Effect of Prolonged Oral Administration of Aqueous *Ficus sycomorus* Stem-bark Extract on Testicular Size of Growing Albino Rat

Efecto de la Administración Oral Prolongada de Extracto Acuoso de la Corteza de *Ficus sycomorus* en el Tamaño Testicular de Rata Albina en Crecimiento

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SUMMARY: Decoction of *Ficus sycomorus* stem bark is used to treat infertility involving low sperm counts, but the reproductive risk associated with its use is uncertain. The aqueous stem-bark extract was orally administered at 200-600 mg/kg for 30 days to post-pubertal growing albino rats. Within the period, the live body weights, scrotal diameters and testicular size parameters were estimated. The mean live body weight, scrotal diameter of the control and treated rats significantly ($p < 0.05$) increased as the animals grew, but the treatment did not significantly ($p > 0.05$) affect the parameters. There were no significant ($p > 0.05$) variations in mean testicular size parameters in treated and control rats, and no dose-dependent nor time-dependent effects occurred. Sections of the testes of the treated animals showed active spermatogenesis in the seminiferous tubules with no apparent histological lesions. It is concluded that the use of the crude extract of the *Ficus sycomorus* within our dose range portends minimal male reproductive risk and underscores the need to study its pro-spermatogenic effect.

KEY WORDS: *Ficus sycomorus* extract; Testicular size; Testicular histopathology; Spermatogenesis; Male reproductive risk.

INTRODUCTION

Ficus sycomorus, a medicinal plant belonging to the class Moracea, is used in Nigeria, Niger, Mali, South Africa, Guinea, Kenya, Tanzania, Somalia, Ethiopia and Ivory Coast as extracts of fruits, leaves, root and stem barks to treat various ailments such as cough, diarrhea, skin infections, stomach disorders, liver disease, epilepsy, tuberculosis, lactation disorders, helminthiasis, infertility and sterility (Palayer, 1977; Heine & Brenzinger, 1988; Gachathi, 1989; Hedberg & Staugard, 1989; Heine & Heine, 1989; Abdurrahman, 1992; Samuelsson *et al.*, 1992; Bekalo *et al.*, 1996; Arnold & Galamain, 2002; Sandabe, 2002; Pakia & Cooke, 2003; Kone, 2005; Moshi-Mainen *et al.*, 2005; Maregesi *et al.*, 2007; AFLORA, 2008; Kone & Atindehou, 2008; Mahenge, 2008; Malgras, 2008). The aqueous extract of the plant had relative safety with LD50 of 720mg/kg, contained pharmacologically active substances such as gallic tannis, saponins, reducing sugars, alkaloids and flavone

aglycones, and caused no haematological, hepatic and renal toxicities (Sandabe). The aqueous extract had some anticonvulsive effect as well as inhibitory effect on muscle contractions (Sandabe *et al.*, 2006). Similarly, other extracts of a related plant species, *Ficus plantyphylla* had sedative, anticonvulsant, muscle relaxant and anesthetic properties (Bokko, 1998; Onyeyili *et al.*, 1998). Because of these nervous system effects, the extracts were used in the management of psychiatric patients (Abdurrahman).

Medicinal plant extract from *Carica papaya* (Lohiya *et al.*, 1994), *Quassia amara* (Raji & Bolarinwa, 1997), *Terminalia catappa* (Ratnasooriya & Dharmasiri, 2000), *Ricinus communis* (Raji *et al.*, 2006), *Garcinia kola* (Akpantah *et al.*, 2003), *Ruta graveolens* (Khoury & El-Akawi, 2005), *Alstonia beonei* (Raji *et al.*, 2005), *Vernonia amygdalina* (Oyeyimi *et al.*, 2008) were reported to have

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adverse effects on male reproduction by reducing the weight of testes. Since *Ficus sycomorus* had been associated with treatment of fertility and sterility in humans (Pakia & Cooke, 2003; Malgras, 2008) and the effects of its extracts on male reproductive organs had not been studied, the present research was designed to assess the effects of the aqueous extract of the stem bark on the testicular size of growing albino rat.

MATERIAL AND METHOD

Animals. Sixty post-pubertal male albino rats, above 50 days of age and weighing 98.0 ± 22.9 g, were sourced from Ayuba Animal Farm, Maiduguri 14 days earlier than the time of commencement of the experiment. They were housed in stan-

dard plastic cages containing 5 rats each with comparable mean live body weights and kept in a room with ambient temperature not exceeding 30°C. They were fed *ad libitum* with commercial pelletized feed (Vital Feed, Jos). Clean drinking water was provided freely in nipple drinkers.

