Antifertility activity of aqueous ethanolic leaf extract of *Spondias mombin* (Anacardiaceae) in rats

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Abstract

Back ground: Despite the availability of modern (orthodox) medicine, many developing countries, especially in the rural areas, still rely heavily on traditional healers and medicinal plants to meet their primary health care needs and that of their domestic animals. This has been attributed to easy accessibility and low cost of herbal medicine. In Eastern Nigeria, fresh leaves of *Spondias mombin* is widely used by the natives to aid delivery and to expel the placenta in small ruminants (sheep and goats), especially during difficult labour.

Objective: The present study was designed to evaluate the *in vivo* effects of leaf extracts of *S. mombin* on reproductive performance of female rats.

Methods: Acute toxicity test of the plant extract was carried out in rats of both sexes. The anticonceptive and abortifacient activity of the extract were investigated, including the Fertility Index or embryo score of control and treated animals. The estrogenic activity was determined using ovariectomized rats.

Results: The results revealed a relatively non-toxic plant extract. The extract displayed anticonceptive but not abortifacient activity as judged by the number of pregnant animals at the end of the third trimester of pregnancy. The extract did not exhibit any oestrogenic activity.

Conclusion: Aqueous ethanol leaf extract of *S. mombin* has significant anticonceptive activity attributed to a direct action of the extract on the uterus.

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Introduction

Spondias mombin is a fruitiferous tree that thrives in the rainforest and coastal areas of Africa. It also has a wide distribution in Southern America and the West Indies but grows to a limited extent in the Indian subcontinent and Indonesia[1].

The plant belongs to the family Anacardiaceae. In Nigeria, it is known by various names (Ibo: Ichikara, Hausa: Tsardarmasar, Yoruba: Akika etikan", Iyeye[2]). The tree grows to about 20 m in height and 60 – 75 cm in diameter. The bark is fissured and thick and its lower branches are whorled. It has a decidous, alternate, pinnate leaves (20 – 45 cm long) and hairy pinkish, pointed leaflets which are inequilateral and oblique at the base. The fruits, hanged in numerous branched clusters, are aromatic, ovoid and oblong in shape. The wood is used for making huts, garden poles, axe and hoe handles in tropical Africa. It is also popular for carving amulets, statuettes, cigarette holders and various ornamental objects[1].

All parts of the plant are reported to be medicinally useful and its traditional use in reproduction have been reported. For example, in Peru, decoctions of the bark and/or leaves are used as a 'child birth aid'. It is also used in postpartum infections of the uterus and following an abortion or miscarriage in women. Women that are pregnant or those seeking to be pregnant are usually advised against the use of the leaf infusion / decoction[3]. In South eastern Nigeria, juice extracts from the leaves are used by the natives to induce delivery in small ruminants with parturient complications (dystocia) arising from uterine inertia. It had been shown in earlier studies, that butanolic leaf extract of the plant contracts isolated uterine muscle of the rat in a concentration dependent manner[4]. The *in vivo* effects of aqueous ethanolic leaf extract of *S. mombin* on fertility of female rats is reported in this communication.

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Materials and methods

Plant material collection and identification

Spondias mombin leaves were collected from the medicinal garden of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria in October, 2001. They were certified and authenticated by Mr. A. Ozioko, a taxonomist in the Department of Botany, University of Nigeria, Nsukka, Nigeria where a Herbarium specimen was deposited, with Herbarium number UNH 241(a). The leaves were sun dried and pulverized into fine powder using a hammer mill (Thomas Wiley mill, Model ED-5, London).

Preparation of Extract

About 262 g of the dried sample was subjected to cold extraction initially, with petroleum ether (60Ú - 80Ú) for two days and subsequently with 70% aqueous ethanol for three days, stirring at intervals. The extracts were concentrated to dryness at room temperature to give a final yield of 1.17% and 13.9% for petroleum ether and aqueous ethanol extracts respectively. The petroleum ether extract was powdery and greenish in colour. However, it was the sticky/gummy and dark brown aqueous ethanol extract that was used for the present bioassay experiments since it was found to contract the isolated uterine muscle of the rat[4]. Given weights (Mettler Toledo Balance, Switzerland) of the extract were reconstituted in appropriate volume of aqueous ethanol and the appropriate volumes administered to the animals based on their body weights as indicated in the respective subheadings.

