Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol 2, No.7, 2012

Antigonadotrophic Effect of *Spondias Mombin* Leaf Extract In Male Wistar Rats

Olaitan R. Asuquo[1*], Theresa B. Ekanem[1], Paul B. Udoh [2], Mokutima A. Eluwa[1]
Otu E. Mesembe[1]
[1] Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Calabar, Cross River State
[2] Department of Zoology, Faculty of Biological Sciences, University of Calabar, Cross River State
*Email of the corresponding author: ola_asuquo@yahoo.com

Abstract
Spondias mombin is a fructiferous tree used for medicinal purposes and is relied on for its contraceptive and abortifacient properties. The aim is study is to investigate the effect of *Spondias mombin* leaf's aqueous extract on the anterior pituitary cells and hormones. 30 mature male Wistar rats (180- 200g) were obtained and divided equally into three groups 1, 2 and 3. Group 1 served as control and received vehicle. Groups 2 and 3 were treated with 400mgkg^{-1} and 800mgkg^{-1} of the leaf extract through oral gavage for 28 days. The animals were anaesthetized by chloroform and sacrificed on the 29th day. Blood was obtained from the hearts of the animals and skull was opened to excise the pituitary gland. Testicular and accessory glandular weights were taken. Regression of gonadotropin cells was observed in the experimental groups compared to the control. Hormonal assay showed significantly reduced levels ($p<0.05$) of FSH, LH and T in experimental groups. Significant ($p<0.05$) changes were recorded in weights of testis, epididymis and prostate gland with no changes in the weight of seminal vesicle. We conclude that aqueous leaf extract of *Spondias mombin* may cause regression of the anterior pituitary cells with decrease in serum levels of FSH, LH and T which supports its use as an herbal contraceptive.
Keywords: Antigonadotrophic, FSH, LH, *Spondias mombin*, Regression.

1. Introduction

Spondias mombin Linn belongs to the family *Anacardiaceae*, a fructiferous tree that thrives in rainforest and the Amazons (Morton, 1987). It is found in West Africa especially in Nigeria and in Brazil (Okwu and Okwu, 2004). All parts of the plant have medicinal and non-medicinal uses; it is traditionally used for reproductive purposes. The leaves and bark are used to aid child birth, pregnant women or those seeking to be pregnant are advised against the use of the leaf infusion or decoction (Taylor, 2004). Its chemical components include tannins, saponin, flavonoids, sterols and quinines (Okwu and Okwu, 2004; Njoku and Akumefula, 2007). The leaves have been reported to contain several salicylic acid derivatives, which explain the traditional use of the leaves for various types of pain (Corthout *et al*, 1994). The bark and leaves also contain caryophyllene, a well known chemical with pain-relieving actions (Moronkonla *et al*, 2003). The leaves are also a significant source of chlorogenic acid (Abad *et al*, 1996). Pharmacological activities of this plant include antiviral (Corthout *et al*, 1992), anti-microbial (Abo *et al*, 1999), anti-malarial (Caraballo et al, 2004), antibacterial (Corthout *et al*, 1994), hypnotic (Ayoka *et al*, 2005), wound-healing (Villegas *et al*, 1997), blood lipid lowering activity (Igwe *et al*, 2008) and hypoglycemic effect (Iweala and Oludare, 2011). Extracts of *Spondias mombin* have been reported to have abortifacient and anticonceptive effect (Offiah and Anyanwu, 1989; Uchendu and Isek, 2008). Its anticonceptive effect led to our investigation on the effect of aqueous leaf extract of *Spondias mombin* on the histology of the anterior pituitary and serum hormonal levels (FSH, LH and T) of adult male Wistar rats.

