Antispermatogenic activity of *Morinda lucida* extract in male rats

Yinusa Raji¹, Olumide S. Akinsomisoye², Toyin M. Salman¹

¹Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria
²Department of Physiological Sciences, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria

Abstract

**Aim:** To investigate the effect of *Morinda lucida* Benth (Rubiaceae) on the reproductive activity of male albino rats.

**Methods:** Two groups of rats were treated with 400 mg/(kg·d) of *Morinda lucida* leaf extract for 4 and 13 weeks, respectively. The control rats received the vehicle. All the treated rats had corresponding recovery groups. At the end of each experimental period, animals were killed and organ weights, sperm characteristics, serum testosterone levels, histology of the testes and fertility were assessed.

**Results:** *Morinda lucida* leaf extract did not cause any changes in body and somatic organ weights, but significantly increased the testis weight (*P* < 0.05). The sperm motility and viability, and the epididymal sperm counts of rats treated for 13 weeks were significantly reduced (*P* < 0.05). Sperm morphological abnormalities and serum testosterone levels were significantly increased (*P* < 0.05). There were various degrees of damage to the seminiferous tubules. The extract reduced the fertility of the treated rats by reducing the litter size. Reversal of these changes, however, occurred after a period of time. **Conclusion:** The extract of *Morinda lucida* has reversible antispermatogenic properties. (Asian J Androl 2005 Dec; 7: 405–410)

Keywords: *Morinda lucida*; sperm; fertility; testosterone; antispermatogenic agents

1 Introduction

*Morinda lucida* Benth (Rubiaceae) is a medium-sized tree about 15 m tall and is widely used as a medicinal plant in West Africa, especially in Nigeria. The leaves are used as an ingredient of “fever teas”, which are usually taken for the traditional treatment of malaria. The plant is also used as a general febrifuge, analgesic and laxative. A weak decoction of the stem bark is used for the treatment of severe jaundice [1]. The major constituents of *Morinda lucida* extract are various types of alkaloids-anthraquinones and anthraquinols. Adewunmi and Adesogan [2] isolated and characterized two compounds, oruwalol and oruwal, and ten anthraquinones from the stem of the plant.

Anthraquinones isolated from *Morinda lucida* have shown to possess potent *in vitro* activity against *Plasmodium falciparum* [3]. Trypanocidal activity of the methanol extract of the leaf of this plant was reported by Asuzu and Chineme [4]. Obih et al. [5] reported the antimalarial activity of *Morinda lucida* against *Plasmodium berghei berghei* in mice. Many antimalarial agents have been demonstrated to have various degrees of anti-fertility activities [6, 7]. The use of *Morinda lucida* ex-
tracts in the treatment of various ailments is increasing, but its impacts on the reproductive system have not been reported. The present study was to investigate the effects of *Morinda lucida* leaf extract on male rat reproductive functions, such as sperm characteristics, fertility and testosterone secretion.

## 2 Materials and methods

### 2.1 Plant material and extraction procedure

The leaves of *Morinda lucida* were obtained in March 2002 from a tree beside the Pharmacognosy Laboratory, University of Ibadan. The plant specimen was authenticated at the herbarium of the Forestry Research Institute of Nigeria. The leaves were air-dried and then grounded into powder that was subjected to Soxhlet cold extraction using methanol as the solvent.

### 2.2 Animals

Adult male Wistar strain albino rats (200 g – 300 g), purchased from the Central Animal House, College of Medicine, University of Ibadan, were used for the study. The animals were housed in wire mesh cages under standard environmental conditions with the provision of 12 h light and 12 h darkness. Rat cubes (Ladokun feeds, Nig. Ltd., Ibadan, Nigeria) and water were provided *ad libitum*.

### 2.3 Experimental protocol

A total of 30 male albino rats were divided into six groups of five rats each, with two treated, two recovery and two control groups. The two treated groups received intragastric (i.g.) administration of 400 mg/(kg·d) for 4 and 13 weeks, respectively. The corresponding recovery groups were also treated for 4 and 13 weeks and allowed to recover for 4 and 13 weeks, respectively. The choice of duration of extract administration for 4 weeks was to mimic the use of the plant in traditional treatment of malaria while that of 13 weeks was intended to cover the spermatogenic cycles in rats. The control groups received the equivalent volume of the vehicle (Tween 80 in equivalent amount of normal saline). The animals were weighed daily throughout the duration of the study.

### 2.4 Autopsy and organ weights

At the end of the treatment and recovery periods, each rat was anesthetized with 25% urethane at a dose of 0.6 mL/100 g (intraperitoneally, i.p.). The testes, seminal vesicles and epididymis were dissected and weighed immediately.

