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Effect of the Aqueous Extract of *Schumanniophyton magnificum* Harms on Sexual Maturation and Fertility of Immature (*K. schum*) Female Rat

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Abstract

The aim of the study was to bring scientific evidence of the curative action of the stem bark aqueous extract of *Schumanniophyton magnificum* (ASMa) on the sexual maturation and fertility of the immature female Wistar rat. Forty immature female rats were randomized and divided into 4 groups of ten animals each and orally treated with the ASMa at doses of 0 (distilled water), 200 mg/kg, 400 mg/kg and 800 mg/kg /BW/day for 30 consecutive days. Body weight and food intake were recorded throughout the experimental period. The precocity of the puberty onset in treated animals was evaluated through the determination of their age at vaginal opening. At the end of the experimental period, 5 animals in each group were sacrificed and blood samples were collected for hormonal assay. Their ovaries and uteri were removed, blotted, weighted and prepared for biochemical analysis. The remaining rats (5 per group) were crossed with males of proven fertility. After laparotomy (ten days after mating) and delivery, fertility and gestational parameters were recorded. It was noticed that, body weight gain increased significantly at all doses although there was no significant difference in food intake. The sexual maturation of treated animal was reduced to 5 days when compared to control. This was associated with the simultaneous elevation of FSH and LH ($p < 0.0001$) at dose of 800 mg/kg and FSH alone ($p < 0.001$) at dose of 400 mg/kg. However, the ovarian cholesterol significantly decreased ($p < 0.0001$) at all doses while the uterine protein significantly increased ($p < 0.05$; $p < 0.0001$) respectively at dose of 400 mg/kg and 800 mg/kg. The animal treated at doses

of 400 mg/kg and 800 mg/kg exhibited an early fertilization (2 - 3 days) when compared to the control one (9 - 14 days). The number of implantation site significantly increased ($p < 0.05$; $p < 0.01$) respectively at dose of 400 mg/kg and 800 mg/kg) after laparotomy as well as the number of alive fetuses after delivery and gestational rate (80% and 100%) respectively. These results provide the strong evidence on the induction of the onset puberty and gonadotropin synthesis, the improvement of the ovarian folliculogenesis and the fertility effect of ASMa in immature rats.

Keywords

Schumanniophyton magnificum, Sexual Maturation, Hormone Level, Fertility

1. Introduction

Medicinal plants have been used for many years in daily life to treat diseases all over the world [1] [2]. According to the world health organization (WHO), more than (70%) of the world's population relies upon complementary and alternative medicine for health care. Many medicinal plants are used to treat various reproductive function ailments, such as female infertility which is a public health concern in African country [3]. Infertility is characterized by the absence of fertilization or pregnancy in a couple which exercises followed-up sexual relations without the use of contraceptives for a period of two years [4]. Infertility is a disease of the reproductive system which affects both men and women with almost equal frequency. However, in many African countries and particularly in Cameroon, women are mostly the ones incriminated [5]. That is why the search for remedy against infertility within a couple is more in women. According to their social status, one can refer either to modern or traditional medicine [6].

Hopefully, many ethnopharmacological studies have proven the implication of medicinal plants or its derived chemical compounds which biological properties regulate some female reproductive function in mammals [7]. These metabolites act on main organs of the reproductive system to inhibit or induce ovarian folliculogenesis. Their biological activities are often evaluated on reproductive organs of immature female rats which have long been used as a model system for studying, *in vivo,* the inducing effect of pharmacological compounds [8] [9] and medicinal plants [10] [11] on ovarian folliculogenesis. In those various studies, the gonadotrophic-like effects of the preparation were characterized by the following biological parameters: increase in the weight of the ovary and uterus; opening and cornification of the vagina; formation of corpora lutea or changes in the histology of the ovary, uterus and vagina; induction of ovulation; increase in ovarian estradiol, progesterone, protein levels; decrease in ovarian cholesterol level.

