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Anti-Spermatogenic Effects of *Ficus sycomorus* Aqueous Leaf Extract on Testes and Epididymis of Adult Male Wistar Rats

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ABSTRACT: The Effects of Aqueous Extract of *Ficus sycomorus* leaves on the testis, epididymis and sperm parameters in adult male Wistar rats was investigated. Eighteen [18] adult male rats were used for the experiment. The rats were randomly assigned into three groups; control group (A) and two treatment groups (B and C) with each containing six rats (n=6/group). Rats in group A were given feed mash and water *ad libitum* daily throughout the experimental period while animals in group B and C received 500mg/kg and 1500mg/kg body weight respectively of the *Ficus sycomorus* leaf extract in addition to the feed mash and water respectively. The rats were weighed before and at the end of the experimental period which lasted for 56 days. They were put under chloroform anesthesia and a mid-line incision was made through the ventral wall of the abdomen. The testis and epididymis were excised, weighed and fixed in Bouin's fluid for routine histological examination while semen was collected from the vas deferens for sperm analysis. Data were expressed as Mean ± SEM. Significant difference between means were determined by t-test and one-way analysis of variance (ANOVA). Results gave a significant decrease in the testicular and epididymal weights, total sperm count and sperm motility in the treated rats when compared with the control rats ($p<0.05$). A non-significant increase in the sperm abnormal morphology of treated rats when compared with the control rats ($p>0.05$) was also recorded. Histological findings revealed a dose dependent distortion of cellular architecture in the seminiferous tubules of the testes and a decrease in sperm quantity in the lumen of the epididymis causing oligospermia in low dose treated rats and azospermia in high dose treated rats. This study revealed that aqueous extract of *Ficus sycomorus* caused an antifertility effect by causing a reduction in the sperm count and motility, an increase in abnormal morphology and distortion of the testicular histology in a dose dependent manner.

Keywords: *Ficus sycomorus,* Antifertility, Testes, Spermatozoa

Introduction

Medicinal herbal use has been long practiced by rural dwellers and in developing countries for the treatment of ailments (Ogunlana *et al.,* 2008). An effective use of such herbal remedies, require an adequate knowledge of its lethal dose, purity, adverse effects and suitable extraction techniques (Murray, 1998). Long term use of herbs without proper monitoring have resulted in a number of health related ailments of which infertility appears to be key (Ako *et al*., 2008). Although infertility has been reported to be a major world problem among couples affecting both males and females, yet it is a major checker to world population explosion threat (Asuquo *et al.*, 2013).

Declining male fertility has become a common worldwide problem accounting for about 30 % of infertility cases (WHO, 1991). The presence of anatomically normal spermatozoa with optimal physiologic action in semen is necessary for fertility; on the contrary, low or abnormal sperm production by the testes, premature ejaculation or testosterone hormonal distortions are the predominant causes of infertility in males (Hamman, 2008). It has been reported that a number of medicinal plants have induced male infertility via different modes of action. Asuquo in 2012 reported that administration of ethanolic leaf extract of Spondias mombin caused impairment of testicular and epididymal structures, Sarathchandiran in 2014 showed that *Clerodendrum serratum* impaired epididymal sperm motility and reduced testosterone production while Anitha *et al.* (2013) demonstrated that *Cynoglossum zeylanicum* reduced testicular and epididymal weights and caused reduced sperm motility, to mention but a few.

Ficus sycomorus is a tropical medicinal plant of the family of *moracea* (Sheikha *et al.,* 2015) found in tropical and sub-tropical regions with low or moderate rainfall. It is commonly called fig or sycamore and has been documented to possess a number of medicinal potentials. The plant extracts have been used to treat sore throat, chest pain, scrofula, cough and snakebites (Malgras, 2008). It has also been shown to possess anti-bacterial activities in the management of

diarrhea (Bello, *et al*. 2013), anti-helminthic and laxative activities (Sofowora, 1993); it has been reported to contain a number of potent phytochemicals and antioxidants responsible for its therapeutic action (Ogunlana *et al.*, 2008). Studies have not yet been carried out on its antifertility effect. The aim of this study is to investigate the effects of *Ficus sycomorus* on the testes, epididymis and sperm parameters of adult male Wistar rats.

