ISSN 0974 – 5211

Journal of Natural Products

Volume 6 (2013)

www.JournalofNaturalProducts.Com

Research Paper

Hormonal variation in gravid does after oral treatment with crude ethanol extract of *Spondias mombin*

A.A. Oloye[1*], M.O. Oyeyemi[2], O.E. Ola-Davies[3], S.A. Olurode[1], A.R. Ajayi[1]

[1]Department of Veterinary Public Health and Reproduction, College of Veterinary Medicine, Federal University of Agriculture Abeokuta

[2]Department of Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine, University of Ibadan

[3]Department of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan

* Corresponding Author

(Received 10 November 2012; Revised 14 - 27 November 2012; Accepted 30 November 2012)

ABSTRACT

Twelve mixed breed gravid rabbits weighing between 4.5-6.0kg body weights were randomly selected to study the effect of oral treatment with leaf ethanol crude extract of *Spondias mombin* on sex steroid hormones. Six were administered 800mg/kg of the extract while six were administered distilled water once every day for 7 days at day 14 of gestation. Blood samples were collected before commencement of treatment and 7 days post treatment in both groups. This was repeated 24 hours after parturition in the control group only. Sera from blood samples were analyzed for sex hormones concentration using ELISA. Results revealed that mean progesterone concentration significantly increased in the treatment group from pre-treatment value of 5.4±0.30ng/ml to post treatment value of 9.5±0.30ng/ml compared to significant decrease from 5.1±0.20ng/ml to 2.5±0.50ng/ml in the control group (P<0.05). All other hormones had no significant changes in both groups comparing pre and post treatment except FSH which significantly reduced from 3.1±0.08mIU/ml to 2.2±0.10mIU/ml in control group. Abortion was observed in the treatment group on days 22 and 23 of gestation, between 24 and 48 hours post treatment while all the rabbits in control group kindled a mean value of 5.7±0.68 kits at a mean gestation period of 30.3±0.33days. The study concluded that crude ethanol extract of *Spondias mombin* has abortifacient tendency by manipulation of steroids important in gestation.

Keywords: Hormone; *Spondias mombin;* Rabbit.

INTRODUCTION

Use of plant products in medicine has gained ground over the years. Many derivatives from naturally occurring compounds are available and many more still in different stages of development (Aiyeloja, 2006). One of such plant which is commonly used by local folks for several purposes is *Spondias mombin* (Family-*Anacardiacae*), this is found in the tropical clime of the world. In Nigeria, the fresh leaves are used by natives to aid delivery during difficult labor and expulsion of retained placenta in

small ruminants (Uchendu and Isek, 2008). In humans, it helps in labor induction, pain relief and control of hemorrhage during and after childbirth in addition to bringing on the flow of breast milk, and usage as vaginal wash to prevent or treat uterine or vaginal infections after childbirth (Taylor, 2006). It is also used as an abortifacient (Offiah and Anyanwu, 1989). Among its various chemical constitutions is the saponin, which is known to have an oxytocitic property, a property believed to be responsible for the plant's abortifacient tendency. (Yeonju Lee, et al, 2012).

This work was aimed at studying the interplay of reproductive hormones in reaction to the effect of oral administration of leaf ethanol extract of *Spondia mombin* in gravid does. The choice of ethanolic medium and 800mg/kg dosage was informed by the work done by Uchendu and Isek (2008) and Offiah and Anyanwu (1989). Uchendu and Isek (2008), working with 800mg/kg aqueous ethanolic extract of *Spondias mombin* leaves reported possible abortifacient effect of the extract going by the low pregnancy percentage in treated albino rats that were used. Offiah and Anyanwu (1989) also reported fetus expulsion in all the mice and albino rats administered 750 and 1500mg/kg of aqueous extract at all stages of pregnancy except the first trimester. No one has reported effect of *Spondias mombin* in gravid rabbits.

MATERIALS AND METHODS

***Plant extract*:** Collection of *Spondias mombin* leaves was done at the Federal University of Agriculture Abeokuta in the month of April, 2012. Identification was done at Federal University of Agriculture and confirmed with voucher number PSC No Ib/2012/1470007841 at the Nigerian Agricultural Quarantine Service. 3.4kg of the powdered leaves were soaked in hexane to reduce the fat. Air dried residue was treated with ethanol for 3 days. Resultant filtrate was then concentrated with the use of rota-evaporator. Dark brownish paste recovered was kept in fume hood to solidify for five days at 25°C. A yield of 60g of dried extract was obtained from which a stock solution of 8000mg of extract in 1ml of propylene glycol was constituted.

Dose was calculated using the formula:

Dose = Weight x Dosage/Concentration

- Weight of gravid rabbit (kg)
- Dosage is the weight of extract administered per kg body weight.
- Concentration is weight of extract dissolved in one ml of solvent.