Schumanniophyton magnificum Harms (family *Rubiaceae)* was selected on

the basis of his ethnomedical studies and endemicity. Stem bark of this plant is widely used in association with the stem bark of *Albizia zygia,* to treat fever, malaria, dysmenorrhoea and some cases of women infertility [12]. Phytochemical investigation of *Schumanniophyton magnificum* plant showed the presence of secondary metabolites, such as flavonoids, alkaloids, polyphenols and steroids [13]. The present study was therefore undertaken to evaluate the effect of the aqueous extract *Schumanniophyton magnificum* (ASMa) on sexual maturation and fertility on immature female rats.

2. Materials and Methods

2.1. Plant Material

Plant collection and extract preparation. The fresh bark of *S. magnificum* was collected from trees in "Eseka" subdivision (southern Cameroon). Botanical identification was performed in the Cameroon national herbarium (HNC) where a voucher N˚65110/HNC has been deposited. These stem bark were ground in a motar and the powder was obtained. Aqueous extract of *S. magnificum* was prepared following the recommendations of the traditional practitioners consulted for treating of sterility. Slight modifications were applied to improve the yield of extraction, 2 kg of *S. magnificum* was soaked in distilled water (6l) and the mixture boiled for 30 min. the heated decoction was taken and allowed to cool at room temperature, filtered using whatman paper N˚3 and oven-dried to give 238.3 g of dried aqueous extract (yield of extraction 11, 9%; w/w based on the dried starting weight). The extracts were prepared in distilled water at concentrations of 8 mg/ml (extract 1), 16 mg/ml (extract 2) and 32 mg/ml (extract 3). Together with distilled water (control group), these preparations (extracts 1, 2 and 3) were orally administered to animals in volume of 1 ml/kg body weight, corresponding to doses 0, 200, 400, 800 mg/kg respectively.

2.2. Animals

The animals used in this study were immature female albino wistar rats of 21 - 22 days old and weighing between 30 - 45 g. They were bred in animal house of the department of animal sciences of the University of Douala- Cameroon), housed under natural conditions of light (12 h cycle) and temperature (22˚C ± 2˚C) and fed with a standard laboratory diet and tap water *ad libitum*.

Puberty onset and fertility assays. The precocity of the puberty onset in treated animals was evaluated through the determination of their age at vaginal opening and the inducing effect of the extract on animal fertility evaluated through the determination of ovarian and uterine weights, protein or cholesterol levels; number of corpora lutea, implantation sites and other gestational parameters. A total of forty immature female rats were randomized, based on their body weight, into 4 groups of ten animals each. They received by gavage, either water or different doses of ASMa for 30 consecutive days. Their body weight and food intake were recorded, throughout the experimental period, at 2 day inter-

vals. After two weeks of treatment, each rat was checked every day for vaginal opening and the vaginal smear collected and stained from the day of opening up to the end of experiment. The vaginal smears were stained by May-Grünwald solution (1% w/v) followed by Giemsa solution (1% w/v) and viewed under low magnification (40×) under a light microscope. This staining helped in characterizing each phase of the rat's estrous cycle, its length and that of complete cycle. At the end of the experimental period, 5 animals in each group were randomly sacrificed. Their ovaries and uteri were removed, blotted, weighted and stored at −20˚C until used.

The remaining rats (5 per group) were crossed the following day, during two weeks, with males of proven fertility. Vaginal smears were collected on daily basis in order to assess for the presence of sperm. A laparoscopy was undertaken under diazepam (5 mg/kg, 5 mg/kg) and ketamin (50 mg/ml, 80 mg/kg) ten days after the day of mating to count the number of implantation sites in uterine cords and the number of corpora lutea in ovaries. After delivery, the fetuses were weighed and resorption sites (number of implantation site - number of live fetuses), implantation index ([total number of implantation sites/number corpora lutea] × 100), resorption index ([total number of resorption sites/total number of implantation sites] × 100), preimplantation loss ([number of corpora lutea - number of implantations/number of corpora lutea] × 100), postimplantation loss ([number of implantations - number of life fetuses/number of implantations] × 100), antifertility activity ([number of females without life fetuses/total number of females] × 100), antiimplantation activity ([number of females without implantation sites/total. number of females] × 100), and gestation rate ([number of females with life fetuses at birth/total number of gestational females] × 100) were calculated according to Musa ToyinYakubu and Isa Fakai Musa, 2012 [14]. Animal handling and in vivo experiments were carried out in conformity with the European Union on Animal Care (CEE Council 86/609) guidelines adopted by the Institutional Ethics Committee of the Cameroon Ministry of Scientific Research and Technology Innovation.