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 300ml of distilled water using maceration extraction method for 24 hours in an air tight container. The extract obtained was filtered through a Whatman paper 1 and the filtrate was concentrated at a low temperature, under reduced pressure using a vacuum rotary evaporator. 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 eighteen (18) adult male 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 declaration of Helsinki on guiding principles in the care and use of animals, approval was received from the Research and Ethics Committee of the College of Medical Sciences, University of Benin, Benin City.

Experimental design: Eighteen male rats were randomly divided into three groups each containing six rats (n=6/group). Group A served as the control. The rats were given normal rat feed and water *ad libitum*. Groups B and C rats in addition to normal feed and water were treated with 500 and 1500 mg/kg body weight respectively, of *Ficus sycomorus* leaves extract. The extract was given orally once daily and the experimental period lasted for eight weeks (56 days). The rats were anesthetized with chloroform and sacrificed. The testes and epididymal weights were recorded.

Sperm count and motility: At the time of sacrifice, sperm cells were collected from the vas deferens. The vas deferens were ligated at both extremities to a length of about 36 mm minimum, and teased open to release the sperm cells into a 35 mm Petri dish containing 2 mL phosphate buffer (0.1 M, pH 7.4). A drop of the solution was put on a Neubauer chamber to assess sperm motility (Amelar *et al.*, 1973). Both motile and immotile spermatozoa were counted in different fields to determine the percentage of motile sperms. Sperm count was determined in the same way, with the exception that 1% formalin solution was used instead of phosphate buffer and the count was expressed as millions of cells/mm^3 (Besley *et al*., 1980). To maintain activity of the viable sperms, all tools, containers and surfaces used in the experiments were kept at a temperature of 37 °C.

Histological analysis: The testes and epididymal tissues from the control and treatment groups were dissected out and fixed in Bouin's fluid. The tissues were processed for histological examination and paraffin sections were stained with hematoxylin and eosin and qualitative microscopic examination was made.

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

*Testicular and Epididymal weights***.** Significant weight loss were observed in the testes and epididymis of all the treatment groups in a dose dependent manner when compared to the control group ($P<0.015$) except in the right testes of Group B (low dose) where there was a non-significant weight loss ($p>0.05$) as shown in Table 1.

*Sperm characteristics***.** Sperm count significantly decreased in a dose dependent manner in treated groups B (low dose) and C (high dose) compared to the control group ($p<0.05$) as shown in Table 1. Abnormal sperm morphology increased in a non-significant fashion in both treatment groups B (low dose) and C (high dose) when compared to the control group ($p<0.05$). Sperm progressive motility results, showed that rats treated with *F. sycomorus* had significantly low total motility compared to the control rats ($p<0.05$).

*Histology***:** In the histological study of the testes, seminiferous tubules of control animals showed sequential arrangement of germ cells at various stages of spermatogenesis with spermatozoa maturation occurring near the lumen (Fig. 1). The histoarchitecture of testes of treated rats showed seminiferous tubule with some degenerative changes marked by the presence of cellular debris and early germ cells released into the lumen. There was mild disruption of the spermatogenic cell series in the seminiferous tubule epithelium of rats treated with low dose of the extract (Fig. 3). While in rats treated with high dose, the lumen of the seminiferous tubule was devoid of spermatozoa with marked disruption of spermatogenic cell series (Fig. 5). The epididymis of control rats showed normal epithelial architecture with spermatozoa in the lumen (Fig. 2). The lumina of treated rats contained scanty spermatozoa in low dose and were devoid of spermatozoa at high dose (Fig. 4 and 6).

Table 1**:** Effect of aqueous leaf extract of *F. sycomorus* on testicular and epididymal weight of rats (Mean ±SEM).

