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Effect of the Aqueous Stem Bark Extract of *Schumanniophyton magnificum* on Reproductive Functions on Wistar Strain Mature Female Rats

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Abstract

In recent years, the rate of infertility has not stopped increasing in the world. The aim of the present study was to evaluate the effects of the aqueous extract of *Schumanniophyton magnificum* (Rubiaceae) on cyclicity, ovulation and gestation in mature rats. **Methods:** After a qualitative phytochemical analysis of these aqueous extracts, the experimental studies carried out were based on the evaluation of the pro-fertility effects of this extract in mature rats. For this purpose, 35 rats were used for the estrous cyclicity test and treated for 21 days at the end of which vaginal smears were taken and the duration, as well as the frequency of the appearance of the phases of the cycle, were evaluated. The ovulation test was performed on 80 female rats, which were divided into two groups of 40 animals and treated respectively in the morning and evening with distilled water, β-oestradiol or plant extract at doses of 200, 400 and 800 mg/kg. At the end of estrus, the rats were sacrificed, the ovaries were removed and weighed, the hemorrhagic points counted and the blood samples were taken for hormonal studies. The last phase of the study consisted in evaluating the effects of these plant extracts on the evolution of gestation. Thus, 42 mature rats were treated during the periods from the 1st to the 10th day (1st stage), and from the 11th to the 17th day (2nd stage). At the end of these two phases, a laparotomy was performed and the number of implantation sites and corpus luteum was counted. And finally, at parturition, from the 18th to the 22nd day (3rd stage), the number of living pups was performed and the gestational parameters were calculated. **Results:** Administration at the begin-

ning of prœstrus allowed a significant increase ($p < 0.0001$) in the number of ovulations, bleeding points and oestradiol levels at doses of 400 and 800 mg/kg of *S. magnificum* extract. On the other hand, a significant decrease in progesterone levels ($p < 0.0001$) was noted. Concerning the effects on the estrous cycle, after 3 weeks of treatment of the rats, there was no disturbance in the total duration of the estrous cycle when compared to the control. However, there was a significant increase in the duration of proestrus coupled with a significant decrease in the duration of diestrus in these treated animals. In pregnant rats, administration of the different extracts from day 1 to day 10 resulted in a significant decrease in the number of implantation sites ($p < 0.01$) at the dose of 800 mg/kg. Similarly, blood discharges were observed during the first six days of treatment when compared to the control. Decreases in progesterone, implantation and pregnancy levels were observed in rats treated with 400 and 800 mg/kg ($p < 0.0001$). Treatment from day 18 to 22 of gestation significantly decreased fetal weight from female rats treated with *S. magnificum* at doses of 200, 800 mg/kg ($p < 0.01$) and 400 mg/kg ($p < 0.05$) when compared to the control. In addition, early parturition was recorded in these animals treated at dose of 400 mg/kg (21 ± 0.25 d) and 800 mg/kg (20.33 ± 0.21 d) ($p < 0.0001$) with *S. magnificum* when compared to the control (23 ± 0.16 d). **Conclusion:** It appears from all these investigations that the aqueous extract of *S. magnificum* promotes fertility in the rat but represents a danger for the good development of gestation. All these results obtained would be closely related to the presence of certain chemical compounds contained in these various extracts; which would justify their use in traditional medicine for the treatment of certain cases of female infertility in Cameroon.

Keywords

Estrous Cycle, Ovulation, Fertility, *Schumanniophyton magnificum*

1. Introduction

Cameroon, like most African countries, is characterized by a relatively high birth rate; a demographic indicator that does little to hide the drama of infertility experienced by many couples [1]. The consequences of infertility are not negligible because they limit the perpetuation of the animal and human species. Therefore, it is considered as a serious public health problem that represents in 15% of the cases studied, the most difficult experience in the life of a human being, causing marital frustration, violence, divorce and sometimes polygamy [2]. Infertility is a disease of the reproductive system that is defined as the inability of a couple to conceive after one or two years of unprotected sex during the woman's fertile period [3]. A variety of plants are used in Cameroon to overcome this infertility problem [4] [5]. In Cameroon, *Schumanniophyton magnificum* has been recognized as a plant relieving infertile women. Subsequent studies have shown the

accelerating and stimulating effect of *Schumanniophyton magnificum* on the growth and fertility of immature rats [6]. Recent phytochemical studies revealed the presence of secondary metabolites, such as flavonoids, alkaloids, polyphenols and steroids [7] [8]. Therefore, the present study was undertaken to investigate the effects of the aqueous extract of *Schumanniophyton magnificum* (Rubiaceae) on cyclicity, ovulation and gestation in mature female rats.

