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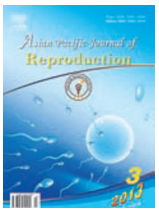

Document heading doi: 10.1016/S2305-0500(13)60141-4

Effects of ethanolic leaf extract of *Spondias mombin* on the pituitary-gonadal axis of female Wistar rats

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ARTICLE INFO

Article history:
Received 3 July 2013
Received in revised form 10 August 2013
Accepted 10 August 2013
Available online 20 September 2013

Keywords:
Blood
Estrogen
FSH
LH
Ovary
Pituitary
Progesterone

ABSTRACT

Objective: To determine the effects of ethanolic leaf extract of *Spondias mombin (S. mombin)* on the histology of the anterior pituitary, ovary and uterus; and on the serum sex hormones of adult female Wistar rats. **Methods:** Twenty-four female Wistar rats were randomly assigned into four groups of six rats per group. The animals in the treatment groups were administered orally ethanolic extract of *S. mombin* leaves at the doses of 250, 350 and 500 mg/kg body weight daily for fourteen days. Rats in the control group received distilled water. The body weights of the rats were determined at the beginning and end of the experiment. Histological analysis of pituitary, ovary and uterus was carried out. Hormonal assay for estrogen, progesterone, FSH and LH were done using Enzyme-Linked Immunoabsorbent Assay (ELISA). **Results:** There was significant ($P<0.05$) decrease in relative organ weights of extract-treated rats compared to the control. Pituitary showed accumulation and aggregation of cells in experimental animals. Uteri of treated groups showed thickening of endometrial lining and presence of cysts, ovarian tissues were damaged. Furthermore, the extract caused reduction in serum concentration of sex hormones of the treated animals relative to the control. **Conclusion:** This study suggests that ethanolic extract of *S. mombin* leaf may have antifertility property, confirming its use as a local contraceptive.

1. Introduction

One of the millennium development goals is to eradicate poverty and hunger; however, this seems to be non-approachable due to population explosion. Increased population growth has been identified as the leading cause of poverty and pollution especially in developing countries [1]. Several methods for induction of infertility to cub human fertility has been investigated [2]. More than half of the world's population rely extensively on traditional medicines for primary health care using plant extracts [3]. In most herbal medicine systems of the world, the leaves of Spondias mombin are widely used for female reproductive tract issues [4]. It is used by midwives to help induce labour, reduce bleeding and pain during and after childbirth and is also used as a vaginal wash to prevent or treat uterine or vaginal infections [5]. The leaves of the plant have been found to have diverse medicinal uses. It is documented to have antiviral abilities [6-7], antimicrobial properties [8-10], anti-malarial [11], anti-epileptic and anxiolytic [12-13], wound healing [14], vitamin C substitute [15], beta-lactamase inhibitor [16] and has haemostatic function [17], abortifacient properties [18]. The antifertility potential of leaf and bark in male rats has been established [19-22].

2. Materials and methods

2.1. Plant material and preparation of the extract

The leaves of *Spondias mombin* (Spm) (*S. mombin*) were procured from a local community in Yakkur, Ugep, Nigeria.

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Taxonomic identification was conducted by Mr Frank Apejoye, Chief herbarium, Department of Botany, University of Calabar, Calabar, Nigeria, with a voucher specimen already existing.

Air-dried leaves of Spm (50 g) were powdered and extracted with 500 mL of 95% ethanol using a soxhlet apparatus. The extract obtained was filtered through Whatmann paper 1 and the filtrate was evaporated to dryness on rotary evaporator (50 ℃). The extract yield was 10% (w/w).

The extracts were preserved in clean glass container for further use.

2.2. Animals

This study was approved by the Departmental Ethics Committee of the University of Calabar, Calabar. Twenty-four virgin female rats with an average weight of 180 g were bred in the animal house of the Department of Anatomy and were used for this study. They were screened and observed to exhibit regular estrous cycle. The rats were maintained on a 12 h light and 12 h dark cycle, provided with pelleted rat chow (Pfizer Nig. Ltd, Lagos, Nigeria) and water *ad libitum*.