Experimental animals

Except for the experiment on oestrogenic activity, sexually matured Wistar rats weighing between 150 and 200 g were used for the study. The animals were purchased from a breeder at the University of Nigeria, Nsukka and housed in the Laboratory Animal Section of the Department of Veterinary Obstetrics and Reproductive Diseases, University of Nigeria, Nsukka. They were provided commercial standard ration (Top Feeds Nig Ltd) containing 24 % crude protein *ad libitum*. Water was provided freely throughout the period of the study.

Acute Toxicity Test

Rats of both sexes were used for the acute toxicity test. They were randomly divided into three groups of five rats each. Animals in group 1 received a single dose of the extract (500 mg/kg) intraperitoneally. Groups 2 and 3 were similarly treated with 1 g/kg and 2 g/kg of the extract respectively. All the animals were allowed access to feed and water and observed for treatment related abnormalities and death over a period of 24 hours.

Anticonceptive / abortifacient activities

In the experiment to determine the abortifacient and/or anticonceptive effect of the extract, a total of fifteen sexually matured female animals were used. Each female was paired with a male rat. A second female rat was introduced into the cage only when mating with the first female rat had been confirmed. Successful mating was ascertained by the presence of a copulatory (vaginal) plug on the floor of the cage the next morning and/or the presence of sperm cells in fresh vaginal smear made on clean microscope slide and observed under the x10 magnification of wide angle eyepiece of the light microscope. Following mating, the female rat was separated from the male and the day copulatory plug and/or sperm cells were found designated Day 1 of pregnancy.

Thereafter, the pregnant animals were randomly assigned to three groups of 5 rats each as follows:

Group 1 received 800 mg/kg of the extract dissolved in 70% aqueous ethanol intraperitoneally for 4 days consecutively starting from the first day of pregnancy. This was regarded as the anticonceptive group.

Animals in group 2 were given the extract (800 mg/kg) intraperitoneally from the 8^{th} to the 11^{th} day of pregnancy. This group was regarded as the abortifacient group

Group 3 were given equivalent volume of 70% aqueous ethanol as in group 1 but without the extract. This group served as the control. All the animals were given unrestricted access to feed and water and closely observed throughout the duration of gestation. The body weights were also recorded on daily basis.

On the 20^{th} day of pregnancy, the animals were sacrificed by stunning and decapitation and the following parameters evaluated according to Wong et al.,[5] (a) Percentage of pregnant females per group (PPF) (b) mean live foetal number (LFN) (c) mean corpus luteum number (d) mean resorbed embryo number per pregnant female (REN) (d) mean day 20 foetal crown-rump length (FC-RL)). The Fertility Index (embryo score) was deduced from the equation:

FI (Fertility Index) =LFN x FC-RL x PPF / CLN

Oestrogenic activity

For studies on oestrogenic activity of the plant extract, 20 sexually immature female rats were used. The animals were prepared for ovariectomy, using intraperitoneal pentobarbital sodium (6%) at the dose of 60 mg/kg to induce anaesthesia. The uterine horns were exteriorized and the ovaries identified and excised via a laparotomy incision. The incision sites were routinely closed.

Fifteen days after ovariectomy, the animals were divided into 4 groups as follows:

Group1 was administered 0.1 mg/kg body weight of stilboestrol suspension in paraffin oil subcutaneously for 4 days. This group served as positive control. Animals in group 2 received the extract at the concentration of 500 mg/kg subcutaneously for 4 days while animals in group 3 were given the same volume of paraffin oil as group 1 but without stilboestrol. This group served as the negative control. Five other rats

which were neither ovariectomized nor treated with the extract or standard drug were kept with animals in groups 1 to 3. These were regarded as normal 'intact' rats

On the 5th day, the body weights of the animals were recorded. They were subsequently sacrificed by stunning and decapitation. The uterine horns were removed after a laparotomy incision, blotted on a filter paper and weighed on a digital balance (Mettler Toledo). The respective uterine weights were then expressed as percentage of the body weight of each rat.

Statistical analysis

Statistical analyses were carried out using Analysis of variance (ANOVA) and the means separated using Duncan's New Multiple Range Test. Data are presented as the mean ± standard error of the mean (SEM).