2. Materials and Methods

2.1. Plant Material

Source of the plant: The fresh bark of *S. magnificum* was taken from trees in the "Eseka" locality (central region of Cameroon). The botanical identification was carried out at the National Herbarium of Cameroon (HNC) at n˚65110/HNC, a sample of which is there.

2.2. Preparation of the Extract

Preparation of the extract: These barks were ground in a blender and the powder was obtained. The aqueous extract of *S. magnificum* was prepared following the recommendations of the traditional practitioners consulted for the treatment of sterility. Slight modifications were applied to improve the extraction yield, 2 kg of *S. magnificum* was soaked in distilled water (6l) and boiled for 30 min. The decoction was cooled to room temperature, filtered through whatman No. 3 paper and oven dried to give 238.3 g of dried aqueous extract (extraction yield 11, 9%; w/w based on 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). These preparations (extracts 1, 2 and 3) were administered orally to animals at the volume of 10 mL/kg body weight, corresponding to the doses 0, 200, 400, 800 mg/kg respectively. The concentrations of 8 mg/mL were obtained by reconstitution from the recipe mainly used by traditional practitioners following the ethnopharmacological survey carried out in the locality of Eséka (south of Cameroon), and the other two doses were its multiples.

2.3. Experimental Animal

The animals used in this study were mature female albino wistar rats, 10 to 12 weeks old and weighing between 150 g and 180 g. they were bred and raised in pet shops of the department of animal sciences of the University of Douala-Cameroon, housed under natural light (12 h cycle) and temperature (22˚C ± 2˚C) conditions and fed a standard laboratory diet and tap water ad libitum.

2.4. Experimental Design

Pre-treatment stage: The animals were acclimated for two weeks. Vaginal swabs were taken to select females with at least four regular estrous cycles as described by some authors [9].

Treatment stage: The experiment was carried out in 3 parts:

1) Estrous cycle study: The estrous cycles of 20 rats were studied using vaginal swabs. 20 female rats in this experiment were divided into four subgroups (1a, 1b, 1c and 1d) of 5 rats each. Rats in subgroup 1b-1c-1d received 200, 400, and 800 mg/kg body weight of extract by gavage for 21 days, respectively, while those in subgroup 1a serving as controls received an equivalent volume of distilled water. The four stages of the estrous cycle were defined using the vaginal smear method. Vaginal smears were collected daily using a small suction pipette and normal saline (0.9% NaCl) between 9 and 10 a.m., then the smears were placed on slides and examined using the light microscope. Rats with a 4- to 5-day estrous cycle of proestrus-oestrus-metoestrus-dioestrus were classified as normal, whereas any deviation from this pattern in duration and sequence was classified as abnormal.

2) Ovulation study

After rat distribution, treatments were administered as follows:

Group 1: 5 rats were treated with distilled water orally and 5 other rats with beta estradiol intraperitoneally in the morning of proestrous (9 o'clock). Another group of animals identical to the previous one was treated rather in the evening (6 p.m.) of the same stage (proestrus). These two groups of animals were considered as controls.

Group 2: 15 rats were divided into 3 subgroups of 5 rats each and treated respectfully with 200, 400 and 800 mg/kg of aqueous *S. magnificum* extract. The extract was administered orally on the morning of proestrus (9 a.m.). The female rats were sacrificed the next day in estrus using chloroform anesthesia.

Group 3: 15 rats were separated into 3 subgroups of 5 rats each and treated respectfully with 200, 400 and 800 mg/kg of aqueous *S. magnificum* extract. The administration of the extract was given orally in the evening of proestrus. The administration of the extract was made orally in the evening of proestrus. The female rats were sacrificed the next day in estrus using chloroform anesthesia.