2.3. Treatment protocol

The animals were equally divided into four treatment groups (6/group). Group I animals were given distilled water for 14 days and they served as control. Groups II, III and IV animals received ethanolic extract at dose levels of 250, 350 and 500 mg/kg body weight/day for 14 days respectively. The animals were anaesthetized under chloroform at the end of the experiment.

2.4. Hormonal assay

Blood samples were collected by cardiac puncture technique in centrifuge tubes. The blood was allowed to stand for 10 minutes to clot at room temperature and centrifuged at 3 000 r/min for 10 minutes. The serum was then tipped into a separate vial and later subjected by ELISA method for assessment of FSH, LH, estradiol and progesterone.

2.5. Determination of body and reproductive organ weights

Final body weights of the animals were recorded a day after the last dose administration. Pituitary and reproductive organs (uterus and ovary) were excised, cleared of supporting tissues and weighed.

2.6. Histological analysis

The tissues were examined for pathological changes before being fixed in 10% buffered formalin solution. The tissues were dehydrated in graded alcohol, cleared in xylene and embedded in paraffin wax. Sections were cut using a rotary microtome at 5 μm thickness. The sections were stained using haematoxylin and eosin. Photomicrographs were taken.

2.7. Statistical analysis

Data were expressed as Mean ± S.E.M. Statistical analysis was carried out by one-way analysis of variance (ANOVA) with significance expressed as $P< 0.05$.

3. Results

3.1. Body weight and organs relative weights

There were no significant ($P>0.05$) in body weights of the treated rats compared with the control group (Table 1). However, treatment with *S. mombin* leaf extract showed dose-dependent significant ($P<0.05$) decrease in the weights of pituitary, ovaries and uterus of the animals as shown in Table 1.

3.2. Reproductive hormone levels

Significant reduction ($P<0.05$) in the hormonal levels of FSH, LH, estradiol and progesterone in a dose-dependent manner was recorded in leaf extract treated groups compared to the control group (Table 2).

Table 1

Effect of *S. mombin* leaf extract on body, pituitary and reproductive organs weight.

Groups	Body weight (g)	Pituitary (mg)	Ovary (mg)	Uterus (mg)
Control I	206.2±0.2	2.14±0.01	32.8±1.0	320.0±6.5
Group II 250 mg/kg	202.7±0.5	1.40±0.05*	24.6±0.6*	285.5±2.8*
Group III 350 mg/kg	200.5±0.1	1.28±0.10*	22.7±1.4*	242.0±4.5*
Group IV 500 mg/kg	200.0±0.5	0.85±0.05*	20.2±0.6*	210.7±3.2*

n=8. Data represents Mean± S.E.M. * $P<0.05$ comparing with the control group.

Table 2

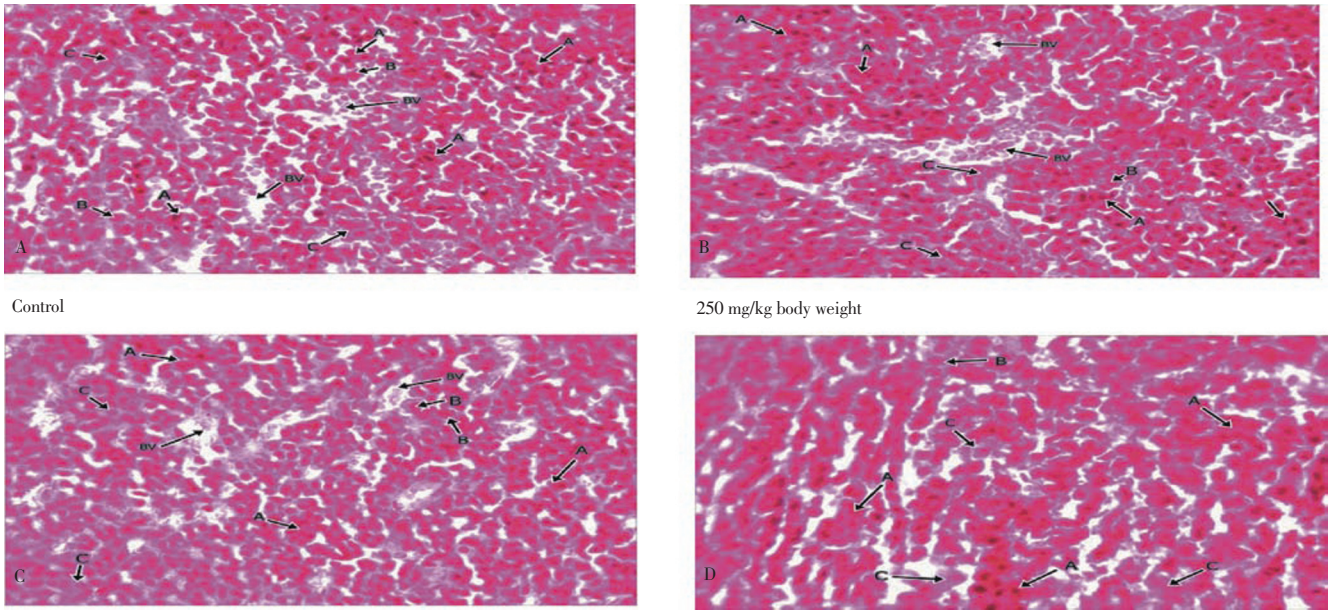
Effect of *S. mombin* leaf extract on the serum levels of reproductive hormones.

