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Original Research Article

Effect of methanolic extract of *Spondias mombin* on oestrous cycle, conception rate and gestation in rabbits

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ABSTRACT

Effect of *Spondias mombin* (SM) on oestrous cycle, conception and pregnancy was evaluated in 25 sexually mature Chinchilla rabbit does (Mean weight: 1.94 ± 1.06kg). In phase one, fifteen rabbits were synchronized with FSH (2 mg/kg i.m.) 12 hourly for three days. They were randomly assigned into three groups of five rabbits each. Group 1 and 2 received 1ml saline solution and 800mg/kg of methanolic extract of SM (meSM) orally for thirty days respectively. Group 3 received a single treatment of melengesterol acetate (MGA; 50mg/kg i.m.). Blood was obtained from the jugular vein at ten days interval for thirty days to determine plasma concentration of LH, FSH, estrogen and progesterone. Thereafter, the does were mated and laparotomised was carried out one week after to determine the number of embryonal sacs. In phase two, ten does were mated and ultrasonographically confirmed to be pregnant seven days post-mating. They were treated with 800mg/kg meSM orally at ten and twenty days post-mating. The does were observed daily until they kidded or aborted. Data obtained were analysed using student's T-test and ANOVA, with significance set at $P \leq 0.05$. Mean values of LH and Progesterone significantly decreased in Group 3 and 2 compared to control group while FSH was lower significantly in Group 3 compared to other groups. The effect of meSM on oestrous cycle was not so obvious in this study. Administration of meSM 10 days post-mating caused abortion in only one doe and caused no abortion at 20 days post-mating. It was concluded that meSM did not adversely affect reproductive cyclicity and pregnancy but has potential to cause abortion during early pregnancy in Chinchilla rabbits. Further studies where hormonal assays are done more frequently than this study to be able to determine the effect of meSM on oestrous cycle are suggested.

Keywords: Chinchilla, contraceptive, hormonal, rabbit, *Spondias mombin*, ultrasound.

INTRODUCTION

Targeted adjustment of the reproductive system is an important requirement for high performance in animal breeding. This is achieved by regulation of the hormonal control of various aspect of reproduction (Asuquo *et al*., 2012). This regulation is done through the use of several synthetic hormones. However, synthetic hormones can adversely alter the morphology of reproductive organs resulting in infertility or accumulate in the tissues of the animal thus affecting their reproductive quality (Asuquo *et al*., 2012). Therefore, increased interest has been paid to some plant-derived chemicals that can influence endocrine activities in both humans and animals due to their possible beneficial and sometimes adverse effects. Some of these plants possess fertility and anti-fertility effects through their actions on hypothalamo-gonadal axis or hypothalamo-pituitary-gonadal axis or through direct hormonal effects on reproductive organs (Ayoka *et al*., 2008).

Spondias mombin L. (Anacardiaceae) is a delicious erect tree, which grows to 15-20 m tall with buttressed, thick and coarse trunk (Baracaldo, 2013). *Spondias mombin* L. is traditionally known for the treatment of a variety of disease conditions. Its bark, leaves, roots and fruits are used in various ways. *Spondias mombin* leaves are among the forages usually fed to domestic animals in South Eastern Nigeria. The young leaves are also cooked as green vegetables for human consumption (Bliedtner *et al*., 2009). The leaves are used in the treatment of bacterial infections, the prevention and inhibition of the progression of viral infections, treatment of candida infections and expelling intestinal parasites such as intestinal worms. The bark is reported to reduce inflammation, relieve pain, reduce

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spasms, kill fungi, kill bacteria, heal rashes, heal wound and stop bleeding. Extracts of *Spondias mombin* have also been reported to have both contraceptive and abortifacient effects (Kistanova *et al*., 2005; Boiti *et al*., 2006).