Performing Pap smears: The vaginal smear was performed as described by Marcondes *et al.* (2002) as follows: Samples of vaginal smears were taken by the aspiration technique. During the experimental period, every morning between 9:00 a.m. and 10:00 a.m., each cage of animals was transported to the experimental room. Vaginal secretion was collected with a plastic pipette filled with 10 µL of normal saline (0.9% NaCl) by slightly inserting the tip into the rat's vagina. The vaginal fluid was placed on different glass slides and brought to observation under a microscope with objective 10 or 40 for the unstained slides or objective 100 for the stained slides.

Determination of the number of ovulations: Animals in the ovulation study were sacrificed on the morning of estrus by brain dislocation. The oviducts of each of the uterine horns were incised and the contents placed delicately in a normal saline solution and then placed on a glass slide and placed for observation under a light microscope (40 and 100 X) at the end of which the number of ovulated oocytes was counted. Similarly, at the level of the ovaries, the hemorr-

hagic points were counted using an electronic magnifying glass.

3) Study on gestational parameters

This study was made according to the three stages of pregnancy. 1st stage: from day 1 to 10 (implantation); from 11th-17th (gestation); from 18th-22nd (parturition).

Effects on implantation:

24 female rats were divided into four subgroups (1a, 1b, 1c and 1d) of 6 rats each, then crossed on the evening of their proestrous phase to rats of proven fertility at the rate of one male for two females per cage. The next day, the presence of sperm clusters in the vaginal smear or a cervical plug in the vagina made it possible to confirm the effectiveness of a coupling the day before. This day was considered the 1st day of pregnancy. The rats of subgroup 1a-1b-1c-1d received respectfully distilled water (10 mL/kg), 200, 400 and 800 mg/kg body weight extract by gavage for 10 days. The following day (11th day), the different groups of rats were laparotomized under ether anesthesia. The number of implants was counted, weighed; fetal resorption sites (if present) were recorded.

Effects on pregnancy: Preparation of the animals for this experiment was the same as for the implantation study of *S. magnificum*. The only difference is that the treated group received the oral treatment from the 11th to the 17th day of gestation to observe possible teratogenic effects. After day 15, a laparotomy was performed and fetal parameters recorded. The placentas were weighed; the length of the umbilical cord and the body of the fetus were measured and the pups were examined for any abnormalities.

Effects on parturition: 20 rats pregnant for 17 days were subdivided into 4 groups of 5 rats each. They were gavaged daily from day 18 to day 22 of the plant extract at doses of 200, 400 and 800 mg/kg body weight and the equivalent of distilled water was administered to the control group. During the treatment period, the rats were inspected minutely for vaginal secretions related to early parturition. Finally, any parturition initiated before day 22 of gestation was recognised as early parturition. Parameters such as the number of pups, their body weight, their antifertility activity, their fertility index and their parturition index were determined.

- **Anti-fertile activity** = Number of females without implantation sites/Number of females crossed × 100.
- **The implantation rate** = Number of implantation sites/Number of yellow bodies × 100.
- **Anti-implantation activity** = number of females without implantation sites/total number of females × 100.
- **Pregnancy rate** = Number of females with live young/Number of pregnant females × 100.
- **Percentage of uterine discharge** =Number of females with uterine discharge/Total number of females × 100.
- **Fertility rate** = Number of pregnant females/Number of crossed females × 100.
- **Resorption index** = Number of resorption sites/Number of implantation

sites × 100.

- **Pre-implantation loss** = Number of corpora lutea – Number of implantation sites/Number of corpora lutea × 100.
- **Post-implantation loss** = Number of implantation sites – Number of live pups at birth/Number of implantation sites × 100 [10].

Hormonal assay

Sex hormone levels were assessed using a competitive indirect ELISA binding technique for estradiol and progesterone. The reagents used for these assays were kits obtained from Cloud-Clone Corp.

2.5. Statistical Analysis

The results were expressed as mean ± standard deviation and subjected to statistical analysis using one-way analysis of variance (ANOVA) followed by a Tukey post-test of the graph pat program. The level of significance was considered at $p < 0.05$.