Groups	FSH (mlu/mL)	LH (mlu/mL)	Estradiol (pg/mL)	Progesterone (pg/mL)
Control 1	1.58±0.08	0.32±0.04	155.75±1.00	16.94±0.95
Group II 250 mg	1.10±0.01*	0.14±0.06*	92.50±0.12*	8.46±0.74*
Group III 350 mg	0.58±0.07*	0.08±0.01*	83.75±0.04*	7.18±0.38*
Group IV 500 mg	0.26±0.05*	0.02±0.01*	68.33±0.08*	3.21±0.02*

n=8. Data represents Mean± S.E.M. * $P<0.05$ comparing with the control group.

3.3. Histological observations

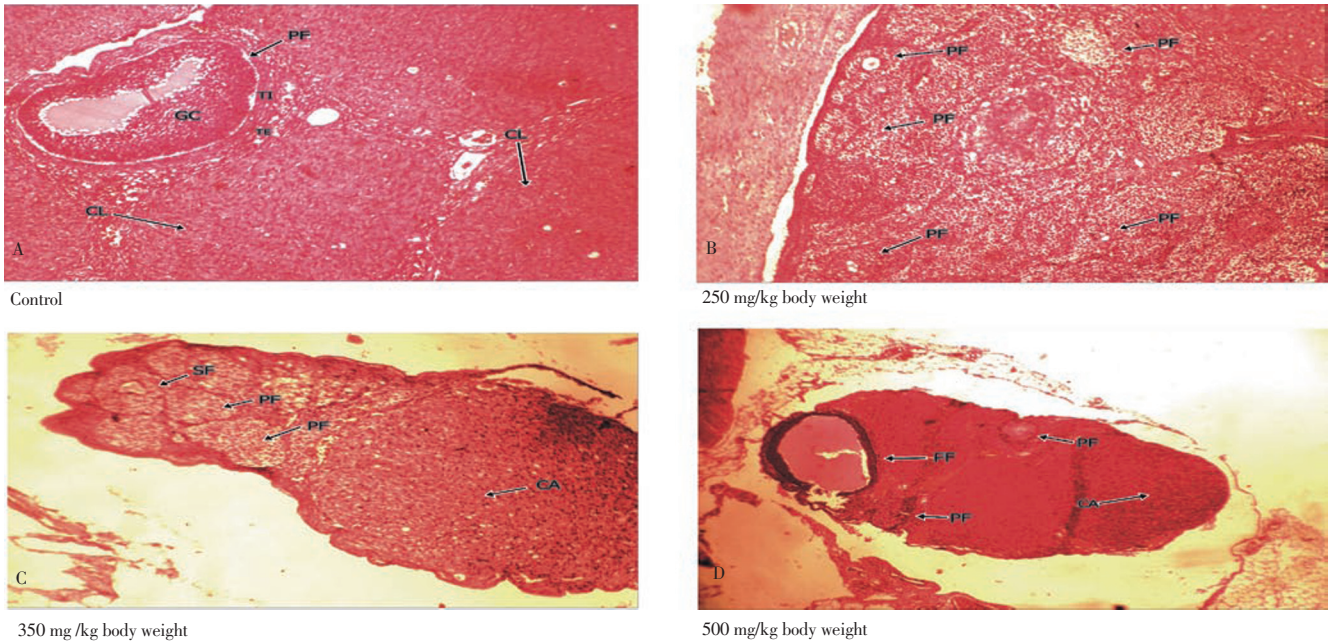
Figure 1 shows the histoarchitecture of the pituitary and gonads of female rats of control and Sp extract treated at doses of 250, 350 and 500 mg/kg body weights for 14 days. The anterior pituitary of control animals showed normal pituitary cells made up of basophils, acidophils and chromophobes (Figure 1A). Treatment with Sp caused hypoplasia of pituitary cells (Figure 1B–D). Control ovary reflected matured graafian follicle, corpus luteum and follicular cells in various stages of development (Figure 2A). However, ovaries of Sp treated animals showed thinning of cell lining, degeneration of follicular cells, atrophied granulose cells and ruptured thecal cells (Figure 2B–D). These degenerative changes were more pronounced in animals administered with 500 mg/kg extract (Figure 2D). Uterine histology revealed distortion of endometrial epithelium of animals treated with leaf extract. Shrunken appearance and reduction in the number of uterine glands were observed (Figure 3B–D) compared to control (Figure 3A).



