

## VAGINAL CYTOLOGY PATTERN AND BIRTH FEATURES OF FEMALE WISTAR RATS TREATED WITH GRADED DOSES OF ETHANOLIC EXTRACT OF SPONDIAS MOMBIN

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### Abstract

Twenty-five virgin female wistar rats weighing between 120g and 200g and divided into five groups of five rats per group were treated with graded oral dosages of ethanolic extract of Spondias mombin. Dosages served were 800mg/kg, 600mg/kg, 400mg/kg and 200mg/kg for groups A, B, C, D respectively and distilled water for group E which served as control. Five untreated proven male were used for copulation, one per group. Vaginal cytology was done daily for two weeks before treatment with extract to establish an oestrous cycle pattern repeated daily for another two weeks simultaneously with extract administration. Mating, pregnancy diagnosis and determination of birth parameters followed immediately after the end of extract treatment. Vaginal smears prior treatment and during treatment contained epithelial cells consistent with the different phases of estrus in the wistar rat and provided a cyclical pattern indicating that Spondias mombin had no negative effect on the estrous cycle of the wistar rat. Pregnancy and birth rates were favoured with groups A (800mg/kg) and E (control). Average litter sizes in all the groups were not significantly different. The average live birth weight of the neonates measured was observed to be highest for Group A with average live birth weight of 6.27g, followed by Groups B and the control with average live birth weight of 5.83g and 5.50g. The Groups C and D had lower average live birth weights of 4.01g and 4.74g respectively. The group A average weight was significantly high compared with other treatment groups and control ( $p < 0.05$ ). It was concluded that ethanolic extract of Spondia Mombin at 800mg/kg fed orally before copulation had no anti fertility effect on female wistar rat instead appeared to potentiate gestation parameters but same could not be said for dosages as low as 200mg/kg.

Key words : Antifertility, copulation, dosage, estrous cyle, litter size, Gestation.

## PROFIL CYTOLOGIQUE VAGINALET CHARACTERISQUES DES MISE-BAS CHEZ LES RATS WISTAR FEMELLES TRAITÉES AVEC DES DOSES PROGRESSIVES DE L'EXTRAIT ÉTHANOLIQUE DE SPONDIAS MOMBIN

### Résumé

Vingt-cinq rats Wistar femelles vierges pesant entre 120g et 200g et réparties en cinq groupes de cinq rats par groupe ont été traitées avec des doses orales progressives de l'extrait éthanolique de Spondias mombin. Les doses servies étaient 800mg/kg, 600mg/kg, 400mg/kg et 200mg/kg respectivement pour les groupes A, B, C, D, et de l'eau distillée pour le groupe E servant de témoin. Cinq mâles non traités ont été utilisés pour la copulation, un par groupe. Une cytologie vaginale a été faite tous les jours pendant deux semaines avant le traitement avec l'extrait, afin d'établir un modèle de cycle œstral répété quotidiennement pendant deux autres semaines en même temps que l'administration de l'extrait. L'accouplement, le diagnostic de grossesse et la détermination des paramètres de mise-bas ont suivi immédiatement après la fin du traitement à base de l'extrait. Des frottis vaginaux avant et pendant le traitement contenaient des cellules épithéliales cohérentes avec les différentes phases de l'œstrus du rat Wistar et ont fourni un modèle cyclique indiquant que Spondias mombin n'avait aucun effet négatif

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sur le cycle œstral du rat Wistar. Les taux de grossesse et de naissance étaient plus élevés chez les groupes A (800mg/kg) et E (témoin). Les tailles moyennes des portées dans tous les groupes n'étaient pas significativement différentes. Le poids moyen le plus élevé à la naissance des nouveau-nés vivants mesurés a été observée chez le groupe A qui était de 6.27g, suivi du groupe B et du groupe témoin, respectivement avec un poids moyen à la naissance de 5,83g et de 5,50g. Les groupes C et D avaient des poids moyens plus faibles, respectivement 4.01g et 4.74g. Le poids moyen chez le groupe A était significativement élevé par rapport aux autres groupes de traitement et au groupe témoin ( $p < 0,05$ ). Il a été conclu que l'extrait éthanolique de *Spondias mombin* à 800mg/kg administré oralement avant la copulation n'avait aucun effet anti-fertilité sur le rat Wistar femelle, au contraire il a semblé améliorer les paramètres de gestation, mais on ne peut pas en dire autant des doses aussi faibles que 200mg/kg.

