

## Anti-Fertility Effects of Leaf Extract of *Hibiscus rosa-sinensis* Linn in Female Wistar Rats

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### Abstract

The effects of ethanolic leaf-extract of *Hibiscus rosa-sinensis* was investigated in thirty mated female rats. The rats were categorized into three (3) major groups containing two (2) sub-groups each of five (5) rats per group. Five male rats of proven virility were used to mate the female rats and those found in proestrus phase of cycle were caged with the males of proven fertility. Vaginal smears from each rat were monitored daily and examined for evidence of copulation. Rats with evidence of thick vagina plugs were examined for clumps of spermatozoa in vagina smears. The uteri and ovaries were excised and these tissues were fixed in formol saline for further histological analysis. The histologic report of the ovaries in Group A1 (control) showed numerous normal ovarian follicles at various stages of maturation containing granulosa and luteal cells surrounded by the ovarian stroma. Group B1 which was treated with *H. rosa-sinensis* showed paucity of ovarian follicles, degenerative changes, vacuolation in the ovarian stroma and lack of germ cell maturation. Group C1 treated with saline had normal ovaries. The uteri of the sacrificed *H. rosa-sinensis*-treated rats were all almost empty with no implants, but in one of the rats from this group, a resorption site was seen. The group treated with the extract that was allowed to carry to term (Group B2) did not litter and no evidence of pregnancy was seen in all during the first month, but they later littered following subsequent month mating. This study generally demonstrates reversible antifertility effect of *H. rosa-sinensis*, which is indicative of its usefulness as possible contraceptive agent.

**Keywords:** Anti- Fertility, *Hibiscus rosa-sinensis*, Leaf extracts, Female, Rats.

### Introduction

All civilizations have always had traditions of using herbs to promote healing. Plants still remain the basis for development of modern drugs and medicinal plants have been used for years in daily life to treat diseases all over the world (1). According to Ayitey-Smith (2), traditional medicine evolved from environmental resources, which the people of a community adapted in desperation for survival from disease. On the African continent, traditional medical practices date as far back as 4000 years. It was the sole medical system for health care before the advent of orthodox or modern medicine. Even in this present technological era, traditional medicine is still the predominant means in the third world for the preservation of health of the rural majority who constitute over 70% of the total population (3). *Hibiscus rosa-sinensis* Linn (Malvaceae) is a glabrous shrub widely cultivated in the tropics as an ornamental plant and has several forms with varying colours of flowers. The leaves are anodyne, emollient and aperients (4,5). The leave juice is considered beneficial in treating gonorrhoea, alopecia and also used for blackening hair and the buds have a sweet odour with bitter taste. It has also been said to be useful in managing uterine and vaginal discharges and promotes the growth of the foetus (6). It has antitumor, antihypertensive and antioxidant properties (7,8). It is considered an antifertility agent and the flowers have been reported to possess anti-implantation activity (9). Root extract of the plant has been reported to exhibit strong anti-implantation (inhibition 100%) and uterotrophic activity at the dose level of 400mg/kg body weight (10). *Hibiscus rosa-sinensis* leaf-extract (common name, obobo in Esan language) has been used traditionally by the Esan people of Edo State, Nigeria, to treat abnormalities related to development of foetus (3). The purpose of this study is to also evaluate the fertility effects of the leaf-extract of this plant in female Wistar rats.

### Materials and Methods

**Collection of Plant and Preparation of Extract:** Leaves of *Hibiscus rosa-sinensis* Linn were obtained from the premises of a compound in BDPA, Benin city, Nigeria and authenticated at the Department of Pharmacognosy, University of Benin, Benin-City. The leaves were air dried at room temperature and pulverized to powdered form. Five hundred (500g) of the powdered substance were subjected to extraction in 1.5 L of ethanol by maceration for 48 hours. The macerated solution was sieved with Whatman no. 4 filter paper. The filtrate was concentrated to semi-solid state using an oven at 56°C to obtain 11.2 % yield for use (11). Two hundred and fifty (250) mg/kg body weight of sample dissolved in saline was administered to the experimental animals.

**Experimental Animals:** Thirty from thirty-five rats found in pro-oestrus phase of cycle were caged with males of proven fertility. The rats were obtained from the Animal House in the Department of Anatomy, University of Benin and kept at the same location for the experiment, in cages at room temperature (25°C). The animals were allowed to acclimatize for three (3) weeks, during which they were fed on livestock growers mash and water *ad libitum*. The rats were categorized into three (3) major groups divided into two (2) sub-groups of five (5) rats per group. The five male rats of proven virility were only used to mate the female rats. Vaginal smears from each rat were monitored daily and examined for evidence of copulation. Rats with evidence of thick vagina plugs were examined for clumps of spermatozoa in vagina smears. Only such were considered pregnant and used for the experiment. Such day was considered as day one of pregnancy (12). The experimental rats were grouped as follows:

**Grouping of Animals**

- **Group A1:** These (normal) non-pregnant female rats were given distilled water, *ad libitum*, they served as control. No extract was given.
- **Group A2:** These Pregnant female rats were given distilled water, *ad libitum*, they served as control. No extract was given. They were allowed to carry pregnancy to term.
- **Group B1:** These pregnant female rats were treated orally with 250 mg/kg of leaf extract of *H. rosa-sinensis* from day 1 of Gestation till the 19<sup>th</sup> day. They were sacrificed on day 19.
- **Group B2:** These pregnant female Wistar rats received oral administration of 250 mg/kg body weight of *H. rosa-sinensis* leaf extract in saline vehicle from day 1 of gestation to full term only and left for another month without extract treatment.
- **Group C1:** These Pregnant female rats were given equivalent volume of saline. They were sacrificed on day 19.
- **Group C2:** These Pregnant female rats were given equivalent volume of saline till term. They were allowed to carry pregnancy to term.

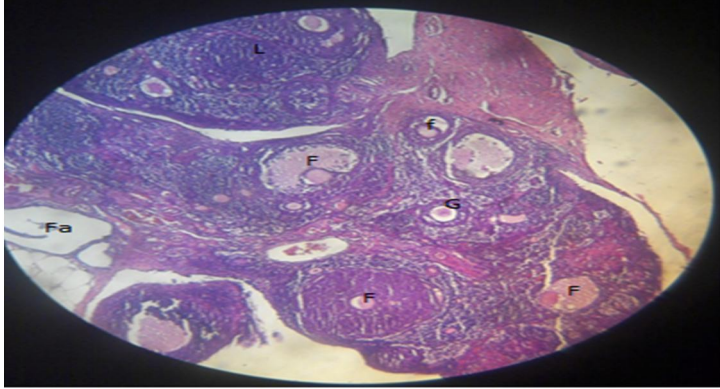
On the 19th day of gestation, groups B1 and C1 rats following mild anesthesia using chloroform inhalation, the rats were sacrificed and the peritoneal cavity was opened. The intact uteri and ovaries were extracted. The uteri were assessed for number of developing fetuses, resorptions, total implantation sites and for any observable developmental anomaly. The ovaries and uteri were fixed in formal saline for histological assessment following standard procedure (13). Prepared sections were examined microscopically for histological remarks. Groups A2, B2 and C2 rats were allowed to carry to term and the litters were assessed for number and size. Group A1 rats were assessed for normal ovary and uterine histological features.

**Data Analysis:** The data obtained were analyzed using the statistical package Genstat Release 8.1 (PC/Windows XP, 2005 and Microsoft excel. All data were expressed as Mean + SEM of number of experiments. Means separation was done using the Duncan multiple range test (14,15). Significant differences between the mean of each group and the control were determined using the student's t- test.

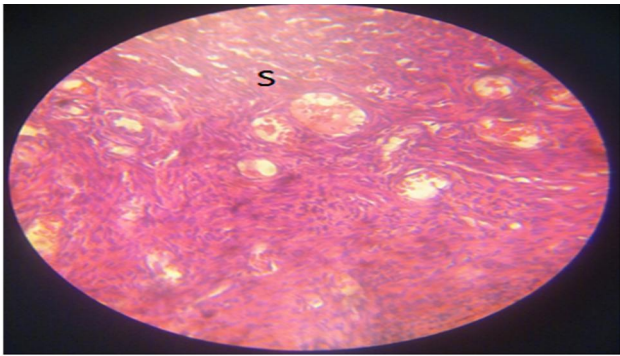
## Result

### Histopathology

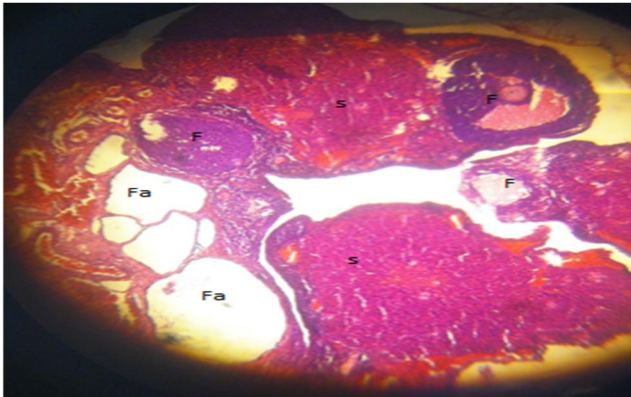
The histologic report of the ovaries of the rats in Group A1 (control) showed numerous normal ovarian follicles at various stages of maturation containing granulosa and luteal cells and surrounded by the ovarian stroma. Fat cells were also present (Plate 1). Group B1 (*H. rosa-sinensis*-treated) rats showed paucity of ovarian follicles, degenerative changes and vacuolation in the ovarian stroma. Also, lack of germ cell maturation was seen (Plate 2). Group C1 (saline-treated) rats had evidence of normal implants with no resorptions while some showed normal ovaries with follicular cells (Plate 3). Just as the uterus of the normal (control) rats (Plate 4), the uterus of the *H. rosa-sinensis*-treated rats were all almost empty with no implants, but in one of the rats from this group, a resorption site was noted (Plate 5). The B2 group treated with the extract that was allowed to carry to term did not litter and no evidence of pregnancy was seen in all during the first cycle. However, following re-mating in the subsequent month, a good number of the rats became pregnant and had 16 litters with two resorptions at the end of the second month. In contrast, the rats of the control groups A2 and the saline-treated ones of group C2 had a total of 21 and 19 normal litters respectively during the first mating that were not significantly different from each other both in size and number.