2. Materials and methods

Thirty adult male Wistar rats of an average weight of 200g were randomly divided into three groups 1, 2 and 3. The animals were acclimatized for two weeks in the Animal House of the Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria. Care of the animals was in compliance with the international guidelines for animal research study. Ethical approval was obtained from the institution's committee on ethics and animal use. Leaves of *Spondias mombin* were washed and air-dried for 3 days. The leaves were blended into powder with the use of an electric blender. Aqueous extraction was carried out using distilled water for 300g of the powder, with a yield of 20% (60g). The extract was dissolved in distilled water and administered orally at 400mgkg^{-1} and 800mgkg^{-1} to the experimental groups. Group 1 animals served as control and received distilled water, while groups 2 and 3 served as the experimental groups treated with

400mgkg^{-1} and 800mgkg^{-1} of aqueous leaf extracts of *Spondias mombin* respectively for 28 days. On the 29th day, the animals were anaesthetized with chloroform and sacrificed. Blood was collected from the heart, and stored in heparinized test tubes for hormonal assay. The pituitary gland was harvested after excision; the anterior pituitary was separated and fixed in Bouins's fluid. The pars anterior was stained with PAS-Orange G method of Pearse (1953). ELISA method was used to determine luteinizing hormone (LH) and follicle stimulating hormone (FSH) in serum using microwell's kits. One way ANOVA was used to determine the relationship of the serum hormonal levels between the control and experimental groups. Differences at $p<0.05$ was significant.

3 Results

The anterior pituitary of the control showed normal cell types of acidophils, basophils and chromophobes with acidophils stained yellow, basophils stained magenta and chromophobes were stained pale blue-grey (Fig 1). The pars anterior of group 2 animals showed reduced acidophils and basophils with degranulated chromophobes. Gonadotrophs (FSH and LH) showed progressive regression compared to control (Fig 2). Group 3 anterior pituitary showed hypertrophy, hyperplasia and vacuolation of gonadotrophs (Fig 3). Table 1 shows the hormonal profile of control and experimental animals administered with aqueous extract of *Spondias mombin*. FSH and LH levels of the experimental animals were significantly ($p<0.05$) lower than control. Serum FSH levels in group 2 and 3 animals were significantly lower ($P< 0.05$) compared to group 1 animals. The values in the experimental animals were 6.46 ± 0.05 in group 2 and 5.76 ± 0.29 in group 3 against 12.48 ± 0.12 in group 1. Similarly the value of LH was significantly reduced in the experimental animals having values of 1.18 ± 0.62 and 0.54± 0.04 in groups 2 and 3 respectively compared to control (3.00±1.17). Serum level of testosterone was also affected by extracts of *Spondias mombin*, in experimental animals (3.70 ± 1.74 and 2.25 ± 1.13) in groups 2 and 3 compared to group 1 with a value of 8.20 ± 0.91.

4 Discussion

The possibility of an effective check on human fertility regulation consisting of contraception and management of infertility is an important aspect of reproductive health (Allag and Rangari, 2002). Exploration of the hidden wealth of medicinal plants for contraceptive use has been discovered over the years. Accumulations of information in regards to the antifertility efficacy of plants have been documented (Brondegaard, 1973; Kamboj and Dhawan, 1989; Udoh and Kehinde, 1999; Bai and Shi, 2002; Sharma *et al*, 2003). In males, androgens play a pivotal role in the development of the reproductive system, phenotypic sex, and are essential for testicular spermatogenesis, spermiogenesis and for the expression of male sex behavior (Akingbemi, 2005; Wang *et al*, 2009; Schulz *et al*, 2010). In this study, pituitary gonadotrophs (FSH and LH) following treatment with *Spondias mombin* were regressed. This may imply that the plant acted directly on the anterior pituitary to inhibit synthesis of gonadotropins. Reduced chromphilic population seen after treatment with doses of extract is in line with results obtained by Akpantah *et al*, (2010) on the effect of neem on pars anterior of rats. This may be due to the deleterious effect of the leaf extract on the various cell types found in the anterior pituitary. Serum hormonal levels of FSH, LH and Testosterone were significantly reduced in the experimental groups. The decrease in testosterone level may be due to decrease synthesis or increased metabolic clearance. It has been stipulated that as testosterone levels decrease, levels of FSH and LH are expected to increase to stimulate the production of more testosterone (Emanuele and Emanuele, 2001). In this study, low serum testosterone levels in animals treated with *Spondias mombin* extract was accompanied by low levels of LH and FSH. This suggests that the hypothalamic cells which produce LHRH may not function correctly to the feedback when testosterone level decreased. The inability of the anterior pituitary to respond to a decline in testosterone may imply that high glucose has a central effect on the interaction between the nervous system and endocrine system as suggested by Maneesh *et al* (2006). The decrease in serum LH and FSH may result from impairment in their production and secretion. We conclude that *Spondias mombin* may have the ability to disrupt the processes necessary for male reproduction.