3. Results

- **Effects of different treatments of *S. magnificum* on the sexual cycle of adult rats associated with hormonal levels**

➢ **Effects of *S.magnificum* on the sexual cycle**

Vaginal smears obtained after 3 weeks of treatment in rats with *S. magnificum* showed a prolongation of the frequency of onset of proestrus (**Figure 1(a)**) at doses of 200 and 800 mg/kg ($p < 0.05$) and 400 mg/kg ($p < 0.01$). Similarly, a significant reduction in the frequency of occurrence of diestrus with the same doses was also recorded. However, no disturbance in total cycle time was felt when compared to controls (**Figure 1(b)**).

➢ **Effects of *S.magnificum* on the hormonal profile (oestradiol, progesterone)**

After 21 days of treatment of rats with *S. magnificum* extract, estradiol concentrations showed an increase at doses of 200 mg/kg ($p < 0.05$) and 800 mg/kg ($p < 0.001$) when compared to controls (**Figure 2(a)**). Similarly, the progesterone level underwent a significant variation marked by a decrease regardless of the dose of extract administered ($p < 0.001$) when compared to the control in **Figure 2(b)**.

- **Schumanniophyton *magnificum* effects on some reproductive parameters**

The animals that received the aqueous extract of *Schumanniophyton magnificum* by gavage on the morning of proestrus (**Table 1**) showed a significant increase ($p < 0.001$) in the weight of their ovaries with β oestradiol and the dose of 800 mg/kg when compared to the neutral control receiving distilled water. A significant increase in the number of ovulations was recorded at doses of 400 mg/kg ($p < 0.01$) and β estradiol, 800 mg/kg ($p < 0.001$) as well as the number of bleeding points at the same doses ($p < 0.05$).

Table 1. Effects of *Schumanniophyton magnificum* on some reproductive parameters.

PARAMETERS	PROESTRUS (9 a.m.)					PROESTRUS (6 p.m.)				
	Doses (mg/kg b.wt)					Doses (mg/kg b.wt)				
	Control	β estradiol	200 mg/kg	400 mg/kg	800 mg/kg	Control	β estradiol	200 mg/kg	400 mg/kg	800 mg/kg
Ovarian Weight	0.038 ± 0.00	0.05 ± 0.001**	0.04 ± 0.00	0.046 ± 0.00	0.052 ± 0.00**	0.041 ± 0.00	0.04 ± 0.00	0.041 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
Number of ova shed	7.4 ± 0.5	9.2 ± 0.37**	8.8 ± 0.37*	9.2 ± 0.2**	9.6 ± 0.24**	8.6 ± 0. 24	9.6 ± 0.24	8.8 ± 0.2	9 ± 0.31	9.6 ± 0.4
Hemorrhagic points	9.4 ± 0.24	11.2 ± 0.2**	10.8 ± 0.37	11 ± 0.44 *	11.2 ± 0.37*	11.2 ± 0.37	11.8 ± 0.2	12 ± 0.54	11.8 ± 0.66	12 ± 0.63

Value are mean ± SEM, female (n = 5). *p < 0.05 vs control; **p < 0.001 vs. control; ***p < 0.0001 vs control group.

