous studies by [19] reported that steroids, alkaloids and glycosides have contraceptive properties.

The estrous cycle in females involves many histological, physiological, morphological and biochemical changes within the ovary. During the estrous cycle the maturation and ovulation of pre ovulatory follicles takes place under the combined and balanced influence of ovarian and extra ovarian hormones [20]. Any imbalance in these hormones leads to irregularity in the function of the ovary and irregular changes in the duration of estrous cycle. [21] Stated that estrous cycle of rats became irregular with prolonged estrus and metestrus phases, and reduced diestrus and proestrus phases, also administration of *Garcinia kola* seed extract partially block ovulation, alters oestrous cycle with a prolonged diestrus and may cause a dose dependent adverse effect on foetal development in Sprague-Dawley rats [22]. These researches agrees with the present studies which showed alteration in the estrous cycle of rats treated with MSBEVD mostly prolong diestrus and metestrus phases and a shortened proestrus and estrus phases.

The formation of the corpus luteum is a direct continuation of preovulatory follicular development. The corpus luteum forms after follicular rupture and is the major ovarian source of progesterone [21]. The decrease in the number of corpora lutea and number of follicles is an indication that the development of preovulatory follicles and their conversion into corpora lutea is been inhibited [21]. The decrease in the number of corpora lutea and number of follicles in the present studies may also be due to inhibitory effect of MSBEVD on preovulatory follicles. Beim *et al*., mentioned that the results of their experiment showed reduced ovarian weight, number of developing follicles, graffian follicles and corpora lutea and an increased number in histological sections of the ovary [23], this agrees with the present study as there was reduction in number of developing follicles and corpora lutea.

Uterine glands especially the endometrium is an important unit of implantation process and involves in hormonal regulation [24]. The variations in uterine glands diameter cause hormones related changes in the uterine that created environment unsuitable for embryonic implantation [24,25]. Reported that increase in the uterine weight, diameter, thickness of the endometrium and myometrium bring about uterotrophic changes which favor implantation, while decrease in endometrium thickness prevents implantation. MSBEVD revealed uterotrophic effect via decreasing endometrium thickness which may be attributed to the anti-implantation observed in the present studies.

In rats and many species estradiol is the main luteotrophic hormone. Gonadotropins (Prolactin, FSH and LH) contribute to the luteotrohpic complex as they enhance estrogen secretion by promoting the growth of large follicles which are controlled by gonadotropin releasing hormone [21]. While corpora lutea are the major source of progesterone hormone, when there is fertilization corpus luteum secrets large amount of progesterone which helps in thickening of the endometrium and makes the environment favorable for implantation [21]. Decrease in oestrogen and progesterone In the present studies may be attributed to the effect of MSBEVD on gonadotropins through inhibiting the activity of both follicle stimulating hormone (FSH) and luteinizing hormone (LH). In addition, [26] mention that at low concentration, estrogen inhibits gonadotropins, but high concentration of estrogen stimulates them. In addition, as more estrogen is secreted, more LH receptors are made by the theca cells, inciting theca cells to create more androgen that will become estrogen downstream [26]. This positive feedback loop causes LH to spike sharply, and it is this spike that causes ovulation [27].

4. CONCLUSION

Vitex doniana is one of the plant used for several medicinal purposes, several species of Vitex genus have been reported with antifertility potential. *Vitex doniana* contain phytochemicals (alkaloids, flavonoids, tannins, steroids, glycosides balsam and volatile oils) and was found to be relatively safe at acute

administration. Also MSBEVD exhibited antifertility properties such as alteration of estrous cycle, reduced number of follicles and curpus luteum, anti-implantation at higher dose, reduction in serum oestrogen and progesterone. However, further stuies especially on isolation, characterization of bioactive component is recommended.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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