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Effects of aqueous extract of *Ficus capensis* leaf on some reproductive parameters in normal adult male wistar Rats.

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Abstract
In view of the traditional belief that, it is traditionally believed that *Ficus capensis* leaf extract boosts an aphrodisiac effect and has been used to treat azoospermia. However, no scientific study has been carried out to demonstrate the effects of this extracthis experiments were performed to determine the effects of aqueous extract of *F. capensis* leaf on some reproductive parameters of male albino rats. The present study is therefore aimed at as literature regarding this seems to be scarce. Twenty eight normal male rats weighing between 180-240g were used for this study. They were grouped into 4 groups (A—D) of 7 rats each. Group A was the control group and was given distilled water only, while groups B, C and D were administered 50, 100 and 200 mg/kg body weight of aqueous extract of *F. capensis* leaves respectively via oral gavage for 28 days. Blood samples were collected for determination of serum testosterone while testicular weight as well as sperm motility and morphology were evaluated. The acute oral toxicity study was also carried out and the LD_{50} was estimated to be above 5000mg/kg. A significant decrease ($P<0.05$) in body weight and serum testosterone levels was observed in all the test groups when compared with the control group. A significant decrease ($P<0.01$) in testicular weight was observed in group B when compared with the control group. There was significant increase ($P<0.01$) in sperm count in groups B and C when compared with the control. Actively motile sperm significantly decreased ($P<0.001$) in groups C and D respectively when compared with the control. Consequently, sluggishly motile sperm was significantly increased ($P<0.01$) in groups C and D respectively, while abnormal sperm increased significantly ($P<0.05$) in group D when compared with the control. The result of this study suggests that the phytochemical constituents of aqueous extract of *F. capensis* may adversely affect reproductive functions in normal rats, thus may not serve as an aphrodisiac as serum testosterone levels were declined. However, it seems that one or more bioactive constituent at a very low concentration may favour spermatogenesis, thus demonstrating a potential to treat azoospermia.

Keywords: *Ficus capensis*, Male-factor infertility, Spermatogenesis, Sperm quality, Testosterone, Toxicity.

1. Introduction
The folklore use of some vegetables, have prompted scientific research to prove or debunk several pro-health claims. And while various vegetables have received recognition for their beneficial effects, others are termed underutilized vegetables essentially due to paucity of scientific data to back up their reported traditional uses (Otitoju *et al.,* 2014). Most researchers have focused on plants that have been traditionally used for various therapeutic reasons as these plants are more abundant or readily available thus, found to be less expensive and seemingly pose lesser side effects than synthetic drugs (Yakubu *et al.,* 2007). Some of these plants also serve culinary purposes. *Ficus capensis*, locally referred to as *Uwaryara* (Hausa), *Opoto* (Yoruba), *Rima bichehi* (Fulani), *Obada* (Edo) and *Akokoro* (Igbo) belongs to the family *Moraceae,* and has been regarded as an underutilized plant. It is used as a vegetable in foods with a reported blood boosting effect (Otitoju *et al.,* 2014) cum an anti-sickling effect of red blood cells (Umeokoli *et al.,* 2013).
Generally, the Genus *Ficus* comprises over a group of about 900 species of trees, shrubs, and vines, commonly called figs. *Ficus carica* (Common fig) is the most popular. Other benefits of *Ficus capensis* have been reported which include antibacterial (Oyeleke *et al.,* 2008), anti-abortifacient (Owolabi *et al.,* 2009), immune-stimulatory (Daikwo *et al.,* 2012), anti-diarrhoea (Owolabi, 2013), antioxidant (Ramde-tiendrebeogo *et al.,* 2012) and pro-fertility in treating azoospermia (Gelfand *et al.,* 1985)—probably the basis for which it is being touted for its aphrodisiac effect (Lumbile and Mogotsi, 2008).

