

ANTISPERMATOGENIC ACTIVITY OF *Morinda morindoides* ROOT BARK EXTRACT IN MALE WISTAR RATS

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ABSTRACT

To investigate the effect of the aqueous extract of the root bark of *Morinda morindoides* (Rubiaceae) on the reproductive activity of male Wistar rats, three groups (A, B and C) of six rats each were treated with 400,800 and 1600 mg/kg/day of *Morinda morindoides* root bark extract respectively for 28 days while 5mls/kg of distilled water was given to the control group D. At the end of the experimental period, animals were sacrificed and sperm characteristics, histology of the testes and epididymis were assessed. *Morinda morindoides* root bark extract caused a significant reduction ($p < 0.05$) in sperm motility, a significant dose dependent reduction in the sperm count and a significant ($p < 0.05$) dose dependent increase in morphological abnormalities of the spermatozoa of the treated rats. Histopathological evaluation of the testis and epididymis revealed varying degrees of degeneration and necrosis of the germinal epithelia cell of the seminiferous tubules and spermiostasis. *Morinda morindoides* root bark extract has significant anti-spermatogenic effects on adult male Wistar rats which could impair reproductive activities in these male Wistar rats.

Keywords: extract, *Morinda morindoides*, rats, sperm.

INTRODUCTION

Morinda morindoides (Rubiaceae) is a plant with a high reputation in the traditional management of malaria, diarrhoea, amoebiasis, haemorrhoids, gonorrhoea and rheumatic pains (Kambu, 1990). Biological studies have supported some of these traditional uses (Tona *et al.*, 1999, 2001; Cimanga *et al.*, 2003, 2006). Previous phytochemical studies

on the leaves of this plant led to the isolation of nine flavonoids and flavonoid O-glycosides (Cimanga *et al.*, 1995). The structure determination of eight iridoid glycosides was also reported (Cimanga *et al.*, 2003).

There have been various reports on the anti-spermatogenic and antifertility effect of antimalarial agents. Chloroquine, an antima-

larial drug, has been discovered to have negative effects on sperm motility and fertility as a whole (Okanlawon *et al.*, 1993); quinine is also known to inhibit spermatogenesis (Osinubi *et al.*, 2004). Furthermore, pyrimethamine was observed to arrest spermatogenesis and cause infertility in a dose-dependent manner. Cessation of administration of the aforementioned drugs resulted in full restoration to normal fertility status (Awoniyi *et al.*, 1993; Consentino *et al.*, 1990).

Many medicinal plants have also been reported to have antispermatogenic effects. *Alstonia boonei*, a tropical plant, reputed in traditional medicine to have anti-malarial, antipyretic, analgesic and anti-inflammatory properties (Ojewole, 1984, Olajide *et al.*, 2000) was reported to cause dose dependent changes in the body weight, organ weights and sperm characteristics in male rats. *Azadirachta indica*, another medicinal plant with very potent antiplasmodial activities in mice, has also been reported to cause mass atrophy of spermatogenic elements and Leydig cells (Gbile, 1986).

There has not been any documented report on the effect of the root bark extract of *Morinda morindoides* on sperm parameters. This study was therefore carried out to investigate the effects of the administration of aqueous extract of *M. morindoides* root bark on the spermogram of male Wistar rats.

MATERIALS AND METHODS

Animals

Twenty four adult male Wistar rats (Mean body weight- 180.0 ± 3.33 g) were used for this study. The rats were housed in the Experimental Animal Unit of the College of Veterinary Medicine, University of Agriculture, Abeokuta, Ogun State, Nigeria. They

were kept in well-ventilated metal cages at ambient temperature and a period of 12 hour light and 12 hour darkness was maintained. The rats were fed standard ration (Vital Feeds Limited, Ibadan) and clean water *ad-libitum*.

Plant material

The root bark of *Morinda morindoides* was purchased from Kuto Market in Abeokuta, Ogun State. Identification and authentication was done at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria.

Plant extraction

The root bark of *Morinda morindoides* was air dried, pulverized, finely sieved and 500g of the plant was soaked in 1 litre of distilled water for 24 hours after which it was filtered. Thereafter, the filtrate was evaporated to dryness and 1g of it was dissolved in 20mls of distilled water to give a concentration of 50mg/ml.