2.3. Biochemical Analysis

To prepare the uterine and ovarian supernatant, ovaries and uteri were homogenized in Tris - sucrose buffer (0.25 M sucrose, 1 Mm EDTA and 10 Mm Tris-HCl, Ph 7.4) at 1% and 2% respectively. The homogenate was then centrifuged at 6000 × g at 4˚C for 15 min, and the supernatants collected were used for protein [15] and cholesterol [16] assays.

2.4. Statistical Analysis

Data are expressed as mean ± standard error of the mean (SEM). Means were compared by one-way and two-way analysis of variance (ANOVA) followed by the Tukey and Bonferonni post hoc test respectively, using the graph pat program. The analysis of percentages was done by (Khi-square) test. $P < 0.05$ was considered significant.

3. Results

3.1. Effect of ASMa on Body Weight Gain and Food Intake

The effect of ASMa on the body weight of female rats during the treatment is presented in Figure 1. There was a linear increase at various rates in their growth. When compared to control animals, a significant drop in the body weight of animals treated with the doses of 400 mg/kg and 800 mg/kg was noticed after 12 days of treatment ($p < 0.0001$). Animals receiving the 200 mg/kg dose instead gained more weight ($p < 0.01$) when compared to control animals, starting from the 24th day of treatment till the end of the experiment. As concerns the monitoring of their food intake, no significant variation, whatever the duration of the treatment was observed between the different experimental groups (Figure 2).

3.2. Effect of ASMa on the Age and Estrous Cycle Phases at Vaginal Opening

Figure 3 shows the mean age of animals at vaginal opening and the percentage of those presenting vaginal aperture at a given age. Female rats that received ASMa the two highest doses presented vaginal opening almost five days earlier ($p < 0.0001$) as compared to the control animals [45 ± 0.36 days (0 mg/kg) *vs* 44.16 ± 0.30 days (200 mg/kg) or 39.66 ± 0.66 days (400 mg/kg) and 38.5 ± 0.56 (800 mg/kg)].

About 14% of 37 days old rats treated at dose of 800 mg/kg (against 0% for its respective control) presented vaginal aperture. This percentage was significantly increased in these animals and in those treated at dose of 400 mg/kg when they were 38 days old (45% and 53% respectively with two highest doses compared to 0% with 0 mg/kg and 10% with 200 mg/kg). Moreover, complete vaginal opening

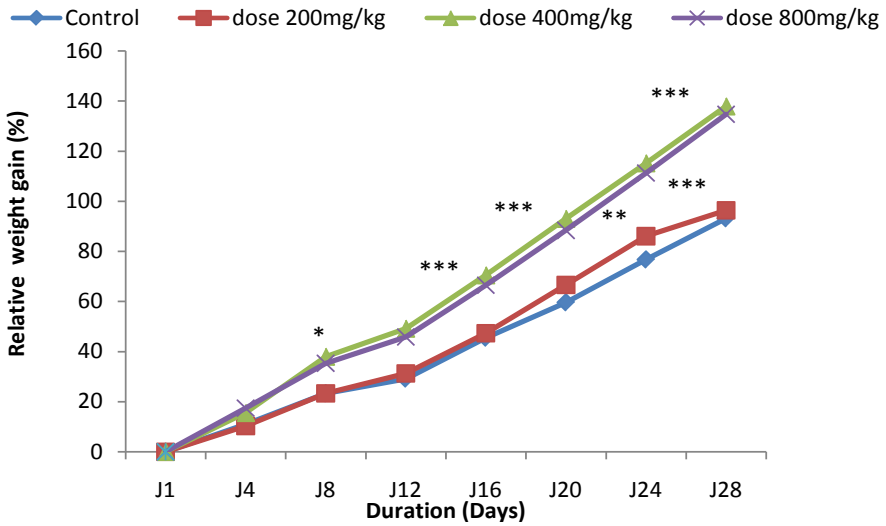

Figure 1. Body weight gain of rats after administration of various doses of ASMa. Each value represents mean ± SEM; n = 10. *; **; *** Values are significantly different respectively at $p < 0.05$; $p < 0.01$; $p < 0.0001$ vs control.