***Experimental* animals:** Twelve gravid mixed breed does weighing between 4.5-6.0kg body weight at day 14 of gestation were utilized. They were diagnosed through abdominal palpation at day 11 of gestation after successful mating. Mating is considered successful when the male is seen mounting the doe, accomplish the rabbit mating, and then fall off the doe with a grunt thumping the cage floor thereafter (Raising-rabbit.com, 2012). Pregnant does were kept in standard hutches and fed formulated feed. Water was given *ad libitum*.

***Experimental design*:** The twelve rabbits were divided into treated group and control group of six rabbits each. Pre-treatment blood samples were collected carefully from the ear vein for hormonal profiling at day 14 of gestation. Ethanol leaf extract was administered orally at dosage rate of 800mg/kg (Offiah and Anyanwu, 1989; Uchendu and Isek, 2008) once daily for seven days to the treated group while the control animals received distilled water at the same period and for the same length of time. Blood samples were collected from both groups again after the last day of treatment for hormonal assay. This was repeated 24 hours after parturition in the control group.

Guiding principles in the use of animals in toxicological studies laid down in July 1989 by Society of Toxicology was adhered to having received approval from

the College Board of the College of Veterinary Medicine, Federal University of Agriculture Abeokuta. Enzyme- linked Immunosorbent Assay (ELISA) was utilised for assay using hormonal kits from Biorex diagnostics (ISO 13485). Batches were: BXEO86OA (Estradiol); BXEO671A (Prolactin); BXEO661A (Progesterone); BXEO631A (FSH); BXE0651A (LH).

***Statistical analysis*:** Data was analyzed into descriptive statistics using Graghpad Prism 6. Means were computed together with Standard Means of Error (SEM) and compared using unpaired t-test. A value of $P< 0.05$ was considered significant

RESULT

There was significant increase in Progesterone concentration in the treatment group comparing pre-treatment to post treatment but significant decrease was recorded in the control group ($P<0.05$). However in both groups there were no significant changes in Prolactin, Estrogen, Follicle stimulating hormone and Luteinising hormone although all were observed to have increased comparing pre-treatment and seven days post treatment (Table 1, 2). Similar non significant increase were observed in the control group comparing last day of treatment and 24 hours post parturition (Table 3). Fetuses were aborted in all the test group animals between 24 and 48 hours post treatment with the crude extract (Figure 1). All Control group animals kindled, having a mean number of 5.7±0.67 kits and an average gestation of 30.3±0.33days

DISCUSSION

Progesterone increased significantly from pre-treatment to post treatment in the test group and decreased significantly in the Control group indicating that ethanol extract of *S. mombin* leaf favored the production of progesterone as opined by Igwe et al., (2011). It has been observed that saponins, one of the components of *S. mombin* leaf, can be converted to progesterone. (Broadhurst and Duke, 1998). However the decrease in the control group is consistent with the normal occurrence at periparturent period. Rebollar et al., (1997) had submitted that progesterone level falls to 6.7±1.4ng/ml at about 29^{th} day of gestation a figure quite higher than the 2.5ng/ml recorded in this study in control group at day 21. Conversely in this same group, non significant increase was observed with serum estrogen, prolactin and LH. FSH in the group however reduced significantly to 2.2 ± 0.10mIU/ml but had increased to a mean value of 8.7±4.70mIU/ml just a day after birth. This could be that as progesterone level reduces, a positive feedback is sent to the hypothalamus for the initiation of FSH and LH production and release. Same increase in the control group, 24h after birth, was observed with estrogen, prolactin and LH but Progesterone level was not affected.

Estrogen concentrations for treated gravid does and control at day 21 of gestation (19.1±2.18pg/ml and 12.0±2.65 pg/ml respectively) were smaller than Ubilla et al., (2001) observation at day 18 of gestation. These authors, working on pregnant does at day 8 and 18 of gestation, haven been separated from their kittens from previous gestation, recorded Mean plasma E2 concentrations of about 26pg/ml and 28pg/ml respectively. The workers also recorded about 6ng/ml for these two days for prolactin which was lower than 7.06 ±5.14ng/ml observed for the treated group but higher than 2.2±0.10ng/ml of control group at day 21 of gestation.

Abortions on days 23 and 24 of gestation in the treated rabbit are explainable from the abortifacient property of the extract reported in rat (Offiah and Anyanwu, 1989) and mice (Taylor, 2006). Saponin has been observed to have oxytotic property

and considered to be phytoestrogen because of its steroid backbone (Yeonju Lee, et al., 2012) just as well as it could be converted to progesterone (Broadhurst and Duke, 1998). This dual conversion appears to potentiate both progesterone-like and oxytotic actions in the uterus which explains why Taylor, 2006 reported both muscular stimulant and relaxant properties of the plant. The oxytotic effect seemed to have an overriding effect in this study. Gruber and O'Brien, (2011) had reported that oxytotic activity of the leaf methanol extract derived from Ca^{2+} ion mobilization from the cytosol and direct musculotropic effects.

CONCLUSION

Spondias mombin produces changes in sex steroid hormone concentration in gravid rabbit which can alter gestation and has abortifacient tendency.