### 2.5 Blood sample collection

Blood was collected from each rat via the left ventricular cardiac puncture. Serum for the estimation of testosterone levels was obtained from the blood.

### 2.6 Sperm characteristic analysis

The testes from each rat were carefully exposed and one of them was removed together with its epididymis. The epididymis was separated and the epididymal fluid was collected from the caudal part. The progressive sperm motility, sperm count, live/dead ratio (viability) and morphology were determined [8, 9].

### 2.7 Testicular histology

The testes of the rats were prefixed in Bouin-Hollandé solution prior to the histologic studies as earlier described [6, 10].

### 2.8 Hormone assay

Serum testosterone assay was carried out using the tube-based enzyme immunoassay (EIA) method [11]. This is a standardized method used by WHO and part of its program for human reproduction research. The EIA testosterone kits were produced by Immunometrics (London, UK) and obtained from Nzemat (Lagos, Nigeria). The procedures for the assay as contained in the manufacturer’s manual were strictly followed. The within assay variation was 8.1% and the sensitivity was 0.3 ng/mL. The optical density was read using a spectrophotometer (Jenway, 6300 spectrophotometer, UK) that was sensitive at wavelengths between 492 nm and 550 nm.

### 2.9 Fertility test

Treated male rats were cohabited with untreated proestrous female albino rats at a ratio of 1:2. Co-habitation commenced on the first day of the last week of extract treatment and the first day of the last week of the recovery period. A single time point fertility test for each rat was carried out using the following formula: percentage fertility success is equal to the number of pregnant females divided by the number of mated females multiplied by 100. The litter size of the pregnant rats was also determined at the end of the gestation period.
2.10 Statistical analysis
Data were expressed as mean ± SEM. Statistical significance between the various groups was determined using unpaired t-test and ANOVA [12].

3 Results

3.1 Effect on body and organ weight of male rats
There were no significant changes in the body weight of treated rats compared with the controls after 4 and 13 weeks of treatment. The recovery group also showed an insignificant change in body weight compared with their control counterpart. Administration of 400 mg/(kg·d) of the extract to the rats for 4 and 13 weeks increased their testicular weights compared with the controls (Table 1). There were also no significant changes in the mean weight of the seminal vesicles in the treated groups (Table 1).

3.2 Effect on spermatozoal indices
The effects on sperm motility, counts, viability and morphology were shown in Table 2.

3.2.1 Motility
Oral administration of Morinda lucida leaf extract for 4 and 13 weeks, progressively and significantly reduced \( P < 0.05 \) sperm progressive motility. There was also a significant decrease \( P < 0.05 \) in percentage sperm motility in the recovery groups compared with the respective control groups (Table 2).

3.2.2 Epididymal sperm count
Daily administration of the extract for 13 weeks significantly reduced \( P < 0.01 \) the mean epididymal sperm counts compared with their controls and the recovery groups, which showed an insignificant increase.

<table>
<thead>
<tr>
<th>Dose of Morinda lucida leaf extract</th>
<th>4 weeks</th>
<th>13 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>Testis (g)</td>
<td>Epididymis (g)</td>
</tr>
<tr>
<td>Control</td>
<td>244.00 ± 18.21</td>
<td>1.37 ± 0.06</td>
</tr>
<tr>
<td>400 mg/(kg·d)</td>
<td>244.00 ± 18.21</td>
<td>1.63 ± 0.06</td>
</tr>
<tr>
<td>(recovery)</td>
<td>256.00 ± 12.18</td>
<td>1.69 ± 0.09</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dose of Morinda lucida leaf extract</th>
<th>4 weeks</th>
<th>13 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motility (%)</td>
<td>Counts ( \times 10^6/\text{mL} )</td>
<td>Viability (%)</td>
</tr>
<tr>
<td>Control</td>
<td>86.76 ± 0.92</td>
<td>67.20 ± 0.71</td>
</tr>
<tr>
<td>400 mg/(kg·d)</td>
<td>61.21 ± 4.60</td>
<td>74.80 ± 8.02</td>
</tr>
<tr>
<td>(recovery)</td>
<td>76.80 ± 1.77</td>
<td>60.40 ± 3.23</td>
</tr>
</tbody>
</table>
3.2.3 Viability (live/dead ratio)

There was a significant decrease ($P < 0.05$) in the mean percentage of live sperm of rats treated with the extract compared with their control counterparts. The mean percentage of live sperm of rats in the 4-week recovery group showed a significant reduction ($P < 0.05$).

3.2.4 Morphology

The commonest morphological abnormality of sperm in rats that received the extract for 4 and 13 weeks was the "curved tail" which accounted for over 70% of the abnormalities observed. Although the recovery groups showed a lesser occurrence of morphological abnormalities, the increase in abnormalities were significantly higher ($P < 0.05$) than those in the controls.