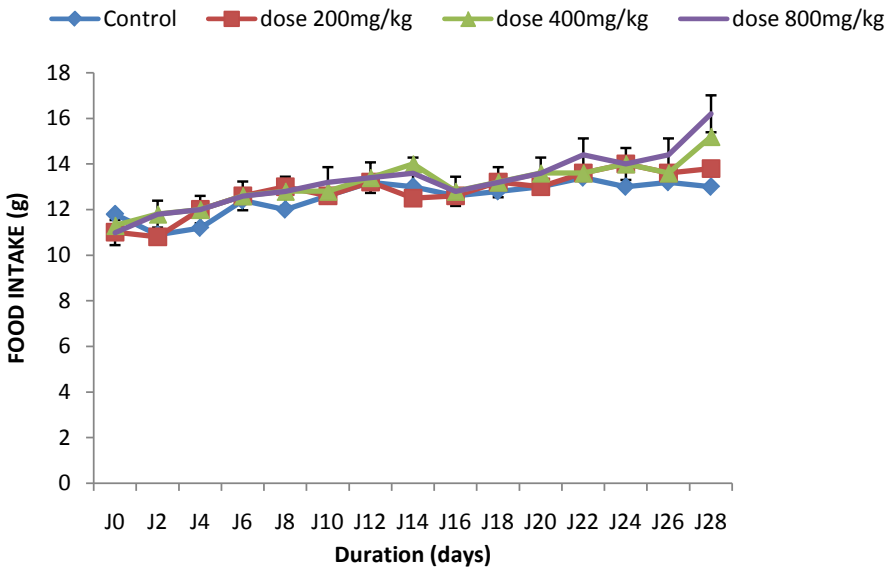

Figure 2: Effect of ASMa on Food intake. Each point represents mean ± SEM; n = 10.