Control | 250 mg/kg body weight

350 mg /kg body weight | 500 mg/kg body weight

Figure 1. Cross section of pituitary gland of control and extract treated female rats at doses of 250, 350, 500 mg/kg body weights for 14 days. A–Acidophils, B–Basophils, C–Chromophos, BV– Blood vessel.



Control | 250 mg/kg body weight

350 mg /kg body weight | 500 mg/kg body weight

Figure 2. Cross section of the ovary of control and extract treated female rats at doses of 250, 350, 500 mg/kg body weights for 14 days. PF–Primary follicle, SF– Secondary follicle, TI– Theca interna, TE–Theca externa, CL– Corpus luteum.

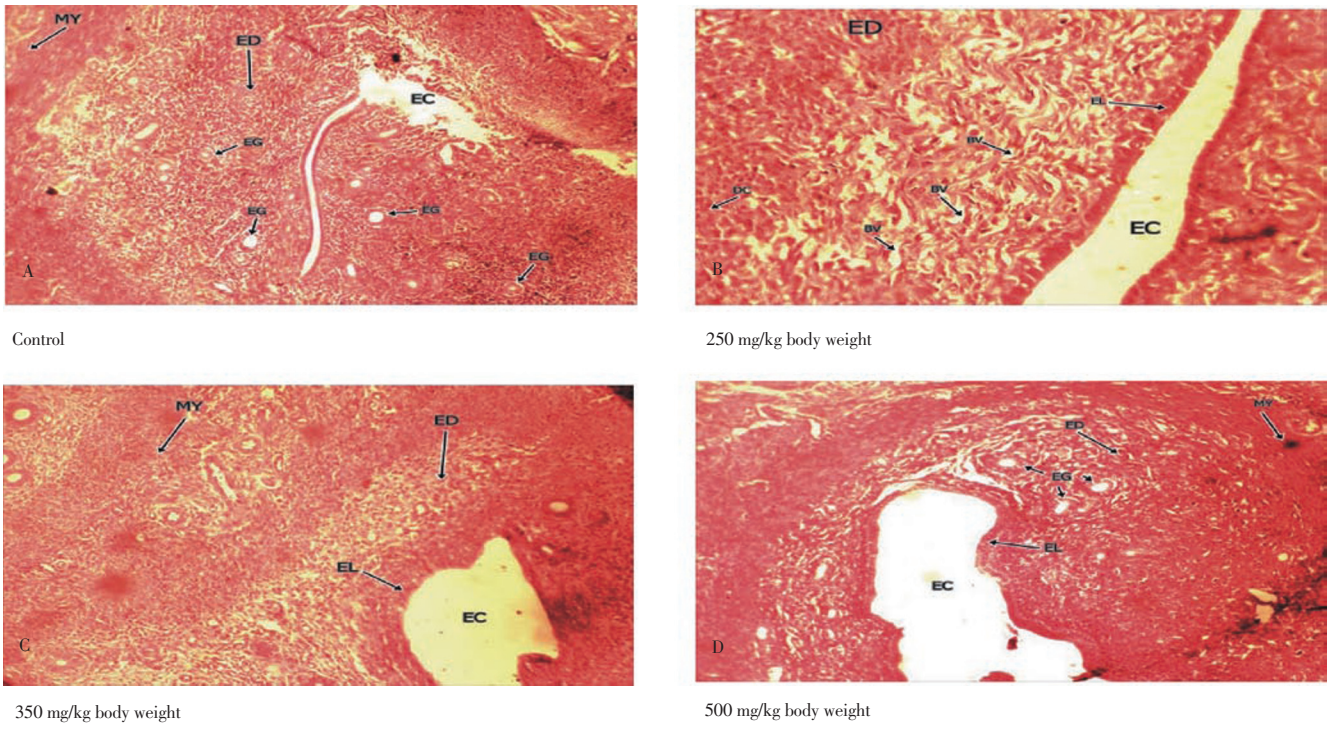


Figure 3. Crross section of the uterus of control and extract treated female rats at doses of 250, 350, 500 mg/kg body weights for 14 days. EC- Endometrial cavity, ED- Endometrium, EL- Endometrial lining, EG- Endometrial gland, BV- Blood vessel, MY-Myometrium.

4. Discussion

The result of this study indicated no significant change in the final body weight of rats after 14 days of oral administration with Sp leaf ethanolic extract at all tested doses. However, a significant reduction in organs weight was observed in all the experimental groups. Since the effect of the plant extract did not alter the body weights of treated rats in comparison with the controls, it is safe to deduce that the Histomorphological changes observed in the pituitaries, ovaries and uteri of the treated female rats may be due to the direct effect of the extract on the organs. Our finding is in line with reports of the effects of plant extracts on female rats' reproductive organs [23-24]. The ruptured thecal cells evidenced in the ovaries of the treated animals may be due to injury to the cell walls by the extract. Administration of *S. mombin* to female rats caused estrogen inhibition which may be due to its antiestrogenic property. The decrease in the weight of the ovary and uterus is indicative of the antiestrogenic nature of the plant