**PLATE1 :** Group A (Control) slide of the ovary, showing numerous normal ovarian follicles (F) at various stages of maturation displayed in the stroma (S), with granulosa cells (G) and luteal cells. Few fat cells (Fa) (H&E x400).



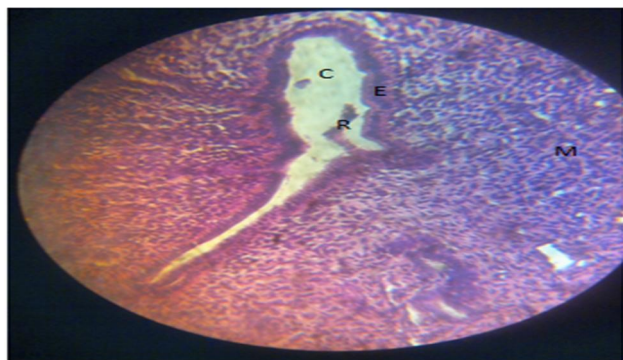
**PLATE 2:** Group B (*H. rosa-sinensis* treated) rat ovary, with paucity of ovarian follicles, degenerative changes and vacuolation in the ovarian stroma (S), lack of germ cell maturation seen (H&E x400).



**PLATE 3:** Ovary of group C (saline-treated) rat, showing normal ovarian stroma (s) with follicular cells (f) and. Fat cells (Fa) (H&E x400)



**PLATE 4:** Group A (Control) Uterus with normal patent uterine canal (C), endometrium (E), myometrium (M) and epimetrium (Ep) (H&E x400).



**PLATE 5: Group B** (*H. rosa-sinensis* treated) Uterus showing resorption site (R) in the uterine canal (C) with normal uterine endometrium (E), Myometrium (M) and Epimetrium (Ep) (H&E x400).

## Discussion

This study revealed that administration of ethanolic leaf extract of *Hibiscus rosa-sinensis* Linn have anti-fertility effects. Histological studies of the ovaries showed sparse germ cell maturation, reduction in the number and degeneration of the follicles. The ovary of *H.rosa sinensis* treated rats showed essentially, paucity of maturing follicles, with most of them seen appearing redundant. Where pregnancy occurred at all, it was associated with impaired embryogenesis which led to the resorption site seen in the uterus. Comparatively, the ovaries of the control group rats not fed with the extract showed numerous normal ovarian follicles (F) at various stages of maturation displayed in the stroma (S), with granulosa cells (G) and luteal cells. Only few fat cells (Fa) were seen. This findings supports previous report (10) who examined with the root extract of same plant and found 100 % anti-implantation as well as uterotrophic effects. Murthy et al. (9), similarly reported irregular oestrous cycle with prolonged oestrous and metestrous. Also remarked in their study was the observation of atretic follicles and absence of corpora lutea which demonstrated the anti-ovulatory effect of the flower extract of *H. rosa-sinensis*. It is possible that the observed findings from our study might be related to hormonal imbalance (9). It is a known fact that the leaf extract of *H. rosa-sinensis* was said to have exhibited significant antioxidant and anticancer activities in previous studies due to the increased flavonoids and terpenoids contents as well as saponins, tannins and glycosides which are adduced to be responsible for its pharmacological effects (16,17). However, in ovarian and uterine cycles, there is a lot of interplay in the pituitary-hypo-gonadal axis which affects the process of fertilization, gestation and birth. Hormonal imbalance resulting from endogenous secretion of oestrogen by atretic follicles and possible oestrogenicity of the extract might be advanced as possible explanation for the observed effects (18,9). It is however remarkable that the rats treated with this extract that could not initially get pregnant which might be related to distortion in the oestrous cycle and ovarian activity in the rats (9) recovered from the inhibitory effects of this extract and littered the following month after successful mating. Also, the observation of no significant difference between the normal (control) rats and the saline-treated rats proves further that the

inhibitory effects on conception and embryogenesis could only have been caused by the extract, a process observed to be only of transient effect. This suggests that the antifertility effect of the plant could be reversible. This study therefore demonstrated that ethanolic leaf-extract of *Hibiscus rosa-sinensis* Linn has the potential to serve as possible contraceptive agent in view of its reversible anti-fertility effect, which could be beneficial in fertility management.

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