As an aphrodisiac, as with some foods or plants like *Allium cepa* (Malviya *et al.*, 2011) and *Mucuna pruriens* (Pratap and Rajender, 2012), *F. capensis* is expected to have a role to play in reproductive health as the decoctions of the leaf extract of *F. capensis* are used locally to treat infertility.. One of the ways aphrodisiacs have been effective in such role is in elevating serum testosterone levels, sometimes demonstrated by increased sexual desire (Gooren and Saad, 2006). The use of aphrodisiac therefore, has proven relevant in the management of some forms of infertility—with about 30-50% of infertility cases attributed to problems with males alone (Ekwere *et al.,* 2007). And while some plants have shown good sexual boosting effect as against the available drugs and sometimes psychological approach to the management of this condition, the search for natural supplements is still ongoing (Yakubu *et al.,* 2007).

These vegetables therefore require scientific scrutiny to validate their safety since many of these may contain anti-nutritional constituents which at varying concentrations can affect normal functioning of the body (Chung *et al.,* 1998). However, the dearth of published information on the effect of this leaf extract on male fertility and the possible mechanisms involved has prompted this research work.

Materials and Method

Plant Materials

The fresh leaves of *Ficus capensis* were hand-picked from a farm in Enugu, Nigeria and identified by Mr. P. O. Ugwuozor, the herbarium curator, Department of Botany, Nnamdi Azikiwe University, Awka, where a voucher specimen was deposited at the herbarium for further reference (N. A. U. H. No. 32).

Preparation of Aqueous Extract of *F. capensis* Leaves

The leaves were washed and air-dried under ambient temperature. The dried leaves were ground using a laboratory mill yielding 800g of powder. This powder was macerated in 4 litres of lukewarm distilled water and poured into a beaker and sealed. The mixture was placed in the mechanical shaker for 24 hours. The mixture was then sieved with filter paper into a clean glass tube, the filtrate was then concentrated using the rotary evaporator.

Breeding of Animals

In this experimental study, twenty eight (28) normal adult male wistar rats weighing between 180-240g were housed in the Animal House of Nnamdi Azikiwe University, Nnewi campus, using standard cages. They were then acclimatized for fourteen (14) days, with water and feed provided *ad libitum.* All procedures used in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the Care and Use of animals (APS, 2002).

Acute Oral Toxicity Study (LD_{50})

The mean lethal dose of oral administration of *F. capensis* was estimated using the Lorke's method (1983). Firstly, nine rats were divided into three groups of three rats each. Each group was administered different doses (10, 100 and 1000 mg/kg) of aqueous leaves extract of *F. capensis*. Simultaneously, three rats which were distributed into three groups of one rat each, were administered higher doses (1600, 2900 and 5000 mg/kg) of the extract. All animals were then observed within 24 hours for mortality. The median lethal dose was then calculated thus:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

where D_0 = Highest dose that gave no mortality

D_{100} = Lowest dose that produced mortality

Experimental Design

At the end of acclimatization, the animals were randomly grouped into four equal groups—Groups A, B, C and D of seven rats each. The aqueous extract of *F. capensis* leaf was administered daily via gavage at 50mg/kg, 100mg/kg and 200mg/kg to groups B, C and D respectively for a period of 28 days while group A rats received distilled water and served as the control group. The body weights of the animals were recorded at the beginning of the experiment and repeated at 7-days interval till the termination of the experiment. At the end of extract administration, the animals were fasted overnight and were sacrificed the next morning under light diethylether anaesthesia. Blood samples were collected by cardiac puncture for determination of testosterone levels.

Determination of Testicular Weight

Dissection was done transversely across the peritoneum and the testes was pushed into the abdominal region and extracted after the ducts were carefully cut. Both testes were weighed using the Electronic balance JA-410 and the average derived.

Serum Testosterone Assay

Blood samples were left for 60 minutes to clot and then centrifuged for 10 minutes at 2500rpm. The obtained clear sera were stored using plain sample bottles, at -4^{o}C. The testosterone level was measured by enzyme-linked immunosorbent assay method using the Testosterone Enzyme Immunoassay Test kit (BC-1115).