Plant identification and extraction. The tree of *Ficus sycomorus* was identified by a taxonomist in the Department of Biological Sciences, Faculty of Science University of Maiduguri. The stem bark was chopped off and air dried away from direct sunlight for eight days. The dried stem bark was pounded into powdered form using pestle and mortar. The powdered extract (550g) was dissolved in distilled water (3000ml) in a round bottom flask. The mixture was refluxed for 2 hours and filtered while hot with No.1 Watman filter paper. The refluxing was done three times using fresh distilled water each time. After filtration,

the filtrate was poured on a tray and placed in a hot air oven at 40-50°C for 2 hours for the water to evaporate. The dried sample left on the tray was weighed and used for the experiment.

Experimental design. The rats were divided into four groups of 15 rats each. The concentration of the extract used was 200 mg/ml (Sandabe). Rats in groups A, B and C were administered orally through an esophago-gastric tube (Onu *et al.*, 2008) with 200 mg/kg, 400 mg/kg and 600 mg/kg, respectively, of the extract for 30 days. Rats in group D served as control and received distilled water only during the period. Five rats from each group were humanely sacrificed on days 10, 20 and 30 of treatment to determine the dose-dependent and time-dependent effects of the treatment on body weight gain, scrotal diameter and testicular size. The live body weights and scrotal diameters of the rats were measured. The testes were dissected out and separated from the spermatic cord by cutting the vascular cone. The testes were weighed without the epididymides and the longitudinal length and mid-circumference of the testes were estimated.

Histopathology. One of the testes of each rat was fixed in Bouin's fluid, processed, cut and stained with haematoxylin and eosin (Akpanatah *et al.*).

Fig. 1. Live body weights and weight gain of albino rats orally administered with aqueous extract of *Ficus sycomorus* stem bark for 30 days.

Fig. 2. Scrotal diameter and percentage increase in the diameter from day 0 of treatment in albino rats orally administered with aqueous extract of *Ficus sycomorus* stem bark for 30 days.

Statistical analysis. The data obtained is presented as means $\pm$ standard deviations and variations in means were assessed by one-way analysis of variance (ANOVA); coefficient of determination, r^2 , for linear trends in relation to time and dose were determined using computer software (GraphPad Instat, 1993 version, [http:// www.graphpadinstat.com](http://www.graphpadinstat.com)).

RESULTS

Body weight and scrotal diameter.The mean live body weights, weight gain, scrotal diameter and increase in scrotal diameter of the control and treated rats significantly ($P < 0.05$) increased during the time of treatment, but the dose of administration of the extract did not significantly ($P > 0.05$) affect the parameters (Figs. 1 and 2). The coefficient of determination, r^2 , assessing time-dependent linear trend of the parameters (Table I) were significant ($P < 0.01$) and comparable at various doses, but no significant ($P > 0.05$) dose-dependent linear trend were observed with all the parameters. The time-dependent linear trend was stronger for the percentage increase in scrotal diameter over time ($r^2 = 0.36-0.56$) than the scrotal diameter per se ($r^2 = 0.09-0.17$).

Testicular size. The effect of oral administration of aqueous *Ficus sycomorus* stem-bark extract for 30 days on the testicular weight, length, mid-circumference and size index of the rats are summarized in Tables II and III. The extract at various doses did not cause any significant ($P > 0.05$) variations in

Table I. Coefficient of determination, r^2 ($P < 0.01$) of all the parameters treated with various doses of *Ficus sycomorus* extract for 30 days: time -dependent linear trends at various doses.

Parameters	Dose (mg/kg)			
	0	200	400	600
Body weight	0.43	0.41	0.44	0.29
Weight gain	0.49	0.46	0.42	0.52
Scrotal diameter	0.17	0.09	0.11	0.10
Increase in scrotal diameter	0.53	0.36	0.56	0.47

testicular size parameters during the period of treatment when compared with the untreated control rats, and no dose-dependent nor time-dependent effects occurred with these parameters.

Fig. 3. Photomicrograph of the testis of a control rat (H&E, x 400)

Fig. 4. Photomicrograph of the testis of a rat treated with *Ficus sycomorus* extract at 600 mg/kg for 30 days.