Experimental procedure

The rats were randomly divided into four groups (A-D) of 6 rats each. The rats in groups A, B and C were given aqueous extract of *Morinda morindoides* root bark at 400mg/kg, 800mg/kg and 1600mg/kg body weight respectively once daily orally for 28 days. Group D rats were the control to which 5mls/kg distilled water was administered orally once daily for 28 days. Thereafter, the rats were euthanized by placing them in a glass chamber containing cotton wool soaked in diethyl ether till they lost consciousness followed by cervical dislocation. A ventral midline abdominal incision was then made using a scalpel blade size 14 to expose the abdominal organs. The testis and epididymis of each rat were identified, carefully removed and a small incision was made on the caudal epididymis to squeeze out the

semen content on a glass slide for semen evaluation. The testis and epididymis were thereafter preserved in Bouins fluid for histopathological evaluation. (Fig I & II).

Statistical Analysis

The mean and standard error of mean were calculated for all the sperm parameters. Analysis of variance (ANOVA) was used to establish any significant difference in all the stated parameters. $p < 0.05$ was considered significant in all cases.

RESULTS

There was a significant decrease ($p < 0.05$) in the progressive sperm motility and percentage sperm live/dead ratio of the male Wistar rats treated with the aqueous extract *Morinda morindoides* root bark when compared with the control (Table 1). In addition, sperm count of the Wistar rats following oral administration of 400mg/kg, 800mg/kg and 1600mg/kg doses of the aqueous extract of *M. morindoides* root bark was significantly reduced in a dose-dependent manner when compared with the control (Table 1).

At lower doses (400mg/kg and 800mg/kg), there was no significant change in the total number of abnormal spermatozoa in the treated groups compared with the control. However, at 1600mg/kg, there was a significant increase ($p < 0.05$) in abnormal spermatozoa such as headless tail, curved tail and curved mid-piece when compared with the control group (Table 2).

Histopathology of the testis and epididymis of rats administered different doses of aqueous extract of *Morinda morindoides* root bark revealed varying degrees of degeneration and necrosis of the germinal epithelia cell of the seminiferous tubules and spermiostasis

DISCUSSION

Administration of the aqueous extract of the root bark of *M. morindoides* caused a reduction in progressive sperm motility, percentage sperm live/dead ratio and sperm count. Some secondary morphological sperm abnormalities such as headless tail, bent tail and curved mid-piece were higher in the treated rats.

M. morindoides has been reported to have a wide safety margin. It was observed that oral administration of the extract of *M. morindoides* to rats up to 6000mg/kg neither showed mortality nor any apparent signs of weakness in the animals (Shoba & Thomas, 2001). Doses ranging from 100mg/kg to 6000mg/kg had been used in clinical trials and in this study, three doses; 400, 800 and 1600mg/kg were tested 400mg/kg is mostly used by herbal practitioners and this dose was used and its multiples.

Sperm motility depends on the coordinated propagated flagella wave under acetylcholinesterase control (Nelson, 1972). Fructose utilization and glucose oxidation are important means by which spermatozoa derive energy for their motility. The reduction in the progressive sperm motility of the treated rats seen in this study could be due to the acetylcholinesterase inhibition and glucose lowering properties of the specie of this plant (Olajide *et al.*, 1999).

The significant decrease in the sperm count supported by the various degrees of degeneration in the histologic sections of the testis and epididymis, suggests that *M. morindoides* root bark extract is capable of permeating the blood-testis barrier (Baldessarini, 1980).

Since several studies have reported the anti-steroidogenesis and fertility in the male (Adeeko & Dada, 1998).
 result is in consonance with previous studies. Chloroquine, quinine and quinacrine have been reported to inhibit Leydig cell
Cimanga, R.K., Kambu, K., Tona, L., Hermans, N., Apers, S., Totte, J., Pieters,

Table 1: Effects of *Morinda Morindoides* root bark extract on sperm characteristics of male wistar rats

Groups	Sperm motility	Sperm live/dead ratio	Sperm volume	Sperm count
Group A	22.50+13.20*	86.3+2.40*	5.15+0.0	45.75+5.10*
Group B	47.50+6.34*	90.00+2.0*	5.13+0.0	40.75+1.10*
Group C	12.50+7.50*	70.00+5.80*	5.18+0.0	35.50+2.40*
Group D (Control)	93.75+1.30	98.00+0.00	5.18.+ 0.0	129.00+ 5.00

Values are mean \pm standard error of mean

*p<0.05.