Results

Acute toxicity test

Apart from occasional clustering of the rats at one corner of the cage, the result of the acute toxicity test showed no lethal or any other treatment-related effect of aqueous ethanolic leaf extract of *Spondias mombin* in all the treatment groups. As a result, the LD_{50} was not taken.

Anticonceptive/abortifacient activity

The effects of intraperitoneal administration of 70% aqueous ethanol leaf extract of *S. mombin* on mated female rats are presented in Table 1. Only two out of the five animals administered the leaf extract for four consecutive days after mating were found to be pregnant when autopsied at the 20th day of pregnancy. The number of pregnant animals in this group represented 40% of the control group where all the rats (100%) were found with foetuses attached to the endometrium. The body weight of the animals increased generally as the experiment progressed but was lower in animals treated with the extract at the beginning of gestation (199.30 ± 13.06) than in the control group (226.20 ± 15.49). All the animals that received the extract at the beginning of the second trimester had live foetuses in their uterine horns. The body weight of this group (220.50 ± 13.63) was comparable to that of the untreated control group that received paraffin oil vehicle.

Table 1: Antifertility activity of aqueous ethanol leaf extract of *Spondias mombin* (800 mg/Kg) in rats. Results are the mean ± standard error of the mean (SEM) with number of animals used in each group indicated in parenthesis.

Gestation (Days)	No Pregnant /No treated	Antifertility % Pregnant	Activity % of Control	Body weight (g)
1 – 4 (5)	2/5	40	40	199.30 ± 13.06
8 – 11 (5)	5/5	100	100	220.50 ± 13.63
Control				
1 – 4 (5)	5/5	100	100	226.20 ± 15.49

Effect on uterine content

The effect of the extract on uterine content is presented in Table 2. The mean day 20 foetal crown-rump length (FC-RL) as well as the mean corpus luteum number (CLN) did not differ significantly ($P>0.05$) between the groups. Similarly, no evidence of foetal resorption was observed as judged by the mean areas of foetal attachment vis-à-vis the number of live foetuses found. However, the fertility index was much lower in the anticonceptive group (125.7) compared with the other two groups (319.0 and 324.0 respectively).

In general, more foetuses were carried in the right uterine horn than in the left irrespective of the group (data not shown).

Oestrogenic activity

Immature ovariectomized rats displayed juvenile uterine horns at autopsy which were smaller in size than that of normal 'intact' control group.

Ovariectomized rats administered the plant extract had the least uterine ratio of 0.985 ± 0.164 which differed significantly ($P< 0.05$) from the uterine ratio of the 'intact'(1.718 ± 0.350) and positive control (1.868 ± 0.231) groups.

Table 2: Effect of aqueous leaf extract of Spondias mombin (800 mg/kg) on fertility index (embryo score) of female rats. Results are the mean ± standard error of the mean (SEM). Number of animals used in each group is indicated in parenthesis. LFN = Live Foetal Number, REN = Resorbed Embryo Number, FC-RL = Foetal Crown – Rump Length, CLN= Corpus Luteum Number

Gestation (days)	Body weight (g)	LFN	REN	FC-RL (cm)	CLN	Fertility Index
1 – 4 (5)	199.30 ± 13.06	*4.11± 0.51	0.0	3.31± 0.08	4.33 ± 0.51	125.7
8 – 11 (5)	220.50 ± 13.63	4.10 ± 0.55	0.0	3.19 ± 0.15	4.10 ± 0.35	319.0
Control						
1 – 4 (5)	226.20 ± 15.49	4.01 ± 0.69	0.0	3.24 ± 0.10	4.01 ± 0.12	324.0

*Values obtained only from pregnant animals in this group.

Table 3: Effect of aqueous ethanol leaf extract of *Spondias mombin* (500 mg /kg) on the uterine ratio of ovariectomized (OVD) and 'intact' female rats. Results are the mean ± standard error of the mean (SEM). Number of animals used in each group is indicated in parenthesis. Stilboestrol (Stb) was administered at the dose of 0.1 mg/kg.