Table II. Effect of oral administration of aqueous *Ficus sycomorus* stem-bark extract for 30 days on the testicular weight and size index of albino rats

Dose (mg/kg)	Testis	Days of treatment		
		10	20	30
0	Right (g)	0.7±0.3 ^a	0.7±0.3 ^a	0.9±0.2 ^a
	Left (g)	0.7±0.3 ^a	0.7±0.4 ^a	0.9±0.2 ^a
	Paired (g)	1.4±0.5 ^a	1.4±0.6 ^a	1.8±0.4 ^a
	†Size index (mg/g)	10.0±2.7 ^a	9.3±3.6 ^a	11.9±1.9 ^a
200	Right (g)	0.7±0.3 ^a	0.8±0.2 ^a	0.9±0.2 ^a
	Left (g)	0.7±0.3 ^a	0.9±0.2 ^a	0.9±0.2 ^a
	Paired (g)	1.4±0.5 ^a	1.7±0.4 ^a	1.9±0.4 ^a
	Size index (mg/g)	11.4±2.6 ^a	11.9±1.0 ^a	11.5±1.3 ^a
400	Right (g)	0.7±0.2 ^a	0.8±0.3 ^a	1.0±0.1 ^a
	Left (g)	0.7±0.2 ^a	0.8±0.3 ^a	1.0±0.1 ^a
	Paired (g)	1.4±0.5 ^a	1.6±0.7 ^a	1.9±0.2 ^a
	Size index (mg/g)	11.1±2.3 ^a	10.6±3.3 ^a	11.7±0.6 ^a
600	Right (g)	0.8±0.2 ^a	0.9±0.1 ^a	1.0±0.1 ^a
	Left(g)	0.9±0.2 ^a	0.9±0.1 ^a	0.9±0.1 ^a
	Paired (g)	1.7±0.4 ^a	1.8±0.2 ^a	1.9±0.2 ^a
	Size index (mg/g)	13.4±1.4 ^a	13.3±1.8 ^a	12.4±1.3 ^a

^a Means ±standard deviations of each parameter with the same superscripts are not significantly different (P<0.05) in columns and rows

†Size index, ratio of paired testicular weight to body weight.

Table III. Effect of oral administration of aqueous *Ficus sycomorus* stem-bark extract for 30 days on testicular length and mid-circumference in albino rats.

Dose (mg/kg)	Testicular parameters (cm)	Days of treatment		
		10	20	30
0	Length:			
	Right	1.3±0.4 ^a	1.7±0.4 ^a	1.9±0.3 ^a
	Left	1.3±0.5 ^a	1.8±0.4 ^a	2.0±0.4 ^a
	Mid-circumference:			
	Right	2.6±0.6 ^a	2.6±1.0 ^a	3.5±0.4 ^a
	Left	2.7±0.6 ^a	2.7±1.0 ^a	3.6±0.3 ^a
200	Length:			
	Right	1.9±0.7 ^a	2.1±0.4 ^a	2.1±0.4 ^a
	Left	2.0±0.3 ^a	2.2±0.4 ^a	2.2±0.5 ^a
	Mid-circumference:			
	Right	3.0±0.7 ^a	3.3±0.5 ^a	3.5±1.0 ^a
	Left	3.1±0.7 ^a	3.3±0.5 ^a	3.5±1.0 ^a
400	Length:			
	Right	1.7±0.5 ^a	2.3±0.7 ^a	2.5±0.6 ^a
	Left	1.8±0.5 ^a	2.3±0.8 ^a	2.6±0.7 ^a
	Mid-circumference:			
	Right	3.0±0.5 ^a	3.2±0.7 ^a	3.3±0.8 ^a
	Left	3.1±0.4 ^a	3.3±0.8 ^a	3.4±1.0 ^a
600	Length:			
	Right	2.1±0.5 ^a	2.3±0.6 ^a	2.4±0.6 ^a
	Left	2.1±0.5 ^a	2.4±0.6 ^a	2.4±0.5 ^a
	Mid-circumference:			
	Right	2.9±0.4 ^a	3.3±0.5 ^a	3.4±0.1 ^a
	Left	2.9±0.5 ^a	3.4±0.5 ^a	3.5±0.3 ^a

^a Means±standard deviations of each parameter with same superscripts are not significantly different (P>0.05).