Table 2: Effects of *Morinda Morindoides* roots bark extract on sperm morphology of male wistar rats

Groups	Headless tail	Tailless head	Rudi-mentary tail	Bent tail	Curved tail	Bent Mid Piece	Curved Mid Piece	Looped Tail	Coiled tail	Total abnormalities
Group A	166.00+48.4	249.50+27.1	13.50+4.8	41017.3+23.65*	3903.8+273.6*	4422.5+57.0	3871.5+263.6	11.0+5.8	13.50+4.8	402.5+2.5
Group B	239.00+27.3	163.75+47.6	16.00+4.9	4629.00+235.5	3822.3+288.5*	4544.8+226.9	4567.00+220.3*	13.50+4.8	11.00+4.1	400.00+0.0
Group C	340.0+27.0*	239.25+27.3*	16.25+4.7	5822.5+255.5*	4433.00+57.7*	4827.5+286.7	5347.3+25.3*	18.50+2.5	8.50+2.5	402.50+1.4
Group D (Control)	116.25+54.9	68.75+47.5	8.50+4.8	2492.00+279.0	2767.00+29.0	3494.2+240.0	2696.25+300.66	13.50+4.8	6.00+2.9	403.75+2.4

Values are mean \pm standard error of mean

*p<0.05.

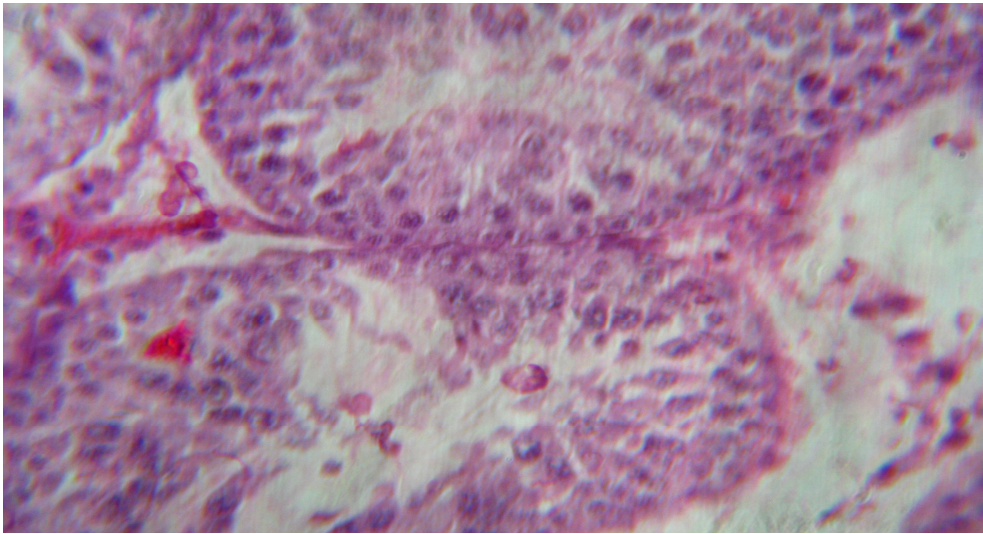


Figure 1: Section of testis with degeneration and necrosis of the germinal epithelial cells of the seminiferous tubules with spermiostasis. X250