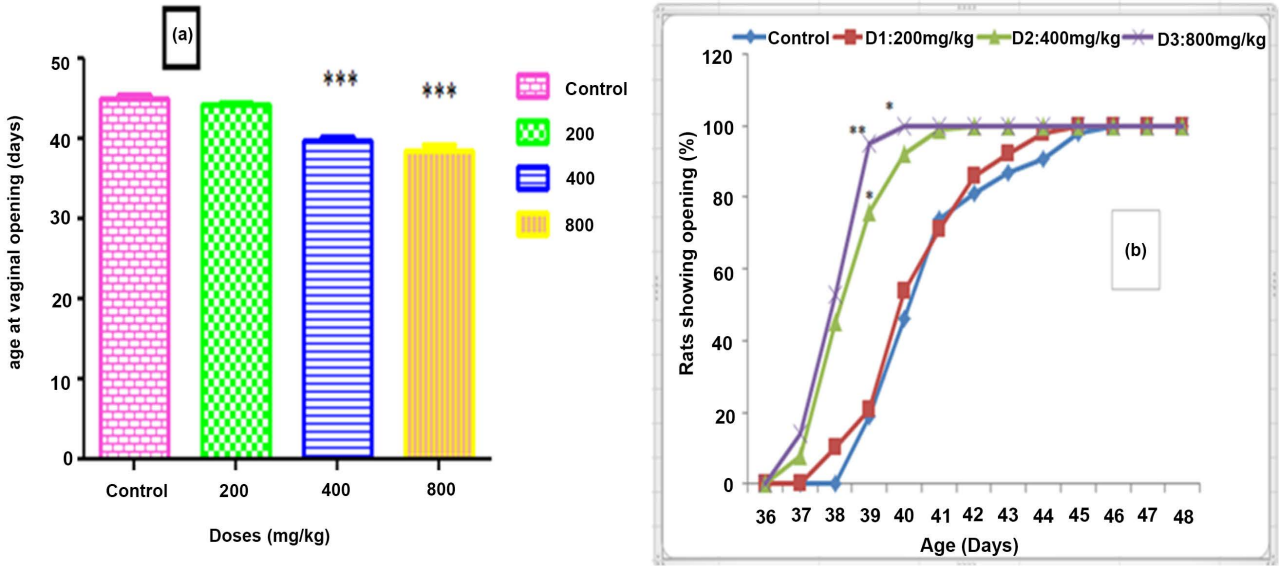

Figure 3. Prepubertal effect of oral administration of ASMa on the timing of the puberty onset. The mean day of vaginal opening in animals (a) and the percentage of animal showing vaginal opening as a function of age (b). *, **, ***Values are significantly different respectively at $p < 0.05$, $p < 0.01$ and $p < 0.0001$ vs control. Each value represents the mean ± SEM, n = 10.

was obtained in all animals of these experimental groups (400 and 800 mg/kg) when they were 39 - 40 days old while those of the control group was obtained at the age of 45.

3.3. Effect of ASMa on Ovarian Weight and Cholesterol Level

The changes obtained in ovaries after 30 days of oral administration of various doses of ASMa to immature rats are presented in Figure 4. We noticed a significant increase ($p < 0.05$ and $p < 0.0001$) of the weight of the ovaries respectively at dose of 400 and 800 mg/kg (Figure 4(a)). Ovarian cholesterol significantly

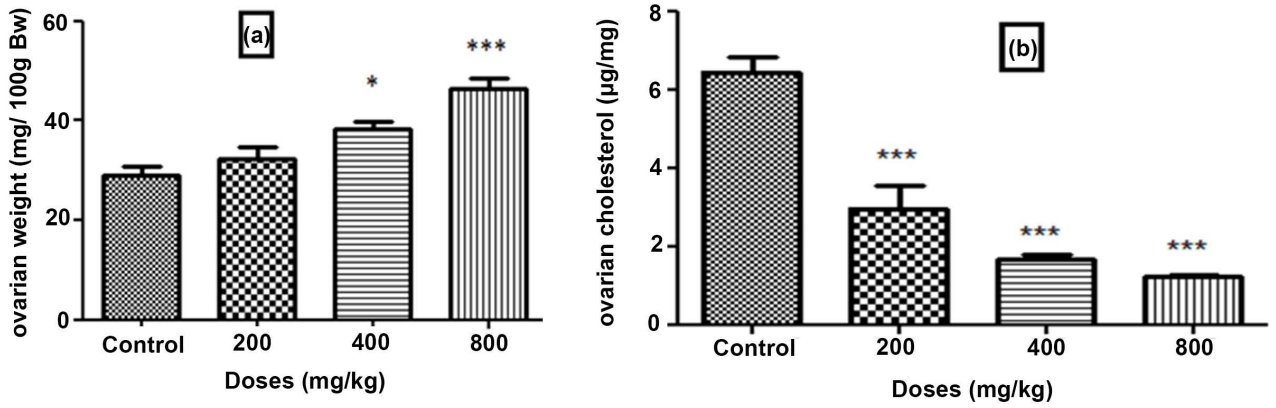

Figure 4. Effect of ASMa on the relative weight of the ovaries (a) and the ovarian cholesterol (b). *; *** Values are significantly different respectively at $p < 0.05$ and $p < 0.0001$ vs control. Each histogram represents the mean ± SEM; n = 5.

decreased ($p < 0.0001$) at all doses when compared to the control group (**Figure 4(b)**).

3.4. Effect of ASMa on Uterine Weight and Proteins Level

Oral administration of ASMa to immature female rats significantly increased the weight of the uteri at dose of 400 and 800 mg/kg (**Figure 5(a)**) while the uterine proteins were also increased ($p < 0.0001$) at all doses when compared to the control group (**Figure 5(b)**).

3.5. Effect of ASMa Gonadotropic Hormones Level (FSH, LH)

Oral administration of ASMa to immature female rats significantly increased ($p < 0.0001$) the FSH and LH level notably at dose of 800 mg/kg when compared to control group (**Figure 6**).