Epididymal Sperm Analysis

The epididymis was separated carefully from the testis and divided such that its tail was trimmed with a scissors and a sample of the semen collected and sufficiently mixed. To estimate the motility, a drop (10-15µl) of well-mixed liquefied semen was placed on a slide and covered with a 20 × 20 mm cover glass. The specimen was then viewed using the 10× objective with the condenser iris closed sufficiently to give a good contrast. Under the 40× objective, a total of 100 spermatozoa were counted such that of this number, both the progressive and slow counts were recorded as percentages. 0.1 ml of the well-mixed liquefied semen was measured into a graduated test tube and 2 ml of sodium bicarbonate formalin diluting fluid was added and mixed together. Using a Pasteur pipette, the Neubauer ruled chamber was filled with the mixture of diluted semen and left for about 5 minutes for the spermatozoa to settle. With the condenser sufficiently closed, the spermatozoa in an area

of 2 sq mm (which is 2 large square) was counted. This figure was then multiplied by 100 000.

The abnormal spermatozoa were estimated using the 100× objective and included the percentage of both abnormal motile and morphology.

Statistical Analysis

All data were tabulated and statistically analyzed using SPSS version 20.0. Results were expressed as Mean ± standard error of mean (M ± SEM). One way analysis of variance (ANOVA) followed by Bonferroni's Post-hoc test were used for data comparison. P<0.05 was taken as statistically significant.

Results

Acute Oral Toxicity (LD_{50}) Study

Table 1. Result of acute oral toxicity of *Ficus capensis*

Phase	Group	Dose	Mortality
A	1	10	0/3
	2	100	0/3
	3	1000	0/3
B	4	1600	0/1
	5	2900	0/1
	6	5000	0/1

The result of this study demonstrates that the acute oral toxicity study of the leaves of *F. capensis* is above 5000 mg/kg in rats.

Effect of Aqueous Extract of *Ficus capensis* Leaves on Body Weight

Fig. 1 shows the observed body weight decrease in all test groups when compared with the control. This reduction in body weight of test animals was significant (P<0.05) at days 7, 14, 21 and 28.

Effect of Aqueous Extract of *Ficus capensis* Leaves on Testicular Weight

A significant decrease (P<0.01) in testicular weight was observed in group B (2.09 ± 0.09) when compared with the control group A (2.74 ± 0.08). No significant decrease (P>0.05) was observed in groups C (2.47 ± 0.15) and D (2.48 ± 0.12) when compared with the control. (See Table 1).

Effect of Aqueous Extract of *Ficus capensis* Leaves on Serum Testosterone

A significant decrease (P<0.05) in serum testosterone levels was observed in all test groups—B (0.23 ± 0.08); C (0.61 ± 0.15) and D (0.41 ± 0.22) when compared with the control group A (1.51 ± 0.38) as shown in Table 1.

Effect of Aqueous Extract of *Ficus capensis* Leaves on Sperm Count

Table 1 shows a statistically significant (P<0.05) increase in sperm count for groups B (70.39 ± 2.69) and C (45.60 ± 2.83) when compared with the control group A (29.31 ± 2.40). Group D (29.66 ± 2.45) however, did not show any significant increase (P>0.05) when compared with the control group.

Effect of Aqueous Extract of *Ficus capensis* Leaves on Sperm Characteristics

Generally, a decrease in the actively motile sperm was observed in all test groups—B (93.86 ± 1.10); C (82.86 ± 3.25) and D (69.00 ± 4.03) when compared to the control group A (94.71 ± 0.36). However, only groups C and D showed a significant decrease (P<0.05) when compared with the control group (See Table 2). An increase in sluggishly motile sperm was observed in all test groups—B (3.71 ± 0.52); C (13.71 ± 2.94) and D (26.29 ± 3.62). However, only groups C and D showed significant increase (P<0.05). The test groups—B (2.43 ± 0.65); C (3.00 ± 0.38) and D (3.57 ± 0.53) showed an increase in the number of abnormal sperm when compared with the control group A (1.86 ± 0.26). Only group D however, was significant (P<0.05).

