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Antifertility Effects of *Ficus sycomorus* Aqueous Leaf Extract on Pituitary Gonadal Axis of Adult Male Wistar Rats

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Abstract
Hormonal regulation, is an important factor affecting fertility. Alteration of hormones at any level of the hypothalamo-pituitary-gonadal axis may affect the fertility outcome observed. Ficus sycomorus (F. sycomorus), known to have a number of medicinal uses is yet poorly investigated in fertility studies. For this reason, its aqueous leaf extract was investigated to determine its effects on pituitary histology and pituitary-gonadal axis hormones in male Wistar rats as well as evaluating fecundity in mated female rats. Thirty rats comprising equal numbers of both sexes were randomly divided into three groups (I, II & III) containing ten rats each (n=10/group), five males and five females separated from each other in separate cages throughout the experimental period. Group I served as the control. All the female and control rats were given normal rat feed and water ad libitum. Group II and III male rats in addition to normal rat feed and water were orally treated with 500 mg and 1500 mg/kg body weight daily of Ficus sycomorus aqueous extract, respectively for 60 days. Towards the end of the experimental period, the male rats in each of the three groups were allowed to mate with the untreated females to test for fecundity in the female rats. The male rats were anesthetized with chloroform and sacrificed while the female rats were kept till day 15 of pregnancy before sacrifice to establish pregnancy success rate. Blood was collected from the inferior vena cava of the male rats for hormonal assay while the pituitary gland from the males in the control and treatment groups were dissected out and fixed in 10 % formal saline. The tissues were processed for routine histological examination with hematoxylin and eosin staining method for light microscopic examination. Data obtained were expressed as Mean ± SEM. Significant difference between means were determined by t-test and one-way analysis of variance (ANOVA). Significant difference was expressed as $P<0.05$. The results from hormonal assay revealed a dose dependent significant decrease in serum testosterone level of the treatment groups, significant increase in FSH, LH and progesterone levels in high dose treatment group and a significant decrease in FSH and estrogen levels in the low dose treatment group. Fecundity test results showed pregnancy success rates of 80 %, 40 % and 0 % for groups I, II and III female rats with an average of 5.6, 2.6 and 0.0 implants, respectively. Histological findings showed cellular hypoplasia in low dose treated rats and cellular hyperplasia in high dose treated rats. It can therefore be concluded that aqueous extract of Ficus sycomorus affected male fertility by altering the hormones of the pituitary-gonadal axis in a dose dependent manner.

Keywords: *Ficus sycomorus,* Fecundity, Pituitary Gland, Hormones, Infertility

Introduction
Population exponential growth in developing and underdeveloped countries has posed a major threat to humanity as the impending cause of poverty and environmental degradation. As a result, various methods have been developed and put to use to induce infertility in men and women for proper regulation and monitoring (1). There exist a number of Contraceptive methods for regulating infertility in women with a number of side effects and unintended conceptions due to intermittent failure of contraceptive devices; this is not the case for men as contraceptive measures and chemical remedies are distant and unreliable (2,3). Hence, facilitating the need for medicinal herbs as alternative methods for contraception in males; their minimal to no side effects has made them become potent therapeutic measures (4).
On the other end, male infertility accounts for 30% of infertility cases worldwide (5) and 20-50 % of the causes of infertility in different parts of Nigeria (6). There exist a number of possible etiologies with the common being reduced hormonal production in the testes as a result of a corresponding decrease in the hormonal level of the hypothalamo-pituitary axis (7). Hormones play an eminent role in male fertility regulated by the hypothalamus, pituitary gland and the testes via positive or negative feedbacks. Therefore, an alteration in the hormones of the hypothalamo-pituitary axis causes a corresponding reductive effect on the testicular hormonal production and generally on fertility (8).
An array of plant extract has been shown to induce antifertility effects in males via different modes of actions. Among the numerous, plants like *Dioscorea esculanta* (9) has been shown to inhibit male fertility while low

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dose of *Allium sativum* (Garlic) crude extract caused a decrease in serum testosterone levels (10). *Capparis aphylla* gave antisteroidogenic effect while *Garcina cambogia* caused degeneration of Leydig cells (11).
Ficus sycomorus is a herbaceous plant found in tropical and sub-tropical areas with low to moderate rainfall. It is commonly called fig or sycamore and has been documented to have a number of medicinal use. The plant extract has been used to treat sore throat, cough, chest pain, scrofula and snakebites (12). It has also been shown to possess anti-bacterial activities in the management of diarrhea, anti-helminthic and laxative activities (13). The plant contains a number of potent phytochemicals and antioxidants (14). Much studies have not been carried out to show the fertility effects of *Ficus* sycomorus.
The aim of this study is to determine the effects of aqueous leaf extract of *Ficus sycomorus* on the histology of the anterior pituitary gland, and the hormones of the pituitary-gonadal axis.