The reproductive effect of *Spondias mombin* was thought to be due to its inhibitory effect on pituitary gonadotropins (Ola-Davies *et al*., 2014). Thus *Spondias mombin* can be a potential candidate for the development of contraceptives for use in humans and companion animals. However, several trials are still required to further understand the mechanism of action of *Spondias mombin* as a contraceptive plant. This study was therefore aimed at determining the effect of *Spondias mombin* on conception, pregnancy and reproductive hormones in rabbits. Also, the effect of *Spondias mombin* was compared with melengesterol acetate which is a known oral contraceptive in humans.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS AND THEIR MANAGEMENT

Twenty five sexually mature female chinchilla rabbits were used for the study. The average body weight of the does was 1.94 ± 1.06 kg. Prior to the arrival of the animals, the house was disinfected thoroughly; drinkers and feeders were cleaned and disinfected properly. The animals were purchased from a breeder at the Federal College of Education, Osiele, Abeokuta. They were quarantined for 15 days during which they were dewormed with a broad spectrum anti-helminthic, ivermetin (Ivomec®, Kepro, Holland) at the rate of 25mg/kg. The rabbits were given Terramycin (LA, Skm Pharm PVT; India) at a prophylactic dose of 1ml/4kg by intramuscular injection to treat any subclinical bacterial or protozoan infections. The animals were housed in individual cages and commercial standard ration containing 24 % crude protein and water was provided ad libitum throughout the period of the study.

Ethical approval (FUNAAB/COLVET/CREC/2018/07/04) for this study was obtained from the Research and Ethics Committee, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

PREPARATION OF METHANOLIC EXTRACT OF *SPONDIAS MOMBIN*

Spondias mombin leaves were sun dried and pulverized into fine powder using a hammer mill. About 860g of the dried sample was soaked in methanol for three days. The extracts were concentrated to dryness at room temperature to give a final yield of 200g of methanolic extracts of *Spondias mombin*. The extract was reconstituted using normal saline and kept in an air-tight container at room temperature until it was needed.

EXPERIMENTAL DESIGN AND PROCEDURE

The study was divided into two phases. In phase one, effect of *Spondias mombin* on oestrous cycle and conception in rabbits were evaluated while the effect of *Spondias mombin* on gestation in rabbits phase was studied in phase.

PHASE I

This was carried out as a simple randomized controlled study comprising of three groups (saline, methanolic extract of *Spondias mombin* (meSM) and melengesterol acetate (MGA) groups). In phase one study, fifteen female rabbits were used. All animals were screened for pregnancy before the commencement of the experiment. They were synchronized with intramuscular injection of Follicle Stimulating Hormone (FSH) (Receptal, Intervet, Salamanca, Spain). Three days after, they were randomly assigned into three groups of five rabbits each. Group 1 (control group) received 1ml of normal saline orally for 30 days. Groups 2 received 800mg/kg methanolic extract of *Spondias mombin* (meSM) as described by Ayoka *et al*. (2008) orally for 30 days. Group 3 received a single intramuscular injection of melengesterol acetate (MGA).

Blood was obtained from the jugular vein immediately after assigning them into the groups and then at ten days interval for duration of thirty days. Thereafter, the rabbits were mated naturally with matured chinchilla proven bucks. One week after mating, they were subjected to laparotomy to determine the number of embryo as well as number of ovulation site.

PHASE II

In phase two, ten rabbits were synchronized and were mated three days after with proven bucks. They were confirmed pregnant seven days post mating using portable ultrasound machine. Thereafter, the rabbits were randomly assigned into two groups. Group one were treated with 800mg/kg methanolic extract of *Spondias mombin* (meSM) orally starting from ten days post mating for thirty days until the rabbits either aborted or kindled, while group two were treated with 800mg/kg methanolic extract of meSM orally beginning from twenty days post mating for thirty days until the rabbits aborted or kindled. Observations were also made daily on the number of rabbits that aborted and the number of bunnies produced.

BLOOD SAMPLING

Three mls of blood were obtained from the jugular vein immediately after the end of synchronization with Follicle Stimulating Hormone (FSH) and at ten days interval for 30 days for the determination of plasma concentration of Luteinizing Hormone (LH), Follicle Stimulating (FSH) Hormone, progesterone and estrogen.