since antiestrogenic substances are known to decrease the wet weight of the uterus [25]. Progesterone and estrogen are known as the most vital hormones for the maintenance of pregnancy and implantation of blastocyst in humans and other mammals [25-28]. The decrease in these hormones was dose-dependent; and they are regulated by pituitary secreted gonadotropin hormones, FSH and LH [29]. The reduced levels of serum LH and FSH in treated animals is indicative of the possible effect of the plant extract on the anterior pituitary, since secretion of these gonadotropins are regulated by the gonadotropic releasing hormone released from the hypothalamus. The reduced concentrations of estradiol, progesterone, LH and FSH may be due to the presence of alkaloids in the plant, since alkaloids have been reported to reduce the concentrations of these hormones [30]. This result tallies with the effect of other medicinal plants [31-35]. Biological agents acting on estrogen and progesterone have been reported to inhibit ovarian function [37-38]. Therefore, the effect of *S. mombin* on these hormones may be responsible for the deleterious effect observed in the ovaries of treated animals. Niswender *et al* [39] has shown that LH stimulates ovulation growth of corpus luteum and release of progesterone. LH also acts on the granulose cells to secrete progesterone which stimulates the release of FSH at midcycle [40]. Several studies [41-46] have confirmed the adverse effect of medicinal plants on female reproductive organs.

The plant extract may enact its effect through the pituitary-gonadal axis evidenced by the diminished gonadotropin levels that led to reduced reproductive organ weights and estrogen-progesterone imbalance. It is therefore safe to deduce that the effects brought by *S. mombin* are antiestrogenic and may lead to antifertility.

Declare of interest statement

We declare that we have no conflict of interest.

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