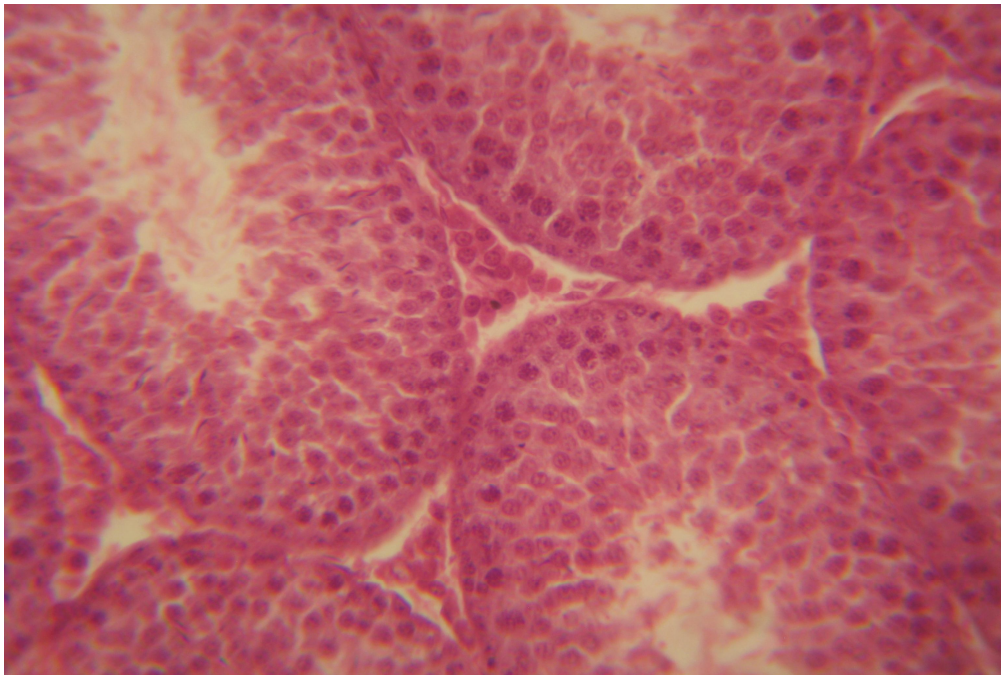


Figure 2: Section of testis with degeneration and necrosis of the germinal epithelial cells of the seminiferous tubules with spermiostasis. H&EX300

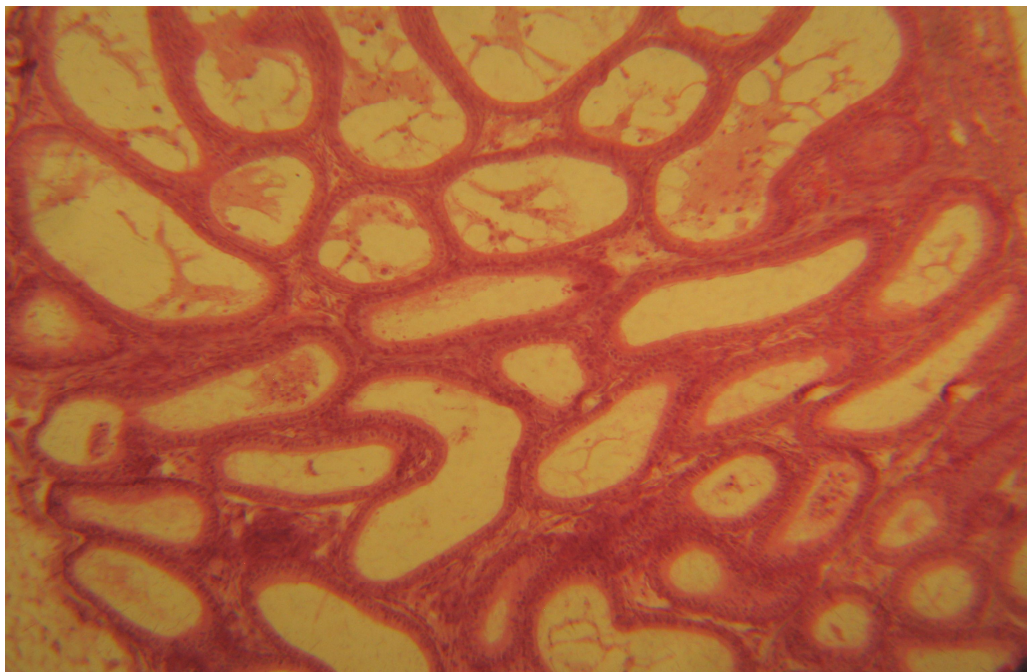


Figure 3: Section of the epididymis that revealed that the epididymis is empty and contains few spermatogonia cells (severe spermiostasis) with no sperm produced at all. X250

CONCLUSION

In conclusion, this study shows that daily oral administration of 400-1600mg/kg body weight of *Morinda morindoides* root bark extract for a period of 28 days has significant anti-spermatogenic effects on adult male Wistar rats as seen with most other anti-malarial herbs. Further studies aimed at elucidating the activities of *Morinda morindoides* root bark extract would be worthwhile.

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