References

Abad, M.J., Bermejo, P., Carretero, E., & Martinez-Acitores, C. (1996). Anti-inflammatory activity of some medicinal plant extracts from Venezuela. *Journal of Ethnopharmacology*, 55(1): 63-68. PMID: 9121169.

Abo, K.A., Ogunleye, V.O., & Asindi, J.S. (1999). Antimicrobial potential of *Spondias mombin*, *Croton zambesicus* and *Zygotritonia crocea*. *Phytotheraphy Research*, 13: 494-497. PMID:10479760

Akingbemi, B.T. (2005). Estrogen regulation of testicular function. *Reproductive Biology and Endocrinology*, 3: 51-54. PMID:16188042.

Akpantah, A.O., Ekong, M.B., Uruakpa, K.C., Akpaso, M.I., Eluwa, M.A., & Ekanem, T.B. (2010). Gonadal histo-morphologies and serum hormonal milieu in female rats treated with *Azadirachta indica* leaf extract. *Iranian Journal of Reproductive Medicine* , 8(4): 185-190

Allag, I.S., & Rangari, K. (2002). Extragenomic action of steroids on spermatozoa: prospects for regulation of fertility. *Journal of Health Population*, 25(1): 38-44

Ayoka, A.O., Akomolafe, R.O., Iwalewa, E.O., & Ukponmwan, O.E. (2005). Studies on the anxiolytic effect of *Spondias mombin* L (*Anacardiaceae*) extracts. *African Journal of Traditional, Complimentary and Alernativet Medicines* , 2(2): 153-165. ISSN: 0189-6016

Bai, J & Shi, Y. (2002). Inhibition of T-type Ca^{2+} currents in mouse spermatogenic cells by gossypol, an antifertility compound. *European Journal of Pharmacology*, 440(1): 1-6. PMID: 11959082.

Brondegaard, V.J. (1973). Contraceptive plant drugs. *Planta Medica*, 23(2): 167-172 PMID 4705796.

Caraballo, A., Caraballo, B., & Rodriquez-Acosta, A. (2004). Preliminary assessment of medicinal plants used as antimalarials in the south-eastern Venezuelan Amazon. *Revista-da-Scciedade-Brasileira-de-Medicna- Tropical*, 37(2): 186-188. ISSN: 00378682.

Corthout, J., Pieters, L.A., Claeys, M., Vanden-Berghe, D.A., & Viletinck, A.J. (1994). Antibacterial and molluscicidal phenolic acid from *Spondias mombin*. *Planta Medica*, 60: 460-463.PMID: 7997478.

Corthout, J, Pieters, L.A., Claeys, M., Vanden-Berghe, D.A., & Viletinck, A.J. (1992). Antiviral caffeoyl: esters from *Spondias mombin*. *Phytochemistry*, 31:79-81. Doi.org/10.1016/0031-9422(92)80344-E.

Emanuele, M A., & Emanuele, N. (2001). Alcohol and the male reproductive system. *Alcohol Reseaarch and Health*,. 25(4):282-287. PMID: 11910706.