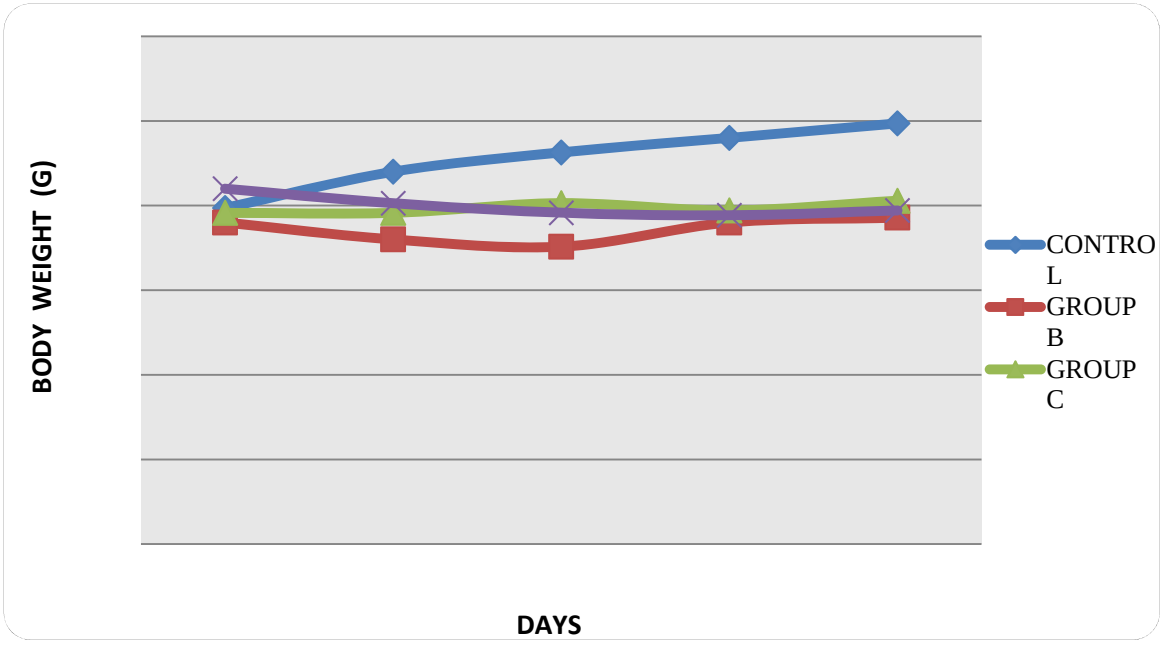

Fig 1. Effect of aqueous extract of *F. capensis* leaves on body weight

Table 1. Effect of aqueous extract of *F. capensis* leaves on testicular weight, serum testosterone levels and sperm count

MEAN ± SEM			
GROUPS	**TESTICULAR WEIGHT (g)**	**SERUM TESTOSTERONE (ng/ml)**	**SPERM COUNT (x10^6)**
A	2.74 ± 0.08	1.51 ± 0.38	29.31 ± 2.40
B	2.09 ± 0.09**	0.23 ± 0.08**	70.39 ± 2.69**
C	2.47 ± 0.15	0.61 ± 0.15*	45.60 ± 2.83**
D	2.48 ± 0.12	0.41 ± 0.22**	29.66 ± 2.45

*P<0.05; **P<0.01; ***P<0.001

Table 2. Effect of aqueous extract of F. capensis leaves on sperm characteristics

GROUPS	**MEAN ± SEM (%)**		
	ACTIVELY MOTILE	**SLUGGISHLY MOTILE**	**ABNORMAL SPERM**
A	94.71 ± 0.36	3.43 ± 0.30	1.86 ± 0.26
B	93.86 ± 1.10	3.71 ± 0.52	2.43 ± 0.65
C	82.86 ± 3.25**	13.71 ± 2.94**	3.00 ± 0.38
D	69.00 ± 4.03***	26.29 ± 3.62***	3.57 ± 0.53*
*P<0.05; **P<0.01; ***P<0.001			

Discussion

The results of this study showed that the aqueous leaf extract of *F. capensis* decreased body weight, testicular weight and serum testosterone levels yet increased sperm counts in normal adult male wistar rats.