HORMONAL ASSAY (LUTEINIZING HORMONE, FOLLICLE STIMULATING HORMONE, PROGESTERONE AND ESTROGEN)

Plasma concentrations of Luteinizing (LH), Follicle Stimulating Hormone (FSH), progesterone and estrogen were carried out using Enzyme linked Immunosorbent assay (ELISA, MyBioSource, San Diego, USA) as described in previous study (Oloye *et al*., 2013).

LAPAROTOMY

Fifteen rabbits from control, meSM and MGA groups underwent laparotomy to examine the state of the reproductive tract at eight days post - mating and the effect of the meSM and MGA on the reproductive tract. Sedation was induced by intramuscular administration of a combination of xylazine and Ketamine (3.5mg/kg). The left and right lateral abdominal walls were shaved. Rabbits were positioned in the lateral recumbency position. They were anesthetized with lidocaine. Lack of muscle tone and loss of ear pinch reflex were used as indicators of anaesthetic depth. The surgical area was prepared for aseptic surgery with multiple swabs of savlon solution and was draped using sterile technique. A lateral flank approach to each ovary was used. A vertical skin incision (2-3 cm long) was made 2cm cranial to the anticipated site of muscle incision so that the sutured skin wound would not directly overlie the translocated ovary. The muscle incision was done approximately half between the last rib and the cranial border of the thigh. The ovary was exteriorized gently, then cut off from the respective sides and immediately fixed into a solution containing normal saline before being observed under a stereo microscope. The muscle layers beyond the trans fixation points were closed with simple continuous sutures of 2.0 catgut. The surgical procedure was performed without intra – or immediate post – operative complications. Visual method using a stereo microscope was used in counting the ovulation sites, number of ovulated and unovulated follicles on the ovary.

STATISTICAL ANALYSIS

Data obtained from these studies were analysed using student's T-test and ANOVA, with significance set at P=0.05 using Statistical Analysis System (SAS Institute, 2000).

RESULTS

The plasma concentration of LH was significantly lower ($P< 0.05$) in MGA-treated rabbits than the control and meSM-treated rabbits by day 20 post treatment (Figure I). Oral administration of meSM in the rabbit's significantly increase ($p < 0.05$) the plasma concentration of LH by day 20 (Fig. I) and thereafter significantly decreased by day 30 post administration. In the MGA treated rabbits, the plasma concentration of LH significantly decreased ($p > 0.05$) by day 20 post administration and thereafter tended to increase (Fig. I). Figure II shows the effect of meSM and MGA on plasma concentration of FSH in rabbits on day 0, 10, 20 and 30 post administrations. There was no significant ($p>0.05$) effect of meSM on plasma FSH concentration in rabbits in day 0, 10, 20 and 30. The plasma concentration of FSH significantly ($p < 0.05$) decreased by day 20 post administration in MGA treated rabbits and thereafter increased by day 30 post administration. The plasma concentration of FSH was significantly ($p < 0.05$) lower in MGA treated rabbits compared with the control and meSM treated rabbit does (Fig. II). Figure III shows the effect of meSM on plasma of Progesterone concentration in rabbits. There was no significant ($p>0.05$) effect of meSM on plasma Progesterone concentration in rabbits on day 0, 20 and 30. However, there were significant ($p< 0.05$) effect of MESM on plasma Progesterone concentration in rabbits on day 10. Treatment had significant effect ($p< 0.05$) on plasma concentration of Progesterone in rabbits on day 10. Figure 4 shows the effect of meSM on Plasma Estrogen concentration in rabbits on day 0, 10, 20 and 30. The plasma estrogen concentration significantly ($p < 0.05$) decreased in both MGA and meSM treated rabbits up to day 20 post administration. In each of the groups, the plasma estrogen concentration tended to increase by day 30. However, plasma concentration of estrogen was significantly higher in the control and meSM – treated rabbits than the MGA – treated rabbits (Fig. IV). Figure 5 shows the effect of meSM on the number of embryonal sac in rabbit does following mating. Treatments had significant ($P>0.01$) effect on number of embryos in the ovaries of the rabbit does. The MGA – treated rabbits did not have embryonal sac while both the meSM and control treated rabbits had embryonal sacs. There was no significant difference in the number of embryonal sac in both the control and meSM – treated rabbit does (Figure V).