Materials and Methods

Plant material and preparation of extract

Fresh leaves of *F. sycomorus* were procured from Egor Local Government area in Edo state Nigeria. They were identified at NIFOR in Benin City Edo State. Air-dried leaves of *F.* sycomorus at room temperature were pounded into fine powder using an electric grinder and extracted with 300 ml of distilled water using maceration extraction method for 24 hours in an air tight container. The extract obtained was filtered through a Whatmann paper size 1 and the filtrate was concentrated at a low temperature, under reduced pressure using a vacuum rotary evaporator (15). The extract was preserved in a refrigerator from which fresh solution was prepared using distilled water when required for use.

Animals

The experiment was performed with thirty (30) adult albino rats weighing between 200-240 g obtained from the Animal house of the Department of Anatomy, University of Benin, Benin City and kept at the Animal Care Unit of the Department. The animals were allowed to acclimatize to the laboratory condition (temperature 24-28°C and 12 hours light-dark cycle) for fourteen days before commencement of the experiment with free access to rat chow(Top feeds Nigeria) and water *ad libitum* throughout the study. All the animals were treated according to the guidelines for the care and use of experimental animals (16). Approval for the study was received from the Research and Ethics Committee of the College of Medical Sciences, University of Benin, Benin City.

Experimental Design

Thirty rats were randomly divided into three groups each containing ten rats (n=10/group) five males and five females separated from each other in separate cages throughout the experimental period. Group I served as the control and the rats were given normal rat feed and water *ad libitum*. Groups II male rats were treated with low dose of the extract of *Ficus sycomorus* (500 mg/kg body weight) while Group III male rats were given high dose of the extract of *Ficus sycomorus* (1500 mg/kg body weight). The extract was given orally once daily throughout the experimental period which lasted for 60 days. The female rats in group I, II and III were given normal rat feed and water *ad libitum*. All animals were weighed at the commencement and end of study period, the weights were recorded as initial and final weights. Towards the end of the experimental period, the male rats in each of the three groups were allowed to mate with the untreated females to test for fecundity in the female rats. The male rats were anesthetized with chloroform and sacrificed while the female rats were kept for some days before sacrifice.

Fertility Test

On day 55 of the experiment, each male rat was cohabited with two adult female rats in mating cages overnight. The females were removed from the cages during the day time to avoid decline of sexual behavior associated with continuous cohabitation with the males. This process was conducted for five consecutive days during which one complete estrous cycle in female rats should have elapsed. Vagina smears were taken the morning after mating for microscopic examination to confirm the presence of spermatozoa and considered day zero of pregnancy. Then on day 15 of the pregnancy, the females were sacrificed. The uteri were examined and the number of implants recorded (17).

Hormonal assay

Blood samples were collected from inferior vena cana in centrifuge tubes. The blood was allowed to stand for 10 minutes to clot at room temperature and centrifuged at 3500 rev/min for 10 minutes. The serum was then tipped into a separate vial and later subjected by ELISA method for assessment of Follicle stimulating hormone (FSH), Luteinizing hormone (LH), Prolactin, Testosterone, estrogen (estradiol) and progesterone.

Histological analysis

The pituitary gland from the control and experimental groups were dissected out and fixed in formal saline. The tissues were processed for histological examination and paraffin sections were stained with hematoxylin and eosin (18). Qualitative microscopic examination was made and photomicrograph taken at x100 magnification.

Statistical analysis

Data were expressed as Mean ± SEM. Significant difference between means were determined by t-test and one-way analysis of variance (ANOVA). Significant difference was expressed as $P<0.05$.

Results

In table 1, it was seen that testosterone level showed a marked dose dependent significant decrease ($p< 0.05$) in the treated groups compared to the control group with values ranging from 0.9565±0.0358 in the control, 0.5670±0.2130 in the low dose treated group and 0.0015±0.0008 in the high dose treated group (Table 1).

Data on follicle stimulating hormone gave a significant decrease ($p<0.05$) in low dose treated group 0.2572±0.0565 compared to control group 0.4163±0.0052 but a significant increase in the high dose treated group 0.5985±0.0784 as shown in table 1.

Luteinizing hormone was slightly decreased in the low dose treated group 0.2930±0.1170 but significantly increased ($P<0.05$) in the high dose treated group 0.7068±0.0705 compared to values from the control group 0.3417±0.0132.