REFERENCES

Aiyeloja, A.A., Bello, O.A., (2006): Ethno botanical potentials of common herbs in Nigeria:A case study of Enugu State. *Educational Research and Review,* 1(1):16-22.

Broadhurst, C.L., Duke, J.A., (1998): Saponin: Natural Steroid. The herb companion. www.herbcompanion.com.

Gruber, C.W., O'Brien, M., (2011): Uterotonic plants and their bioactive constituents. *Plant Med.*, 77(3): 207-220. .

Igwe, C.U., Onwuliri, V.A., Onyeze, G.O.C., Osuagwu, C.G., (2011): Spasmogenic Activity of Ethanolic Leaf Extract of *Spondias Mombin* Linn on Isolated Uterine Muscle Strips of Rat: Possible Hormonal Mechanism of Action. *Res. J. of Agri. and Biol. Science*, 7(2): 228-233.

Offiah, V.N., Anyanwu, I.I., (1989): Abortificient activity of an aqueous extract of *Spondia mombin* leaves. *J. Ethnopharmacol*., 26 (3): 317-320.

Raising-rabbit.com, (2012): Rabbit mating. http://www.raising-rabbits.com.

Rebollar, P.G., Alvarino, J.M.R., Illua, J.C., Silvan, G., Lorenzo, P., (1997): Changes in Plasma progesterone and oestradiol during natural prostaglandinF2&-induced parturition in the rabbit. *World Rabbit Science*, 5 (1): 13-16.

Taylor, L., (2006): Tropical plants Database: Database file for Ubos (Spondias mombin) Pub. Raintree Nutrition Inc.,Carsoncity,NO 89701.Rainforestdatabase.com/plant/ubos/htm. Last updated 5-14-2012.

Ubilla, E., Rebollar, P. G., Pazo, D., Esquifino, A., Alvarifio, J. M. R., (2001): Endocrine profiles during doe-litter separation and the subsequent pregnancy in rabbits. J. *Physio. Biochem.*, 57 (1): 23-29.

Uchendu, C. N., Isek, T., (2008): Antifertility activity of aqueous ethanolic leaf extract of *Spondias mombin* (Anacardiaceae) in rats. *Afr Health Sci.*, 8 (3): 163-167.

Yeonju Lee, Jae-Chul Jung, Zulfiqar Ali, Ikhlas, A. Khan, Seikwan, Oh**.**, (2012): Anti-Inflammatory Effect of Triterpene Saponins Isolated from Blue Cohosh (*Caulophyllum thalictroides*). *Evidence-Based Complementary and Alternative Medicine,* Article ID 798192 (In press).

Table-1: Pretreatment and seven days post-treatment of gravid rabbit with 800mg/kg crude ethanolic extract of *Spondias mombin* in test group.

Hormone	**Pre-treat** (day 14 of gestation)	**Post-Treat** (day 21 of gestation)	**P-Val**
Progestron (ng/ml)	5.4±0.30	9.52±0.30	<0.0001
Estrogen (pg/ml)	16.7±0.25	19.12±2.18	0.29
FSH (mIU/ml)	2.06±0.60	6.68±2.45	0.10
LH (mIU/ml)	2.88±1.53	3.04±0.67	0.93
Prolactin (ng/ml)	1.24±0.24	7.06±5.14	0.29

- P<0.05; significant (Unpaired test).
- Values are given as Mean ± SEM.

Table- 2: Pre-treatment and seven days post-treatment of gravid rabbit with 800mg/kg crude ethanolic extract of *Spondias mombin* in control group.

Hormone	Pre-treat (day 14 of gestation)	**Post-Treat** (day 21 of gestation)	**P-Val**
Progestron (ng/ml)	5.1±0.21	2.5±0.50	0.01
Estrogen (pg/ml)	17.33±2.2	12.0±2.65	0.20
FSH (mIU/ml)	3.1±0.07	2.2 ±0.10	0.002
LH (mIU/ml)	1.2±0.03	1.9±0.55	0.29
Prolactin (ng/ml)	1.4±0.34	2.2±0.10	0.20

- Footnotes are same as given in Table-1.

Table- 3: Seven days post-treatment and 24 hours post parturition of gravid rabbit with 800mg/kg crude ethanolic extract of *Spondias mombin* in Control group.

Hormone	**Post-Treat** (day 21 Gestation)	**24hours** (post parturition)	**P-Val**
Progestron (ng/ml)	2.5± 0.50	3.0±0.58	0.55
Estrogen (pg/ml)	12.0±2.60	29.0±7.37	0.10
FSH (mIU/ml)	2.2 ± 0.10	8.7±4.70	0.24
LH (mIU/ml)	1.9± 0.55	3.2±1.00	0.31
Prolactin (ng/ml)	2.2±0.10	5.17±2.97	0.38

- Footnotes are same as given in Table-1.

Figure-1: Aborted fetuses after treatment with 800mg/kg body weight of *Spondias mombin* for the seven days.