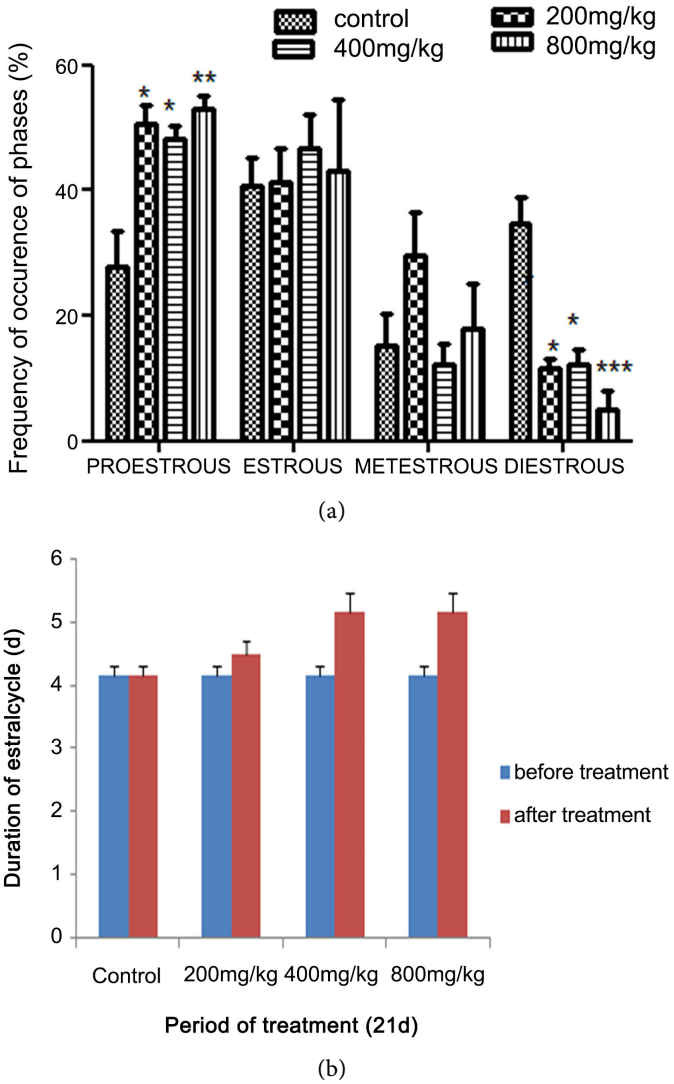
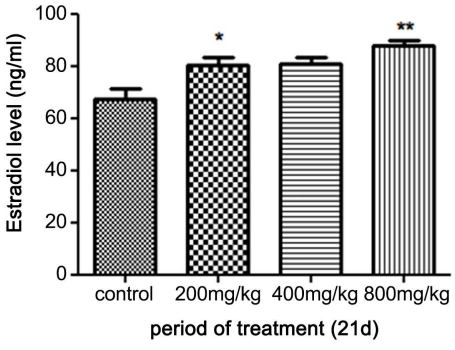
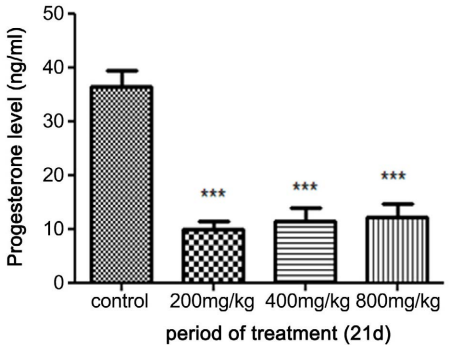


Figure 1. Frequency of appearance of the different phases of the estrous cycle after treatmenton the onset of proestrous (a) and the total cycle time (b) with aqueous extract of *Schumanniophyton magnificum* for 21 days. Each histogram represents the mean ± SEM. n = 5. *p < 0.05; **p < 0.001; ***p < 0.0001: significant differences from the control group.



(a)



(b)

Figure 2. Effects of *S. magnificum*on variation in serum estradiol (a) and progesterone (b) level. Each histogram represents the mean ± SEM; n = 5. *p < 0.05; **p < 0.001; ***p < 0.0001: significant differences from the control group.

➢ **Effect of *S.magnificum* on hormone levels depending on the period of treatment in proestrus**

Figure 3 presents the level of serum hormones in rats after treatment in the morning and the evening of proestrus. The level of estradiol significantly increased in rats treated at doses of 200, 400 ($p < 0.001$) and 800 mg/kg ($p < 0.0001$) (Figure 3(a)) while the level of progesterone significantly decreased at all doses ($p < 0.0001$) (Figure 3(b)).