Following the oral administration of methanolic extract of *Spondias mombin* by day 10 post mating, all the rabbits were found to have vaginal bleeding (Table 1) about 24 hours after. Abdominal ultrasound (Plate I) by day 14 post mating revealed four out of five rabbit does still have embryonal sac while one did not show any evidence of pregnancy. The four rabbits later kidded with a mean litter size of 3.25 kits (Table 1). Rabbit does that were treated with methanolic extract of *Spondias mombin* leaves at 20 days post mating did not show any vaginal bleeding. All the five were confirmed pregnant following abdominal ultrasound at day 21post mating with an average litter size of 6.4 kits (Table I).

DISCUSSION

The result of this study showed that oral administration of meSM in female rabbits did not adversely affect the plasma concentrations of reproductive gonadotropins, estrogen and

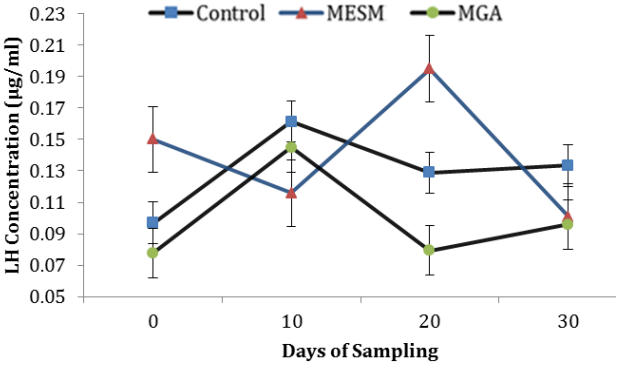

Figure I: Effect of methanolic extract of *Spondias mombin (MESM)* and melengesterol acetate (MGA) on plasma concentration of Luteinizing Hormone in rabbits on day 0, 10, 20 and 30

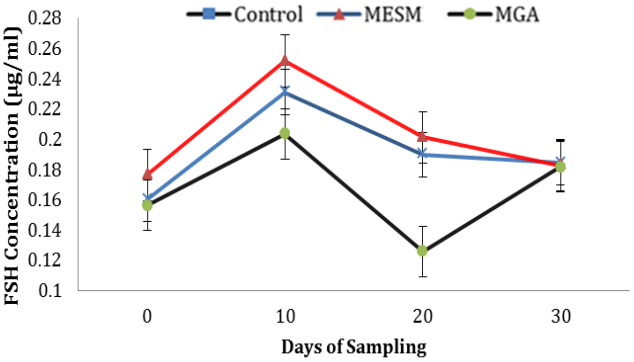

Figure II: Effect of methanolic extract of *Spondias mombin* (MESM) and melengesterol acetate (MGA) on plasma concentration of Follicle stimulating hormone in rabbits on day 0, 10, 20 and 30

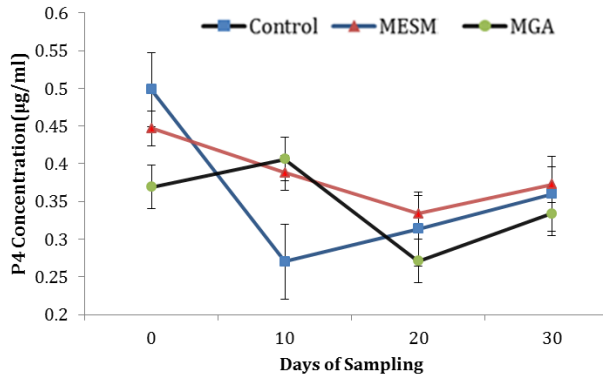

Figure III: Effect of methanolic extract of *Spondias mombin* (MESM) and melengesterol acetate (MGA) on plasma concentration of Progesterone in rabbits on day 0, 10, 20 and 30