Igwe, C.U., Ojiako, A.O., Nwaogu, L.A., & Onyeze, G.O.C.(2008). Lipid lowering effect of aqueous leaf extract of *Spondias mombin* Linn. *Internet Journal of Pharmacology*, 6(1): 1-9. ISSN: 1531-2976.

Iweala, E.E.J., & Oludare, F.D. (2011). Hypoglyceamic effect, biochemical and histological changes of *Spondias mombin* and *Parinari polyandra* Benth seeds ethanolic extracts in alloxan induced diabetic rats. *Journal of Pharmacology and Toxicology*, 6(2): 101-112. Doi: 10.3923/jpt.2011.101.112.

Kamboj, V.P., & Dhawan, B.N.(1989). Fertility regulating plants on Indian scene update In: contraceptive today and tomorrow by Toteja, G.S, Mokkapati, S, Singh, B.K, Sharma, R. S and Saxena, B. N (Eds). ICMR, New Delhi. Pp 115-123.

Maneesh, M. Jayalakshmi, H., Singh, T.A.E., Chakrabarti, A. (2006). Impaired hypothalamic-pituitary-gonadal axis function in men with diabetes mellitus. *Indian Journal of Clinical Biochemistry*, 21(1): 165-168. doi: 10.1007/BF02913088.

Moronkonla, D.O., Adeleke, A.K., & Ekundayo, O. (2003). Constituents of the *Spondias mombin* Linn and the comparison between its fruit and leaf essential oils. *Journal of Essential Oil Bearing Plant*, 6(3): 148-152. ISSN: 0972_ 060X.

Morton, J. (1987). Fruits of warm climates. Yellow mombin. 1st edition. Miami, USA. Pp245-248.

Njoku, P. C., & Akumefula, M. I. (2007). Phytochemical and nutrient evaluation of *Spondias mombin* leaves. *Pakistan Journal of Nutrition*, 6(6): 613-615. ISSN: 1680-5194.

Offiah, V.N., & Anyanwu, I.I. (1989). Abortifacient activity of an aqueous extract of *Spondias mombin* leaves. *Journal of Ethnopharmacology*, 26: 317-320. PMID:2615412.

Okwu, D.E., & Okwu, M.E.(2004). Chemical Composition of Spondia Mombin plants *Journal of Sustainable Agriculture and the Environment*, 6: 140-147.

Pearse, A.G.E.(1953). Histochemistry, theoretical and applied. 1st edition. London. Churchill.

Schulz, R.W., de Franca, L.R., Lareyre, J.J., Le, G.F., Chiarini-Garcia, H., Nobrega, R.H., & Miura,T.(2010). Spermatogenesis in fish. *General and Comparative Endocrinology*, 165(3): 390-411. PMID:19348807.

Sharma, A., Verma, P.K., & Dixit, V.P.(2003). Effect of *Semecarpus anacardium* fruits on reproductive function of male albino rats. *Asian Journal of Andrology*, 2: 121-124. ISSN: 1008-682X.

Taylor, L. (2004). The healing power of rainforest herbs: a guide to understanding and using herbal medicines. Square one publishers Inc . pp 1-2.

Uchendu, C.N., & Isek, T. (2008). Antifertility activity of aqueous ethanolic leaf extract of *Spondias mombin* (*Anacardiaceae*) in rats. *African Health Sciences*, 8(3): 163-167 . PMCID: PMC2583269.

Udoh, P., & Kehinde, A. (1999). Studies on antifertility effect of pawpaw seeds (*Carica papaya*) on the gonads of male albino rats. *Phytotherapy Research*, 13: 226-228. doi: 10.1002/(SICI)1099-1573(199905)13:3<226::AID-PTR396>3.0.CO;2-E.

Villegas, L.F., Fernadz, T.D., Maldonado, H., Torres, R., Zavalet, A., Vaisberg, A.J., & Hammond, G.B. (1997). Evaluation of wounds healing of selected plants from Peru. *Journal of Ethnopharmacology*, 55: 193-200. PMID:9080340.