- **Effects of aqueous extract of *Schumanniophyton magnificum* on gestational parameters**

➢ **Effects on the first stage of pregnancy and implantation; from day 1 to 10)**

The follow-up of the weight evolution during the first 10 days of gestation in adult rats treated with *S. magnificum*, made it possible to note a uterine discharge materialized by blood flows from the 6th day of gestation until the end of the treatment. These blood flows were very significant with the high doses of 400 and 800 mg/kg ($p < 0.0001$) when compared to the controls. Similarly, a significant decrease in body weight from the 7th day, at the same doses as before of 400 mg/kg ($p < 0.01$) and 800 mg/kg ($p < 0.001$) and a decrease in the number of implants at all these doses were recorded with great significance at the dose of 800 mg/kg ($p < 0.001$) when compared to the controls. A significant decrease in the rate of implantation and pregnancy was noted at all doses of this extract ($p <$

0.0001). Consequently, this lead to an increase in the anti-implantation activity which was generalized regardless of the dose administered ($p < 0.0001$) as well as the resorption number at dose of 800mg/kg ($p < 0.05$) (Table 2).

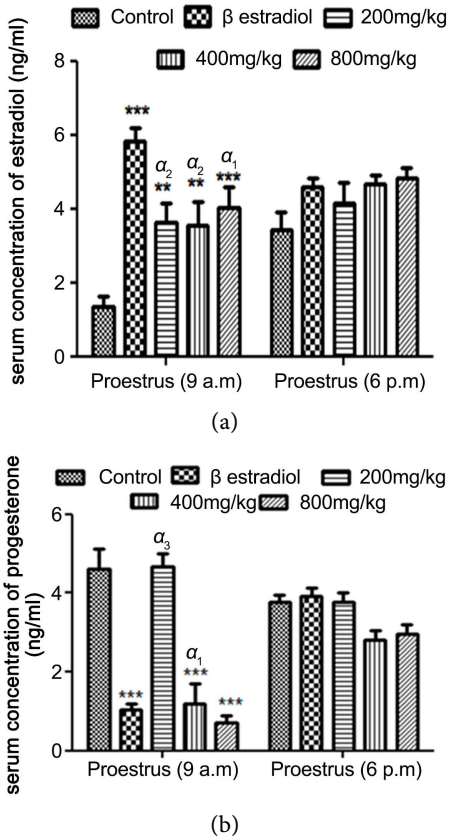


Figure 3. Effects of the aqueous extract of *Schumanniophyton magnificum* on the hormonal level (estradiol and progesterone) during the proestrus period (morning/evening) of female rats. Each histogram represents the mean ± SEM; n = 5. *$p < 0.05$; **$p < 0.001$; ***$p < 0.0001$: significant differences from the neutral control group a_1: $p < 0.05$; a_2: $p < 0.001$; a_3: $p < 0.0001$ significant differences when compared to the positive control.

Table 2. Effects of aqueous extract of *S. magnificum* on implantation.

PARAMETERS	Doses (mg/kg b.wt)			
	Control	200 mg/kg	400 mg/kg	800 mg/kg
N° Implantation sites	7 ± 0.44	5.5 ± 1.20	4.0 ± 1.90	2.16 ± 1.37**
Number of Corpora Lutea	10.33 ± 0.55	10 ± 0.24	9.6 ± 0.61	9.5 ± 0.42
Nidation rate (%)	67.76	55**	41.66***	22.70***
Antifertility Activity (%)	0	16.66***	50***	83***
Gestation Rate (%)	100	83.30**	50***	16.66***
Preimplantation Loss (%)	32.23	45	58.33	77.26
Resorption Index (%)	0	0	8.25	53.70*
Vaginal discharge and bleeding (%)	0	0	60***	100***

Value are mean ± SEM, female (n = 6). *$p < 0.05$ vs control; **$p < 0.001$ vs control; ***$p < 0.0001$ vs control group.

➢ **Effect of *S.magnificum* on progesterone levels (from 1st to 10th day)**

The administration of the extract of *S. magnificum* to albino rats on the first ten days of pregnancy reveals through Figure 4 a significant decrease ($p < 0.0001$) in the level of progesterone observable at doses of 400 and 800 mg/kg when compared to the control.

➢ **Effect of *S. magnificum* on the 2nd stage of pregnancy (Gestation; from 11th-17th day)**

The effects of *S. magnificum* on the gestational parameters of adult rats treated from day 11 to day 17 of pregnancy showed no significant variation recorded from the control in the gestational parameters assessed.