3.6. Effect of ASMa on Some Fertility and Gestational Parameters

Table 1 illustrates effect of the aqueous extract of ASMa on some fertility and gestational parameters. It is found that the number of implantation site significantly increased ($p < 0.05$; $p < 0.01$) respectively at dose of 400 mg/kg and 800 mg/kg) after laparotomy as well as the number of alive fetuses after delivery and gestational rate (80% and 100% respectively).

4. Discussion

The present study was conducted to evaluate the effects of ASMa on female Wistar rat sexual maturation, in order to bring scientific evidence to the traditional use of this plant for the treatment of woman sterility. *S. magnificum*, which is the plant of interest in this study, is used by some people in Africa for its pharmacological properties. As decoction, the bark is well used among some tribes of Cameroons as a remedy for dysentry and as enema while other tribes use it only as lotion after circumcision [12] [17] [18]. Its effect on the onset of puberty (age and phase of the estrous cycle at vaginal opening), ovarian

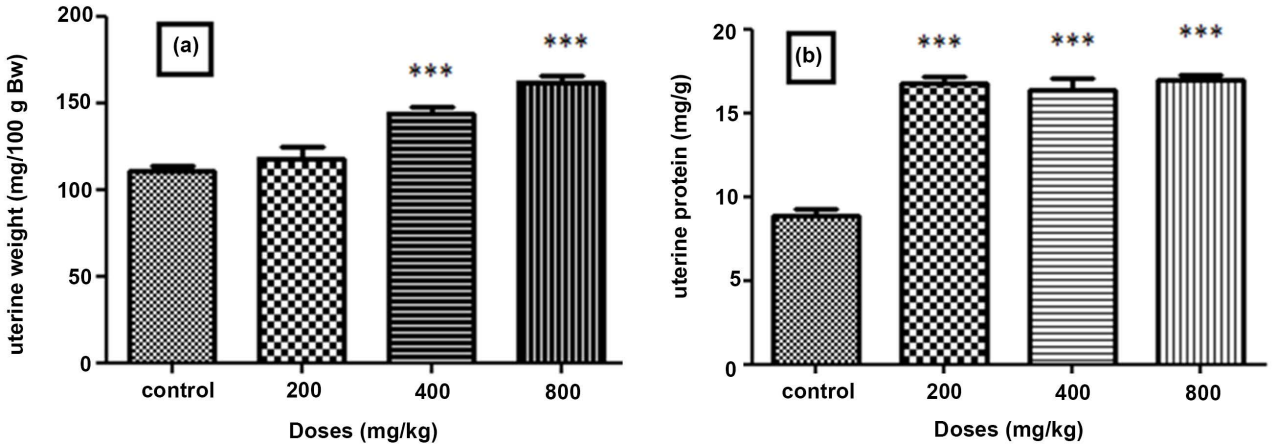

Figure 5. Effect of ASMa on uterine relative weight (a) and uterine proteins level (b). *** Values are significantly different respectively at $p < 0.001$ vs control. Each histogram represents the mean ± SEM; n = 5.