Table 1: Mean hormonal values of experimental rats

Hormones	Group I (Control)	Group II (Low dose of *F. Sycomorus)*	Group III (High dose of *F. sycomorus)*
Testosterone (mg/ml)	0.9565±0.0358	0.5670±0.2130 *	0.0015±0.0008 *
FSH (U/L)	0.4163±0.0052	0.2572±0.0565 *	0.5985±0.0784 *
LH (U/L)	0.3417±0.0132	0.2930±0.1170	0.7068±0.0705 *
Estrogen (ng)	35.1000±12.8000	19.7000±8.3000 *	36.8500±9.3200
Progesterone (ng)	3.0827±0.0346	17.4000±8.7000	29.4000±12.2000 *
Prolactin (ng)	0.2365±0.0160	0.2237±0.0375	0.2384±0.0358
Testosterone/Estrogen Ratio	0.0273	0.0288	0.0004

**(p<0.05) significantly different from the control. FSH - Follicle stimulating hormone, LH - Luteinizing hormone*

The estrogen level was significantly lowered ($P<0.05$) in low dose treated group 19.7000±8.3000 but became slightly raised in the high dose treated group 36.8500±9.3200 compared to control group 35.1000±12.8000.

The progesterone level gave a dose dependent significant increase ($P<0.05$) in low dose treated group 17.4000±8.7000 and high dose treated group 29.4000±12.2000 compared to the control group 3.0827±0.0346.

The prolactin values were not much altered, it showed slight variation which were non-significant ($P<0.05$).

Testosterone/Estrogen Ratio was slightly raised in low dose treated group 0.0288 but was markedly reduced in the high dose treated group 0.0004 compared to the control group 0.0273.

Fecundity test results (table 2) showed a pregnancy success rate of 80 % in group I rats (control), 40 % in group II rats (low dose) with no pregnancy recorded among group III rats (high dose). The number of implantation sites for the control group was 5.6 ± 1.05 while that for the low dose was 2.6 ± 0.01.

Table 2: Mean value of fecundity test of female rats mated with experimental rats.

Treatment groups	Number of implants	Positive mating (%)	Fertility (%)
Control	5.6 ± 1.05	100	80
Low dose (500mg/Kg)	2.6 ± 0.`01	100	40
High dose (1500mg/Kg)	0.0 ± 0.0	100	0

Histological findings revealed that the anterior pituitary gland of the control group showed normal histo-architecture and differential arrangement of acidophils, basophils and chromophobes (Figure 1). Those of low dose treated group revealed mild cellular distortion (Figure 2). In the group treated with high dose of the plant extract, there was cellular hyperplasia (Figure 3).

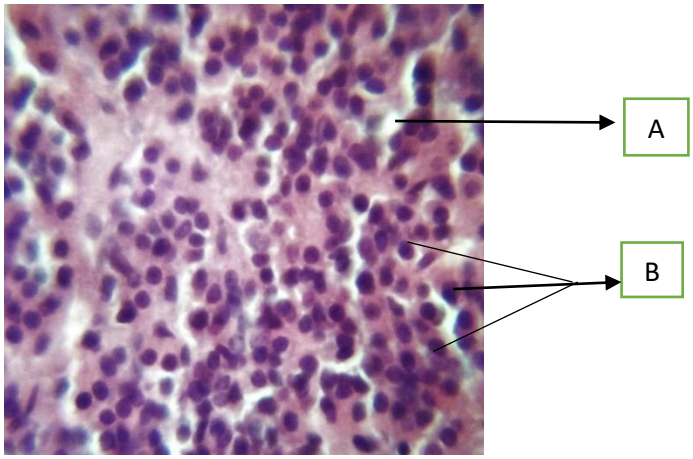

Fig.1 Control: A, showing interstitial space and B showing cell nests containing acidophils, basophils and chromophobes. (H & E X100)

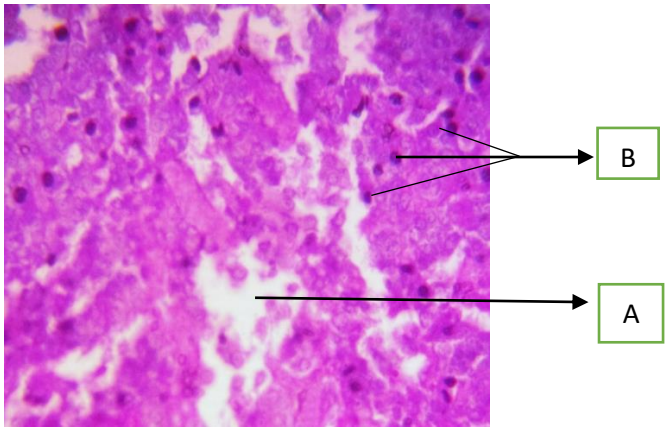

Fig. 2 Low dose: A showing increased interstitial space and B showing cellular hypoplasia within the cell nest. (H & E X100)