➢ **Effects of *S. magnificum* on the 3rd stage of pregnancy (Parturition; from 18th-22nd)**

Table 3 illustrates the effect of *S. magnificum* on some fertility and gestational parameters. The treatment of adult rats from the 18th to the 22nd day of gestation did not lead to any particular difference when compared to controls concerning the number of live pups, the length of the cord, the pregnancy rate and post-implantation losses. However, a significant difference was noted in the weight of the fetuses which, when compared to the control, experienced a significant reduction at all doses respectively 200 and 400 mg/kg ($p < 0.01$) and 800 mg/kg ($p < 0.05$). A very important parameter noted in this table was early parturition which occurred at day 20 of gestation for the dose of 800 mg/kg ($p < 0.0001$), at day 21 for the dose of 400 mg/kg ($p < 0.0001$) and at day 22 for the dose of 200 mg/kg ($p < 0.05$).

4. Discussion

The objective of the present study was to evaluate the effect of aqueous extracts of *Schummaniophyton magnificum* on the fertility of adult rats through the estrous cycle, ovulation and gestational parameters, a medicinal plant whose previous studies have proven its stimulatory effect on fertility in immature rats [6].

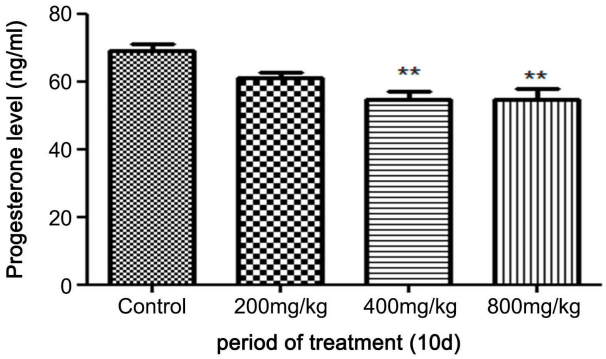


Figure 4. Effects of aqueous extract of *S. magnificum* on progesterone level after 10 days of treatment. Each histogram represents the mean ± SEM, female (n = 6) *$p < 0.05$ vs control; **$p < 0.001$ vs control; ***$p < 0.0001$ vs control group.

Table 3. Effects of *Schumanniophyton magnificum* on parturition.

PARAMETERS	Doses (mg/kg b.wt)			
	Control	200 mg/kg	400 mg/kg	800 mg/kg
Number of alive fetuses	8.16 ± 0.70	8.33 ± 0.55	8 ± 0.68	9.33 ± 0.88*
Mean Weight of the pups	5.08 ± 0.04	4.03 ± 0.26**	4.13 ± 0.17*	4.07 ± 0.26**
Length of umbilical cord	1.61 ± 0.06	1.51 ± 0.10	1.65 ± 0.08	1.5 ± 0.08
Gestation Rate (%)	100	100	100	100
Post-implantation Loss (%)	1.66 ± 1.66	3.18 ± 2.01	3.5 ± 2.21	3.33 ± 3.33
Delivery day	23 ± 0.16	22.33 ± 0.21*	20.33 ± 0.21***	21 ± 0.25***

Values are mean ± SEM, n = 5. *p < 0.05; **p < 0.01; ***p < 0.0001.

Concerning the estrous cycle, the extract did not disturb its total duration. However, a significant decrease in the frequency of appearance of diestrus and a prolongation of the proestrus was observed during the periods of treatment and post treatment. This indicates a stimulating effect of the extract on the follicular growth mechanism and/or ovulation and wich lead to high mitotic activities to the uterus. These results are not agreed with those reported by Westwood (2008) [11] who found mitotic activity from the end of estrus, but are in accordance to those reported by some authors [12] who indicated the amplification of the mitotic activities rather during the proestrus phase. Indeed, Soto *et al.* (2002) [13] investigated that systemic and local IGF-I play an important role in the effect of estrogen on growth and epithelial proliferation of rat uterus. However, our work has shown that the aqueous extract of *Schumanniophyton magnificum* has estrogenic properties by causing vaginal opening in immature rats [6]. It is therefore clear that the aqueous extract of *Schumanniophyton magnificum* could stimulate the production of IGF-I and induce the effects observed in the treated rats. It should be noted that all cellular proliferative activity that takes place in the rodent uterus depends on direct action of estrogens [14]. The extract could therefore directly stimulate cell proliferation by behaving like estrogen or by stimulating the release of estradiol as observed in **Figure 2** at the dose of 200 mg/kg and 800 mg/kg when compared to control. Definitively, one could conclude that the aqueous extract of *Schumanniophyton magnificum* could have interfered with hormonal synthesis, which could have led to changes in the cycle mechanism and cell cytology during the proestrus phase: This plant extract in addition to inducing folliculogenesis would also induce ovulation.