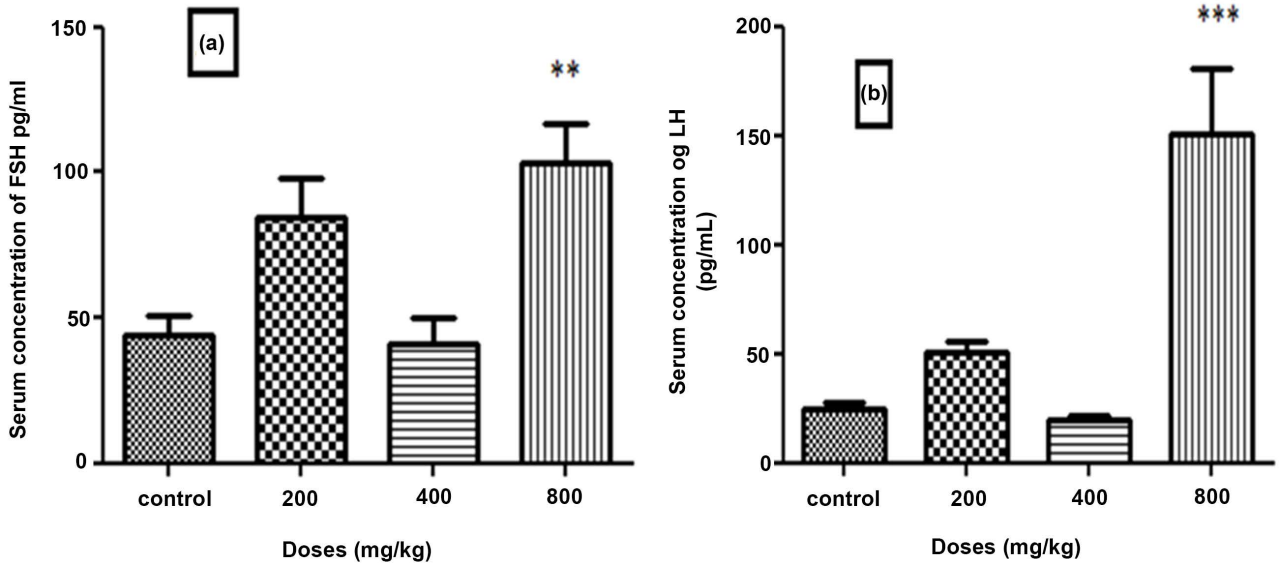

Figure 6. Effect of ASMa on gonadotropic hormones level FHS (a) and LH (b). **; *** Values are significantly different respectively at $p < 0.001$ and $p < 0.0001$ vs control. Each histogram represents the mean ± SEM; n = 5.

folliculogenesis and the fertility of immature female rats were evaluated. The choice of these parameters was not only guided by the influence of the gonadotrophic hormones (FSH, LH) on the precocious onset of puberty and the induction of the follicular growth in immature female rats, but also by the clinical usage of these hormones in the treatment of various forms of infertility (ovulatory defects or hypogonadal infertility) [19].

We first determined the age of sexual maturation of experimental animals. Most of the assessed parameters (body weight, uterine and ovary wet weight, serum levels of gonadotropins and uterine level proteins) were found more elevated in animals who received 400 mg/kg and 800 mg/kg of ASMa than the control animals associated with a precocious onset of vaginal opening. This shows that the plant extract could contain molecules acting, as one of the above

Table 1. Effect of ASMa on some fertility and gestational parameters.

Studied parameters	Dosage (mg/kg/Day)			
	0	**200**	**400**	**800**
N˚ Implantation sites	3.4 ± 2.08	3.2 ± 1.95	5.8 ± 1.46*	7.2 ± 0.37**
N˚ Fetuses Alive	2.8 ± 1.71	2.6 ± 1.6	5.0 ± 1.2*	6.2 ± 0.2**
Mean weight of fetuses (g)	4.09 ± 1.44	4.23 ± 0.23	5.1 ± 0.64	5.22 ± 0.11
N˚ Resorption sites	0.8 ± 0.48	0.6 ± 0.40	0.8 ± 0.37	1.00 ± 0.31
Resorption Index (%)	5.8	6.25	6.8	2.77
Postimplantation Loss (%)	29.41	18.75	13.79	13.88
Antiimplantation Activity (%)	60	60	20**	0***
Antifertility Activity (%)	60	60	20*	0***
Gestation Rate (%)	40	30	80**	100***

Each value represents the mean ± SEM; n = 5. *; **; *** are significantly different respectively at $p < 0.05$; $p < 0.01$ and $p < 0.001$ vs control.