Testicular Weight	Group A (Control)	Group B (500 mg/kg b.wt)	Group C (1500 mg/kg b.wt)
Right testes (g)	1.51±0.02	1.44±0.07	1.18±0.07 *
Left testes (g)	1.52±0.04	1.41±0.06 *	1.21±0.04 *
Right epididymis (g)	0.22±0.02	0.17±0.02 *	0.18±0.07 *
Left epididymis (g)	0.21±0.01	0.16±0.02 *	0.14±0.01 *

**(p<0.05) significantly different from the control*

Table 2. Summary of mean sperm parameters in rats treated with aqueous leaf extract of *F. sycomorus.* Values given represent the Mean±SEM

Semen Parameters	Group A (Control)	Group B (500 mg/kg b.wt)	Group C (1500 mg/kg b.wt)
Total Sperm count ($\times 10^6$)	44.67±3.56	18.17±1.47 *	13.33±1.75 *
Sperm morphology (% Abnormal)	18.30±11.10	26.67±3.44	28.67±1.21
Sperm PM (%)	81.67	67.50	68.00

**(p<0.05) significantly different from the control, PM-progressive motility*

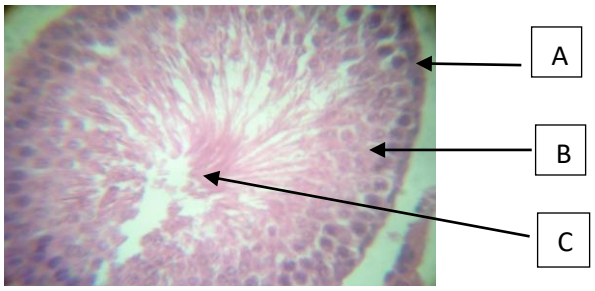

Fig. 1: Photomicrograph of the control of testis showing basement membrane A, Seminiferious epithelium with germ cells in sequential arrangement B, lumen of seminiferous epithelium containing tufty tails of spermatozoa C (H&E X 400).

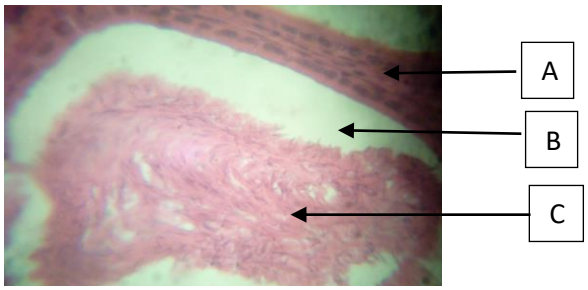

Fig. 2: Photomicrograph of control of epididymis showing normal epithelial lining A, normal luminal gap B, spermatozoa within the lumen C (H&E X 400).

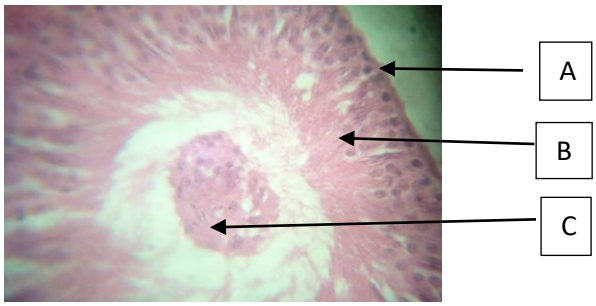

Fig. 3: Photomicrograph of testis treated with low dosage of aqueous *Ficus sycomorus* leaf extracts showing seminiferous tubules with basement membrane A, mild disruption of the spermatogenic cell series in the seminiferous epithelium B, lumen of seminiferous epithelium containing cellular debris with early germ cell series C, (H&E X 400)

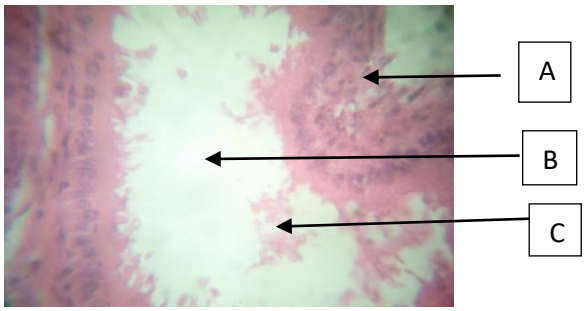

Fig. 4: Photomicrograph of epididymis treated with low dosage of aqueous *ficus sycomorus* leaf extracts showing epithelium of the epididymis A, widened luminal gap B, and lumen containing scanty spermatozoa C (H&E X 400).