3.2.5 Serum testosterone levels

The mean serum testosterone level of rats treated with 400 mg/(kg·d) of the extract for 4 and 13 weeks significantly increased ($P < 0.01$) compared with the controls (Figure 1).

3.2.6 Histologic evaluation

Administration of 400 mg/(kg·d) of the extract for 4

![Figure 2. Light micrograph of testes of rats treated with Morinda lucida extract for 4 and 13 weeks. (A): control; (B): control recovery (no visible lesions in both); (C): rats treated for 4 weeks showing mild degeneration of seminiferous tubules; (D): evidence of regeneration in the corresponding recovery; (E): rats treated for 13 weeks showing severe degeneration of seminiferous tubules; and (F): some degree of regeneration of the tubule in the corresponding recovery. Arrows indicate locations of seminiferous tubules (×40).](image-url)
and 13 weeks caused visible lesions within the seminiferous tubules compared with the controls (Figure 2). There was degeneration and disorganization of the plasmalemma in the basal portion of some of the seminiferous tubules. However, regenerative changes were observed in the recovery group.

3.2.7 Fertility tests

The fertility of the treated rats in terms of percentage mated female rats was unaffected. However, the fertility of these rats was significantly affected by the extract treatments in terms of the number of litters born by the cohabited female rats. Female rats cohabited with treated male rats bore significantly reduced ($P < 0.05$) number of litters: 6.20 ± 0.32 and 4.00 ± 0.27 for 4 and 13 weeks, respectively (control: 9.40 ± 0.44).

4 Discussion

Chronic administration of Morinda lucida leaf extract could impair reproductive activities in male albino rats. The extract caused an increase in the weight of the testes, which was accompanied by an increase in the serum levels of testosterone. Similar changes have been reported with the extract of Zingiber officinale and Pentadiplendra brazzeana in rats [13, 14]. Others have reported testicular weight reduction accompanied by decreased serum testosterone levels in male rats treated with the extracts of Quassia amara [7], Azadirachta indica [6] and gossypol, a phenolic compound extracted from the cotton seed [15]. Both Quassia amara and Azadirachta indica have been demonstrated to possess potent antimalarial properties [6, 7]. Since all organs of male reproduction are androgen dependent, they serve as indicators of the Leydig cell function or androgen action [16].

Testosterone in association with follicle stimulating hormone normally acts on the seminiferous tubules to initiate and maintain spermatogenesis [16]. However, a significant decrease in the epididymal sperm count of rats treated with the extract for 13 weeks was recorded despite the high serum testosterone level. This was supported by the various degrees of degeneration in the histologic sections of the testes, suggesting that Morinda lucida extract administration for a long period was capable of permeating the blood–testis barrier [17]. This probably led to tubular fluid retention, hence non-restoration of testicular weights during recovery. The appreciable increase in the epididymal sperm counts of rats in the recovery groups suggests that the extract could be responsible for the observed decrease in the first instance. This was further corroborated by the fact that no primary abnormalities were recorded in sperm morphology, which normally occurs during the process of spermatogenesis.

Reduction in the progressive epididymal sperm motility of the treated rats could be responsible for the decrease in the average number of litters born by the female rats cohabited with the extract treated male rats leading to the suspicion for the presence of a spermatoxic agent on maturing or mature spermatozoa [18] in the extract. Sperm motility depends on the coordinated propagated flagella wave under acetyl cholinesterase control [19]. Fructose utilization and glucose oxidation are important means by which spermatozoa derive energy for their motility. Morinda lucida has been shown to possess hypoglycemic and anti-hyperglycemic activities [20]. The reduction in motility recorded in this study could be due to the acetylcholinesterase inhibition and glucose lowering properties of this plant. The progressive motility improved in the recovery groups, suggesting that the deleterious effect was reversible.

Phytochemical analyses revealed that Morinda lucida contains various types of anthraquinones and anthranquinols [2]. Koumaglo et al. [3] and Sittie et al. [21] reported the strong activity of anthraquinones isolated from Morinda lucida against the growth of Plasmodium falciparum. Furthermore anthraquinones isolated from Morinda lucida extract have been reported to possess potent activities against the growth of the chloroquine resistant Plasmodium falciparum. Since several studies have reported antifertility effects of antimalarial agents including chloroquine, the antifertility activities exhibited by Morinda lucida in this study could be associated with anthraquinones presented in the extract. Further studies aimed at elucidating the activities of Morinda lucida extract would be worthwhile.

Acknowledgment

This work was partly supported by the University of Ibadan Senate Research grants SRG/COM/2000/11^A to Yinusa Raji and we would, therefore, like to express our gratitude to the University.
References