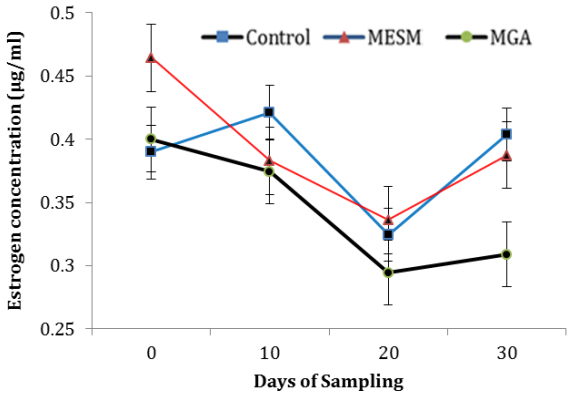

Figure IV: Effect of methanolic extract of *Spondias mombin* (MESM) and melengesterol acetate (MGA) on plasma concentration of Estrogen in rabbits on day 0, 10, 20 and 30

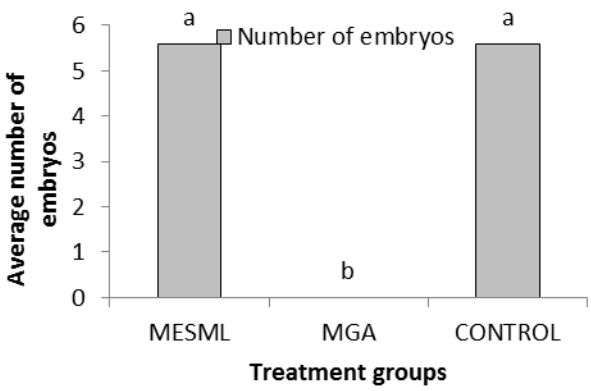

Figure V: Comparison between the numbers of embryos in methanolic extract of *Spondias mombin*, melengesterol acetate, and control groups

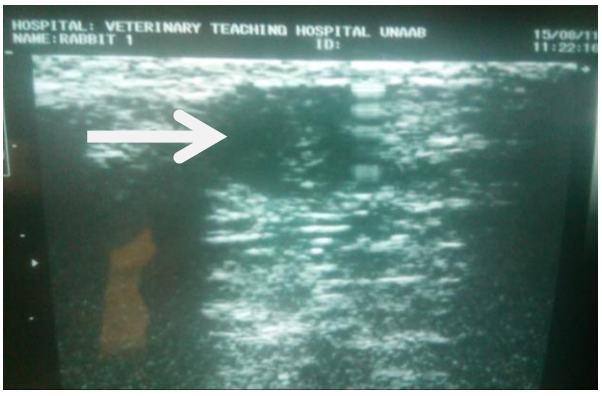

Plate I: Transcutaneous abdominal ultrasound in a rabbit doe showing presence of gestational sac (arrows)

Table 1: Effect of methanolic extract of *Spondias mombin* leaves during pregnancy in rabbit does

Events	Treatment 10 days post mating	Treatment 20 days post Mating
Bleeding	Occurred 24 hours after treatment	No bleeding was noticed
Number of complete abortion	1	0
Number of rabbits that kindled	4/5	5/5

progesterone which are associated with the oestrous cycle, neither did it affect conception rate. In addition, administration of meSM at day 10 of gestation resulted in vaginal bleeding and partial abortion, while administration at day 20 of gestation did not affect gestation in the rabbits.