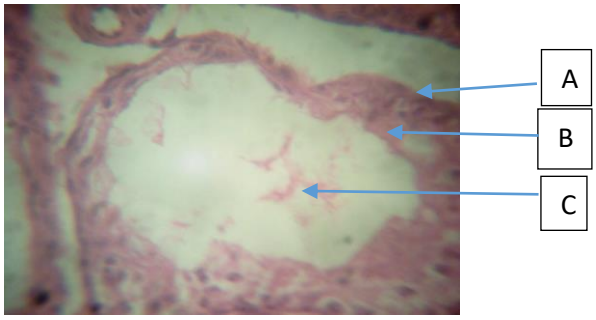

Fig. 5: Photomicrograph of testis treated with high dosage of aqueous *ficus sycomorus* leaf extract showing basement membrane of seminiferous tubules A, disruption of spermatogenic cell series B, lumen of seminiferous tubules devoid of sperm cells C (H&E X 400).

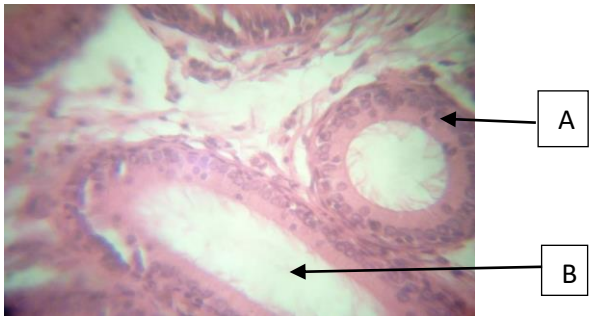

Fig. 6: Photomicrograph of epididymis treated with high dosage of aqueous *ficus sycomorus* leaf extracts showing the epithelial wall of epididymis A, lumen of epididymis devoid of spermatozoa B (H&E X 400).

Discussion

In the present study oral administration of aqueous extract of *F. sycomorus* at doses of 500 and 1500 mg/kg body weight for 56 days produced a dose dependent adverse effect on fertility parameters of adult male rats. The decreasing weights of the testes and epididymis in extract-treated male rats, indicated that the extract altered the structural architecture and corresponding physiological balance in the organs. Reduction in the weight of testes and epididymis might also be due to low level of androgen production, which was insufficient to maintain its weight. These findings support similar works done by other researchers (Sakar *et al.*, 2000; Sharma and Jacob, 2001).

Sperm count and motility were found to be decreased in this study but with an increase in abnormal morphology among the treated rats. The abnormal forms observed consisted of both primary and secondary abnormalities according to the classification of Noarkes (2004). Sperm count is usually correlated with fertility as one of the vital tests for spermatogenesis. Low sperm count and inactive spermatozoa are majorly responsible for infertility in males (Joshi *et al.*, 2012; Udoh *et al*. 2005; Braide *et al*., 2003). The significant decrease in the sperm motility of rats treated with *F. sycomorus* at doses of 500 and 1500 mg/kg body weight suggest that the extract was able to permeate the blood-testis barrier as was reported in a similar work carried out by Baldessarini (1980). It may also have created a different milieu between the inner and outer part of the walls of the seminiferous tubules (Bloom and Fawcett, 1975). Alterations in sperm motility and viability might also have resulted from disturbances in epididymal function (Gupta, 2012). Reduced sperm count in testes and epididymis may be due to the suppressive effect of the extract on spermatogenesis or the inhibition of meiotic division in spermatocytes (Obianime *et al*. 2009; Akbarsha *et al*., 2001)

The histological distortions observed in testicular epithelium and a concomitant decrease in sperm quantity in the lumen of the epididymis were not of the same magnitude in the different study groups. Rather, they were more marked in the group treated with high dose of the extract and less evident in the group treated with low dose. The degree of damage to the testicular epithelium has been described as dose dependent (Gupta *et al*., 2004; Tellez-López *et al*., 2013). Our findings however, suggest that an increased dose of *F*. sycomorus could distort the cyto-architecture of the testes and reduce the luminal content of the epididymis. This result was similar to that obtained from *Carica papaya* where the extract caused a suppression of spermatogenesis, architectural distortion and reduced testicular weight (Uche-Nwachi *et al.*, 2011).

Conclusion

The progressive dosage dependent damage in testicular epithelium, and the adverse effect on sperm quantity and quality parameters caused by extracts from *F*. sycomorus, makes it a potent herb for male fertility control. However, further studies are required to elucidate the mechanism of action.

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