compounds, on the precocious onset of puberty. The simultaneous elevation of gonadotropins and consequently ovarian hormones on treated animals indicates the activation of the hypothalamic-pituitary-ovarian axis. That's why the age of the sexual maturation of our experimental animals was set at 39 - 40 days old when compared to control (45 days). Uterine and vaginal growths observed in these animals may result from the action of endogenous hormones particularly estradiol which was found to stimulate uterine growth and vaginal epithelial proliferation [20] [21]. This could be due to the presence of high level of compounds such as flavonoids in our extract [13] one of the most prevalent classes of phytoestrogens [22]. Indeed, phytoestrogens are plant-derived chemicals with structural similarities with 17β-estradiol, the most potent endogenous estrogens [23] [24] [25]. They have diverse biological activities resulting from their ability to mimic endogenous estrogen actions, to inhibit hormone action, to modulate hormone production, or to alter hormone receptor populations [26] [27]. The opening of the vagina on attainment of the pubertal age of rats resulted from the increase of the serum levels of FSH and LH (400 mg/kg; 800 mg/kg) afterwards secretion of estrogens by ovarian follicles. The vaginal cells are also keratinized in this high estrogenic environment [28], that is why the vaginal smear of pubertal rats the day of vaginal opening corresponds to the estrus phase of the cycle or to the nearest phase following it (metoestrus). These results are in accordance with the observations of Ojeda S and Urban ski H (1988) [29] who reported that these elevations of gonadotropins especially FSH was to stimulate ovarian follicles growth and maturation, therefore promote simultaneously the production of oestradiol agreement and finally initiate a new ovulatory cycle [9] [30]. In the other hand, estrogen are reported to promote the FSH-stimulated expression of mRNA of P450 aromatase, FSH receptor, LH receptor and the production of cAMP by rat granulosa cells in follicles with oocytes [31]. That's why the weight

of ovaries in this study significantly increased at high doses particularly at dose of 800 mg/kg.

The data on uterine parameters presented in this study has shown an increase in uterine proteins and decreased in ovarian cholesterol at all the doses as reported (Lee and Lee, 1996) and (Kouakou and Benie, 2003) [32] [33]. This increase in uterine proteins and weight may have resulted not only from the uterine cell proliferation induced by the oestrogenic effects of some chemical components of ASMa, but also from the increase in their water imbibition effect, especially at high doses age in these cells. The decrease of cholesterol could be due to hormonal synthesis since cholesterol constitutes the main precursor of steroids hormones during their biosynthesis. Its reduction clearly proves its utilization for the biosynthesis of oestradiol which will then contribute into the stimulation of ovarian cells growth. Besides, it is well established that follicular development ends at ovulation. This important physiological landmark of the ovarian function is followed by a stratum transformation of broken follicles into hemorrhagic points [34]. Altogether, the above observations suggest the inductive effects of the aqueous extract of *S. magnificum* on ovarian folliculogenesis or steroidogenesis [12] [35]. This induction is not related to the amount of extract administered, but it may probably result to the synergistic effect of various biochemical compounds in the plant aqueous extract. To better appreciate the inductive effects of the plant extract, fertility test was then carried out.

As concerns the test on gestational parameters, the increase of implantation sites and number of fetuses alive was noticed especially in animals treated with high doses (40 mg/kg and 800 mg/kg) respectively $p < 0.05$ and $p < 0.001$. During our study, these treated animals presented an early fertilization (2 - 3 days) compared to the control animals which could be fertilized by the males only after several days (9 - 14 days). This result implies the induction of a favorable milieu for zygote implantation and development generated by the plant extract components. Indeed, several lines indicated that, implantation of the mammalian embryo into the wall of the uterus is regulated by a timely interplay of the ovarian hormones, estrogen and progesterone [36] [37]. In this work, it could then be suspected that exposure of very young female rats to treatment with extracts of *S. magnificum* for 30 days prior to the encounter of males may have contributed to the implementation of some changes in the uterine endometrium that transformed it from a non-receptive state to a receptive phase allowing the implantation and development of blastocyst. Consequently, this was followed by a remarkable high rate of gestation (100%) at the highest dose .The other parameters did not present any significant differences among the control and treated animals.

5. Conclusion

From the results of the study, it could be concluded that, aqueous extracts of stem bark of *S. magnificum* have promising estrogenic effect. This study has

proven the implication of some active compounds present in this plant on the rapid maturation of ovarian follicular cells leading to a precocious puberty onset (39 vs 45 days) and the improvement of fertility. These sexual pharmacological properties of ASMa support the traditional use of this plant for the treatment of sterility. Further investigations are needed to determinate the above-mentioned active compounds of ASMa.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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