Group	Body weight (g)	Weight of Uterus (mg)	Uterine ratio
S. mombin (5)	100.38 ± 2.61	99.0 ± 9.05	*0.985 ± 0.164
Control			
OVD (4)	109.30 ± 6.01	151.75 ± 28.32	1.358 ± 0.343
OVD + Stb (4)	121.50 ± 5.52	228.50 ± 23.63	1.868 ± 0.231
'Intact' (5)	102.20 ± 4.18	174.40 ± 18.41	1.718 ± 0.350

*P< 0.05 compared with the 'intact' and positive control (OVD + Stb) groups.

Discussion

In the present study, it was found that rats administered aqueous ethanolic leaf extract of *Spondias mombin* intraperitoneally occasionally clustered at one corner of the cage. A similar observation had been made in an earlier study by Ayoka et al[6] although with lethal effects at a much higher concentration of the extract (3.2 g/kg) and over a two day observation period.

Administration of the extract (800 mg/kg) at the immediate postcoitus period (1 – 4 days) resulted in failure of 3 out of the 5 rats used in this group to conceive. At autopsy, no observable gross morphological defects were detected on the endometrium nor evidence of early embryo resorption found in the non-pregnant rats. We attribute failure of the animals to conceive to a direct effect of the extract on uterine cum oviductal muscle function which may have caused accelerated transport of the blastocyst at this period. In addition to the prostaglandins ($PGF_{2\dot{\alpha}}$), a number of agonists including endothelin[7] and angiotensin II[8] which can modulate oviductal muscle contraction have been found to influence the transport of preimplantation embryos through the ampulla and isthmus of the oviduct. Such accelerated movement could result in early arrival of the embryo to a non receptive uterus, and failure of the blastocysts to implant as reported in the rat[9] and women[10].

A synchronized development of the conceptus and differentiation of the uterus to a receptive state (defined as a hormonally regulated period at which the uterine milieu is conducive to embryo acceptance and implantation) are considered essential to the implantation process. Our previous *in vitro* experiments showed that butanolic leaf extract of *S mombin* contracts uterine muscle strips of the rat in a dose-related manner[4] thus lending support to accelerated blastocyst transport as the anticonceptive mechanism proposed in the present study. This may explain why women seeking to be pregnant are advised against the use of the leaf infusion/decoction in Peru[3], presumably due to this potential anticonceptive effect. The low conception rate was reflected in the wide disparity in the fertility index (embryo score) between the anticonceptive group (125.7) and the untreated control (324)(Table 2). The study also revealed that the plant extract was relatively non embryotoxic as judged by the data on foetal crown-rump length (FC-RL) (Table 2) and the absence of any observable, treatment related morphologic defects in the live foetuses.

The conception rate of animals treated with the extract (800 mg/kg) on days 8-11 of gestation compared well with that of the control group (Table 1). The body weights similarly increased as the pregnancy

progressed confirming the continuous growth and development of uterine contents. Offiah and Anyanwu[11] had earlier reported abortion of foetuses in rats and mice given aqueous leaf extract of *S. mombin* at all stages of gestation except the first trimester. The reason for our inability to observe any abortion at the 8 – 11 day trial period is uncertain but may be related to the presence of an increasing plasma progesterone levels at this period[12,13] which may have blunted the effect of the extract on the myometrial cells, especially at a time when the process of embryo implantation had already commenced in this specie[14]. The study on estrogenic activity revealed uterine ratio in the following order: ovariectomized, extract (500 mg/kg) treated < ovariectomized negative control < normal 'intact' control < ovariectomized positive control (treated with stilboestrol, 0.1 mg/kg) The increase in uterine ratio of the latter group (i.e. positive control) relative to others is not surprising. The uterus exhibits signs of increased blood flow following estrogen administration in ovariectomized rats and rabbits and may increase in weight due to the accumulation of extracellular fluid[13].

Estrogen also initiates cellular alterations (in terms of cell size) in the endometrium and in the activity of the smooth muscle of the uterus[15]. These may explain the relative increase in uterine ratio in the estradiol treated group than in others, and the higher uterine ratio in the 'intact' control than in the ovariectomized, the primary source of estrogen (ovary) having been removed in the latter group. Apart from the increased water content and hypertrophy of the tissue, the increase in weight may also be due to increase in number of cells present (hyperplasia).

In conclusion, the work reported herein has shown that aqueous ethanol leaf extract of *Spondias mombin* administered intraperitoneally possesses anticonceptive activity. The potential of this medicinal plant as a useful source of anticonceptive agent in the species warrants further investigation.

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