Wang, R.S., Yeh, S., Tzeng, C.R., & Chang, C. (2009). Androgen receptor roles in spermatogenesis and fertility: Lessons from testicular cell-specific androgen receptor knockout mice. *Endocrine Review*, 30(2): 119-132. doi:10.1210/er.2008-0025.

Table 1: Serum hormonal levels of FSH and LH in control and experimental groups administered with aqueous leaf extract of *Spondias mombin*.

Hormones	Group 1 (control)	Group 2(400mgkg^{-1})	Group 3(800mgkg^{-1})
FSH	12.48±0.12	6.46±0.05*	5.76±0.29*
LH	3.00±1.17	1.18±0.62*	0.54±0.04*
T	8.29±0.91	3.70±1.74*	2.25±1.13*

Results are presented as mean ± SEM. *significantly different from control at $p<0.05$. FSH- follicle stimulating hormone, LH-luteinizing hormone, T-testosterone

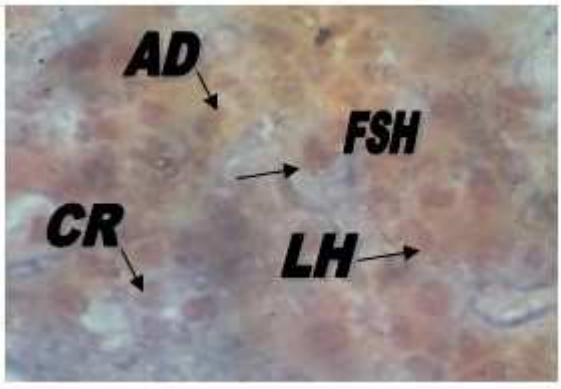

Figure 1: Photomicrograph of the control section shows normal anterior pituitary cell types; acidophils (AD), basophils showing gonadotrophs (FSH & LH), chromophores (CR). Mag X 400. PAS-Orange G.

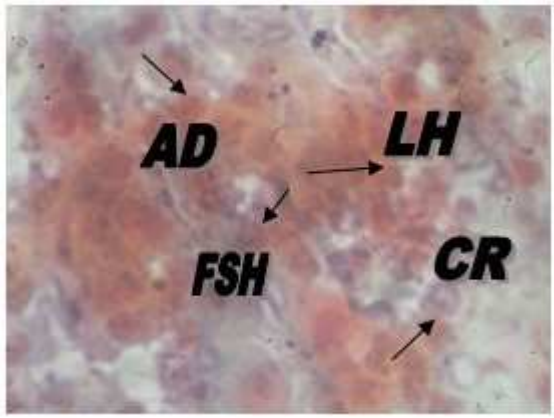

Figure 2: Photomicrograph of group B section treated with 400kg of Spondias mombin leaf extract shows reduced acidophils (AD) and regressed gonadotrophs (FSH & LH). Mag X 400. PAS-Orange G.

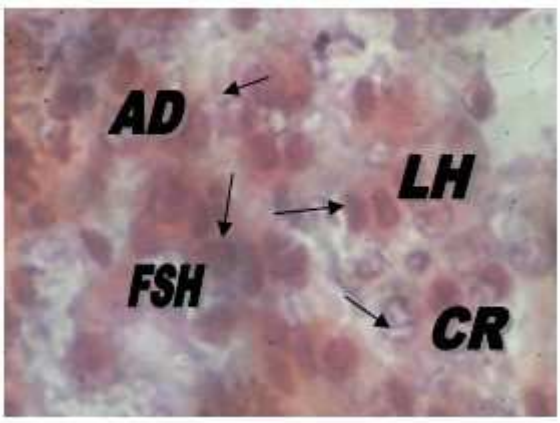

Figure 3: Photomicrograph of group C section treated with 800kg of Spondias mombin leaf extract shows hypertrophied and vacuolated gonadotrophs (FSH & LH) and reduced population of chromophores (CR). Mag X 400. PAS-Orange-G.