DISCUSSION

Aqueous extracts of *Ficus sycomorus* stem-bark, roots, leaves and fruits have been reportedly used to treat infertility and sterility in humans and animals in parts of Africa (Pakia & Cooke; Kone & Atindehou). The oral administration of aqueous extract of *Ficus sycomorus* stem-bark at 200-600mg/kg to male albino rats for 30 days did not affect body weights, scrotal diameter and testicular size parameters. The increasing body weights of the growing rats correlated with their testicular size parameters and scrotal diameters (Igbokwe, 2010), but the means of the testicular size parameters marginally rose over the period, with neither significant changes in their means nor linear trend. Mean scrotal diameter varied significantly without the testicular size parameters in the growing rats, indicating that the

scrotum housing the testes was affected more by growth changes than testicular size parameters. It was expected that any effect of the extract administration would influence the scrotal diameter more than the testicular size parameters, having shown that the scrotal diameters directly correlated with testicular size parameters (Igbokwe). Testicular weights are relatively stable and vary minimally within a given species and change in testicular size parameter is an outcome of a significant alteration in structure of the testis (Collins *et al.*, 2006).

The morphology of the testes of the rats was not adversely affected by the treatment, an observation that corroborated the lack of decrease in the testicular size

parameters. This indicated that the extract had little or no toxicity for the testes. Several plant extracts that caused histological damage of the seminiferous tubules invariably elicited decline in testicular size parameters and were accompanied by reduced function of the testes (Lohiya *et al.*; Raji & Bolarinwa; Ratnasooriya & Dharmasiri; Akpatah *et al.*; Khouri & El-Akawi, 2005; Raji *et al.*, 2005; Raji *et al.*, 2006; Oyeyimi *et al.*). On the other hand, extract of *Lepidium meyenii* (Maca) increased sperm production without change in testicular size (Chung *et al.*, 2005). At the present dose of administration of *Ficus sycomorus* extract, the seminiferous tubular epithelium of the testis was not depleted and there was evidence of active spermatogenesis. The extract increased testicular and epididymal sperm cell counts on day 30 of the treatment (Igbokwe), suggesting a pro-spermatogenic effect on the spermatogenic stages of the germinal epithelium. Thus, those who use decoctions of *Ficus sycomorus* stem bark at

200-600 mg/kg for treatment of low sperm count may be exposed to minimal reproductive risk.

In conclusion, the oral administration of aqueous extract of *Ficus sycomorus* stem bark did not affect testicular size parameters and the related scrotal diameters of rats, because it caused no obvious histopathological change in the epithelium of the seminiferous tubules.

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IGBOKWE, N. A.; IGBOKWE, I. O. & SANDABE, U. K. Efecto de la administración oral prolongada de extracto acuoso de la corteza de *Ficus sycomorus* en el tamaño testicular de Ratas Albina en crecimiento. *Int. J. Morphol.*, 28(4):1315-1322, 2010.

RESUMEN: La decocción de la corteza del tallo de *Ficus sycomorus* se usa para tratar la infertilidad relacionada al bajo conteo de espermatozoides, pero es incierto el riesgo reproductivo asociado con su uso. El extracto acuoso de la corteza se administró, en ratas albinas, por vía oral, en una dosis de 200-600 mg/kg, durante 30 días post-crecimiento puberal. Durante el período, fueron controlados los siguientes parámetros: peso corporal *in vivo*, diámetro escrotal y tamaño testicular. El promedio de peso corporal *in vivo* y diámetro escrotal de las ratas control y tratadas, tuvieron un incremento significativo ($p < 0,05$) en los animales, pero el tratamiento no afectó significativamente ($p > 0,05$) esos parámetros. No hubo variaciones significativas ($p > 0,05$) en la media de los parámetros tamaño testicular en las ratas tratadas y control, ni tampoco se produjeron efectos dependientes de la dosis, ni en función del tiempo. Las secciones de los testículos de los animales tratados mostraron una espermatogénesis activa a nivel de los túbulos seminíferos, sin lesiones histológicas aparentes. Se concluye, que el uso del extracto crudo de *Ficus sycomorus*, dentro de una dosis de 200-600 mg/kg, presenta mínimo riesgo en la reproducción masculina, sin embargo, se hace necesario estudiar su efecto pro-espermático.

PALABRAS CLAVE: Extracto de *Ficus sycomorus*; Tamaño testicular; Histopatología testicular; Espermatogénesis; Riesgo reproductivo macho.

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