The reduction in body weight in the test animals as a result of oral administration of *F. capensis* may be due to the phytochemical constituents of the leaves—anthocyannins, flavonoids, and tannins. These phytohemicals are generally regarded as polyphenols and have been reported to cause weight loss in experimental animals (Badimon *et al*., 2010). Anthocyannins and flavonoids have been demonstrated to suppress growth of the adipose tissue through their anti-angiogenic activity and by modulating adipocyte metabolism (Badimon *et al*., 2010; Mulvihill and Huff, 2010). Tannins are considered anti-nutritional phytochemicals which have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals (Acamovic *et al.*, 2005). However, the mechanism by which tannins exert this action was suggested to be by its ability to decrease efficiency in converting the absorbed nutrients to new body substances rather than its previously believed role in inhibiting food consumption or digestion (Chung *et al*. 1998).

Another constituent of the leaf extract identified as phytates has been indicted in weight loss of test animals. Sometimes called phytanic acid, a compound produced from the metabolism of phytol. Phytates are reported to possess hypoglycaemic and hypolipidaemic effects as shown by Heim *et al*. (2002). In rats, phytanic acid increases the expression of glucose transporters and glucokinase and hence increases glucose uptake in the hepatocytes (Heim *et al*., 2002). The presence of these phytochemicals in the leaf extract of *F. capensis* as reported by François *et al*., (2010) may therefore be responsible for reduction in the weights of the test animals.

The weight of the testes is a useful index in evaluating the efficiency of steroidogenesis. A reduction in the testicular weight was observed in all the test group animals and according to Michael *et al*. (2007) this may infer that the leaf extract is toxic to the organ. Morton (1988) reported that in sacrificed animals, decreased weight of the testes indicates a widespread or diffuse loss of seminiferous epithelial cells such that the testis which possesses a greater number of sertoli cells is heavier and produces more spermatozoa than the testis with fewer sertoli cells. The reduction in testicular weight may be as a result of a reduction in body weight. The sum total of the actions of the individual phytochemical constituents of *F. capensis* to cause a decreased body weight of the test animals may also be collaborated with the reduction in the testicular weight also observed from this study. Thus, agreeing with da Luz *et al*. (2013) that there is a positive relationship between body weight and testicular weight.

Similarly, Yakubu (2012) in another study showed that alkaloids affect spermatogenesis by altering the bioavailability and production of androgens. He suggested that the mechanism by which alkaloids reduced testicular weight may include causing atrophy, spermatogenic arrest, inhibition of steroid synthesis in the Leydig cells, and/or reduced tubule size. The inhibition of steroid synthesis by some of the phytochemicals is also collaborated by the result of this research as a general reduction in testosterone levels was observed in the test animals. Testosterone is required for the development and maintenance of the germ cell and proliferation of the epithelium of the seminiferous tubules. Thus, the observed reduction in testicular weight may also be a function of the decreased testosterone levels possibly indicating atrophy of the epithelium of the seminiferous tubules.

Testosterone is essential to maintain spermatogenesis and male fertility. The decreased serum testosterone observed from this research in all test animals suggests that the leaf extract may not be used as an aphrodisiac as documented by Lumbile and Mogotsi (2008). And while testosterone has been reported to be useful for the histo-morphometric development and maintenance of the testes and ultimately the biochemical process of sperm production (Morton, 1988; Walker, 2010), low serum levels may have adverse effect on fertility. A phytochemical worth mentioning here is the phytates—contained in the leaves of *F. capensis.* This phytochemical is reported to be the form in which phosphates occur in plants thus it is believed to combine with various minerals like iron and zinc thereby exerting an inhibitory activity on the absorption of these minerals from

plants (Halberg, 1987). Some of these minerals have a major role to play in reproduction. Jana *et al.* (2008) stated that dietary zinc is directly related to the synthesis of testosterone by the Leydig cells of the testes. Zinc deficiency, which may have been possible in this study due to the phytate content of *F. capensis*, may therefore induce primary testicular failure and altered stereoidogenesis (Kim and Kim, 2006).