Regarding ovulation, the significant increase in the number of excreted oocytes and estradiol level in rats given the extract in the morning of proestrus; indicate that estrogen is involved in controlling the rate of ovulation [15]. In addition, Dierschke *et al.* (1983) [16] showed that estradiol can have a direct action on the rat ovary. Furthermore, several lines [17] [18] reported that the administration of the extract in the morning of proestrus could partially or completely block ovulation whereas the administration of this extract in the evening of

proestrus had no effect on ovulation. But, our results rather showed an increase in ovulatory activity in the morning of proestrus and had no effect in the evening of proestrus. These differences observed between our results and those of these authors could be attributed to the fact that plants like *Schumanniophyton magnificum* having an estrogenic property, could directly act on the pituitary gland by peripheral modulation of LH and FSH, thus increasing the secretion of these hormones and particularly of estradiol which would stimulate the growth of the uterine lining, causing its thickening during the preovulatory phase of the cycle. In synergy with FSH, estradiol stimulates the proliferation of granulosa cells during follicular development by promoting ovulation [8]. Ultimately, the aqueous extract of *Schumanniophyton magnificum* would have induced folliculogenesis by increasing the number of follicles at different evolutionary stages and would also have induced ovulation by behaving like an endogenous estrogen [19].

The treatment of female rats with the aqueous extract of *Schumanniophyton magnificum* presented us with unexpected results in the different stages of pregnancy. Indeed, the post-coital administration of the aqueous extract of *Schumanniophyton magnificum* during the first ten consecutive days of gestation of the rats caused an implantation failure marked by vaginal bleeding from the 6th day. At the same time, a tendency for the progesterone level and the number of implantation sites to decrease was noted, particularly in animals treated at doses of 400 mg/kg and 800 mg/kg. These results indicate that hormonal imbalances could be caused by many chemical agents contained in plant extracts [20] [21]. The failure of certain implantation sites observed in this study could be attributed to an alteration or interference in the production of hormones (estrogen and progesterone). Thus, the uterine lining has not developed sufficiently to support or nourish the fertilized eggs, thus preventing implantation [22] [23]. This implantation failure could also be related to the deleterious effect of the extracts on the blastocysts or on the final stage of implantation, probably due to an altered endometrial environment or a combination of both. Indeed, the studies of Paria *et al.* (2001) [24] reported that implantation can only be successful when activation of the blastocyst coincides with the receptive state of the uterus.

We have also noticed in this work the decrease of the weight of pups after parturition. Indeed, the presence of phytoestrogens in this extract [4] would have induced chemical interactions that produced alterations in the uterine milieu and created hostile conditions in the uterus [25]. It is these chemical components when given at the third stage of pregnancy that induced early parturition to female rats. In the other hand, some [26] [27] indicated that endogenous estradiol activates oxytocin receptors in the uterine myometrium at term by altering the sites of binding of the calcium ion (Ca^{2+}) which increases reactivity to oxytocin which would thus have facilitated parturition by initiating the onset of labour. This indicates that the aqueous stem extract of *Schumanniophyton magnificum* is also toxic when it is administered in the late period of pregnancy. It is

these chemical compounds that would have acted synergistically causing the death of the pups, abortion and vaginal bleeding in the early stage of pregnancy of treated female rats.

5. Conclusion

In conclusion, these studies show that, although the administration of *Schumanniophyton magnificum* extract stimulates ovulation, it has adverse effects throughout the period of pregnancy development until term. This confirms its traditional use to stimulate fertility in young girls and as a contraceptive for those who do not want to have children.

Conflicts of Interest

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

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