Rabbit does do not have a well - defined oestrous cycle because they are induced ovulators (Dalbosco *et al.,* 2011). They grow waves of follicle that continuously develop to the antral stage under the influence of follicle stimulating hormone (Shibeshil *et al*., 2006). The presence of large antral ovarian follicles increase plasma estrogen concentration which initiates sexual receptivity in rabbit does and this last for several days (Uchendu & Choudhary, 2004). The follicular waves regress at about approximately 7 – 10 days interval. In this study, two phases of oestrous cycle can be depicted from the pattern of the hormonal changes demonstrated by the rabbits. The first phase which appeared to last for ten days is characterized by gradual rise in follicle stimulating hormone (FSH), Luteinizing hormone (LH) and estrogen with a gradual decline in the plasma concentration of progesterone. This phase will appear as the follicular phase and may coincide with the period of receptivity as described by Boiti *et al*. (2006) and Baracaldo (2013). This phase was followed by a second phase characterized by a decline in FSH, LH and estrogen and a gradual increase in the plasma progesterone level. It thus appears that the oestrous cycle in rabbit does although not well defined last approximately about twenty days before another waves of follicle resumes.

The result of this study showed that meSM produced a gradual rise in the plasma concentration of FSH with a concomitant drop in the plasma concentration of LH, estrogen and progesterone. Previous studies have shown that oral administration of *Spondias mombin* (SM) appears to favour folliculogenesis in rabbits (Boiti *et al*., 2006). This might explain the gradual increase in the plasma FSH concentration observed in the first ten days of treatment in the rabbits. In addition to this, oral administration of meSM also resulted in a gradual decline in the plasma concentration of estrogen and LH. Although one would have expected that the plasma concentration of estrogen should increase concomitantly with the follicular wave in other to initiate receptivity in the does, the opposite was observed in the plasma concentration of estrogen. It may be that meSM have adverse effect on the growing follicles, thus negatively influencing the secretion of estrogen. There are actually contrasting reports about the effect of SM in rabbits on reproductive hormones in rabbits. Oloye *et al*. (2013) reported no significant change in the levels of FSH, LH and estrogen in rabbits treated with SM and suggested that the plant does not interfere with oestrous cycle however Asuquo *et al*. (2012), reported that treatment with SM in rats resulted in the regression of pituitary FSH and LH. This thus necessitates the further work should be done to establish the true effect of SM in female animals. The poorly defined pattern of oestrous cycle in rabbits makes it difficult to conclude on the exact effect of SM in female rabbits.

Conception is defined as the formation of viable zygote by the union of males and females ovum followed by the implantation of the zygote in the uterine horn. Pre and post implantation loses have been reported in rabbits following mating or artificial insemination (Uchendu & Isek, 2008). There is presence of maximum number of ova that can become fertilized or implanted, and this varies according to the strains of rabbits and may bear a relationship to the litter size of the strain. Laparotomy was performed in the rabbit does to determine the numbers of implanted embryo owing to the possibility of post implantation loses and early foetal resorption.

In this study rabbits treated with MGA did not have any embryonal sac while both the control and meSM treated rabbits had an average of 6.4 embryonal sacs. This finding showed that meSM did not interfere with conception in rabbits and probably does not have any contraceptive role in rabbit does. This finding is also supported by Oloye (2013) who reported that rabbits treated with oral extract of SM produced an average litter of 5.7 kits. The absence of embryonic sac in rabbits treated with MGA further confirms the contraceptive effect of the drug. MGA is thought to inhibit the secretion of pituitary gonadotropins and prevents follicular maturation and ovulation (Boddener *et al.,* 2005). This might explain the lower plasma levels of LH and FSH in the MGA treated rabbits in this study. Finally oral administration of meSM resulted in partial abortion in rabbit does by day 10 of gestation, but did not cause abortion when administered at day 20 of gestation. This is contrary to early reports by Oloye *et al*. (2013) who observed abortion by day 23 and 24 of gestation. The exact reason for abortion in the first trimester of pregnancy is not known because the hormonal changes were not adequately monitored. However,

it may be due to the oxytocic properties of the saponins in the SM leaves (Yeonju *et al*., 2012). Further studies where hormonal assays could be done every day or every alternate day are suggested to be able to monitor the trend in the hormonal changes during oestrous cycle and conception.

CONCLUSION

In conclusion meSM did not influence the oestrous cycle nor does affect conception in rabbit. However, it resulted in partial abortion during the first trimester of pregnancy.

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