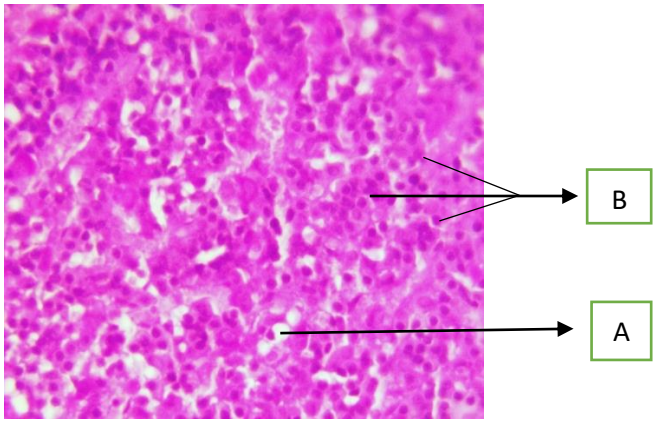

Fig. 3 High dose: A, showing reduced interstitial space and B showing cellular hyperplasia within the cell nest. (H & E X100)

Discussion

Hormonal regulation plays an important role in fertility with the hypothalamo-pituitary gonadal axis serving as the major site of control. In this light, alterations of hormones at any level of the axis may result in a corresponding response that may generally affect fertility (8).

Histopathological results from the anterior pituitary gland in rats treated with low dose of *Ficus sycomorus* revealed the presence of reduced cell population (hypoplasia) within the cell nests. The cells observed in the micrographs are representative of acidophils, basophils and chromophobes which limited by this study, requires histochemical evaluation to distinguish between the cell types. Despite the non-utilization of morphometric studies on the counts, it is seen from the micrographs that the cells in group II are far less in number compared to the control. On the other hand, the micrographs of rats treated with high dose of the extract in group III contained more preponderant cells (hyperplasia) than the control. Pituitary hyperplasia has been linked to an increase in the number of one or more adenohypophyseal cell subtypes marked histologically by hypercellular population and enlarged acini (19), whereas hypoplasia marked by reduced cell numbers are usually due to congenital causes, vascular insufficiency as occurs in Sheehan's syndrome, autoimmunity and other etiological causes which may result in single hormone insufficiency or total hypopituitism (20, 21)

In this study, serum hormonal profile showed significant dose dependent decrease in testosterone level in group II and III rats compared to control. This was also associated with poor pregnancy success rates and decreased number of implantation sites in both group II and III compared to control, a situation which correlates with the lower testosterone levels. This is in tanderm with a similar work on male infertility which demonstrated that decreased testosterone levels were associated with decreased pregnancy success rate in mated female rats (22).

The low dose extract treatment in group II rats resulted in cellular hypoplasia in the anterior pituitary gland which might explain the observed decrease in the gonadotropin hormones FSH, LH and prolactin. The decrease was associated with low testosterone and estrogen levels but raised progesterone level. Progesterone is a necessary substrate in the synthesis of testosterone while estrogen on the other hand is a by-product obtained from the final conversion of testosterone (23). The increased progesterone level in this group may be as a result of non-conversion to its end products, testosterone and estrogen.

The cellular hyperplasia observed in the photomicrograph of group III rats that received the higher dose was associated with significant increase in FSH and LH levels and insignificant increase in prolactin level. These observations might as well be the influence of feedback response to the observed low testosterone levels. Decreased testosterone level has been noted to stimulate the production of gonadotropins by negative feedback mechanism (24). Furthermore, group III rats showed a significant increase in progesterone level and a non-significant increase in estrogen level. The decreased testosterone production may have resulted in these increases as previously alluded. This also resulted in decreased testosterone estrogen ratio which is associated with infertility in males (25).

From this study, it can be deduced that aqueous extract of *F. sycomorus* caused histopathological changes in the pituitary gland with perturbation of the pituitary gonadal axis hormones in treated male rats and associated decreased fecundity in females mated by the males. Further studies is recommended to be carried out on the histology of the pituitary gland using histochemical methods to distinguish the cell lines affected by the histopathological changes as well as morphometric studies of the cells.

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