Similarly, the alkaloidal content (Yakubu, 2012) of the leaves of *F. capensis* suggested to inhibit the synthesis of steroidal hormones such as testosterone may also explain the decreased levels of the test animals in this research while the relationship that exists between testicular weight and serum testosterone levels may also explain this decline.

Sperm indices are a vital tool for assessing fertility. The evaluation of the effect of *F. capensis* on sperm parameters as prompted by the documentation of Gelfand *et al.* (1985) in treating azoospermia may be supported by the result of this study as sperm counts were observed to be increased in all test groups. However, this increase was observed to be negatively related to the increasing concentration of the leaf extract of *F. capensis.*

While the results of this research suggest that serum testosterone is retarded by the administration of aqueous extract of *F. capensis*, the increase in sperm counts in all test groups when compared with the control group is one requiring further research. Testosterone is necessary for spermatogenesis; however its role is reported to be in maintaining this biochemical process. The role of other endocrine factors may therefore explain the sperm count increase observed as spermiogenesis is under the control of follicle stimulating hormone (Barrett *et al.,* 2010). Possibly, the administration of the aqueous leaf extract of *F. capensis* may have a positive effect on follicle stimulating hormone, thereby promoting spermatogenesis. And though the serum testosterone levels were decreased, it was not reduced below the lower border of the reference value (0-7mg/ml) for rats. Thus, the testosterone levels may have been sufficient enough to maintain the maturation of the sperm cells activated by a possible elevation of follicle stimulating hormone.

Though sperm counts are important in assessing fertility, the ability for these sperm cells to swim forward towards the ovum portends a better chance of fertilization especially when there are fewer abnormal sperm cells in the semen sample. The actively motile cells regressed with an increasing concentration of the extract while the sluggishly motile and abnormal sperm cells were greatly increased. Yakubu (2012) demonstrated that alkaloids adversely altered the maturation stages of spermatogenesis and was responsible for most of sperm abnormalities such as multiple heads and tails as well as increased sluggish motility as observed in this research. Similarly, the role of the various phytochemicals especially the polyphenols (tannins, terpenoids and flavonoids) have been implicated as they may offset the balance of the reactive oxygen species of cells at higher concentrations and ultimately induce negative activity on fertility (Taitzoglou *et al.,* 2001; Mennen *et al.,* 2005). These studies back the results of this research as increased doses of the extract induced a corresponding increase in both abnormal as well as sluggishly motile sperm cells while decreasing the actively motile cells. Similarly, the potential antifertility activities of *F. capensis* in this study may also collaborate with the toxic effect of saponin in normal rats as reported by Francis *et al.* (2002) in which it was stated that saponins may exert a negative effect on reproductive functions in normal rats with sterility reported, while it may be beneficial in cases of infertility.

Conclusion

This study suggests that the phytochemical constituents of aqueous extract of *F. capensis* may adversely affect reproductive functions in normal rats, thus may not serve as an aphrodisiac as serum testosterone levels were observed to be decreased. This is not in agreement with its traditional use as an aphrodisiac as earlier reported. However, it seems that one or more bioactive constituent of the aqueous extract of *F. capensis* at a very low concentration may favour spermatogenesis, thus demonstrating a potential to treat azoospermia.

This may therefore justify its ethnomedical use in the treatment of azoospermia. In the same regard, ingestion of large amounts of this extract is ill-advised as this has been shown to cause a decline in sperm count, eventually. Furthermore, the use of this leaf extract in normal conditions (e.g. as a vegetable for culinary purpose) should be highly avoided.

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