**Mots-clés :** Anti-fertilité, Copulation, Dose, Cycleœstral, Taille des portées, Gestation.

## Introduction

The reproductive health of female animals is essential in productivity. This informs the need to constantly protect the integrity of the different elements that combine to ensure non occurrence of reproductive failure.

Naturally occurring medicinal preparation from medicinal plants are utilised as therapy for various disease condition being preferred to synthesised drugs for the obvious reason of cost effectiveness and less exposure to chemical toxicity. *Spondias mombin* is one of such medicinal plants used by many. It is among the forages given to domestic animals in Nigeria. The young leaves are also cooked as green vegetables by local folks (Ayoka et al, 2008). It is a fruitiferous tree that thrives in the rainforest and coastal areas of Africa. It is known by various names in Nigeria (Ibo: Ichikara, Hausa: Tsardarmasar, Yoruba: Iyeye). The therapeutic effect of leaf extract of the plant has been reportedly linked with its constituent (Njoku and Akumefula, 2007). Saponin, one of the constituents, has relationship with sex hormones like oxytocin. Oxytocin is a sex hormone involved in controlling the onset of labour in female animals and the subsequent release of milk (Okwu and Okwu, 2004).

Steroidal saponins and alkaloids such as ergot alkaloids have been reported to elicit uterine muscle activity (Gwotmut and Nwafor, 2001). These phytochemicals may be associated with the reported oxytocic and abortifacient activity of the plant's leaf extract (Offiah and Anyanwu, 1989). This is why the leaves of *Spondias Mombin* are given to expectant ruminant animals and those that

delivered without the release of their placenta (Okwu and Ekeke, 2003). Other constituents include Flavonoids and some other Phenolic derivatives, Alkaloids, and Tannins. Tannins has astringent properties. It hastens the healing of wounds and inflamed mucous membrane. Flavonoids, alkaloids and tannins observed in the plant have also been associated with the observed antimicrobial effects in various studies involving plant extracts (Nwaogu et al, 2007). These also account for the plant's reported molluscicidal (Corthout et al, 1994), anti-viral (Corthout et al, 1992), anti-malarial (Caraballo et al, 2004) and anti-helminthic (Ademola et al, 2005) activities. Major nutritional compositions of *S. mombin* leaves include carbohydrates, moisture, proteins and crude fibre and vitamins C and A. The good distribution of nutrients in the leaves may explain its use as one of the forage feed given to domestic animals. When compared with some other common vegetables domestic animals graze on, *S. Mombin* leaves contain fairly good quantities of carbohydrates (68.92%), proteins (11.04%) and fats (4.82%). Intake of the plant for these various uses do not take into cognizance the effect it might have on the reproductive pattern of the animas.

The reproductive cycle of female wistar rats is characterized as proestrus, œstrus, metoestrus and dioestrus. Noakes et al (2001). In studies about reproductive system as well as studies about the influence of the estrous cycle on non-reproductive functions, vaginal smear cytology is used for the determination of the estrous cycle phases (Long & Evans, 1922). The haracterization of each phase is based on the proportion among three types of

cells observed in the vaginal smear: epithelial cells, cornified cells and leukocytes. From the onset of sexual maturity up to the age of 12 months, the mean cycle length in the female rat is 4 days (Noakes *et al* 2001), and this short cycle length makes the rat an ideal animal for investigation of changes occurring during the reproductive cycle (Caligioni 2009).

This work was carried out to evaluate the effect ethanolic extract of spondias mombin leaves has on the vaginal cytology pattern of rats treated with graded doses and ultimately on litter size and birth weight of pups.

### Materials and Method

#### Plant Collection

Fresh leaves of Spondias mombin plant were air-dried under room temperature until they crumbled to touch. The dried leaves were grounded into fine powder, defatted with hexane before soaking in ethanol for three days. The resultant filtrate was distilled in rota-evaporator.

#### Experimental Animals

Twenty-five virgin female wistar rats weighing between 120g and 200g and five proven male were used for the study. The rats were fed with commercially prepared feed and water ad-libitum.

#### Experimental Design

Rats were allowed to acclimatize for two weeks. Female rats were divided into five groups (A, B, C, D, and E) of five rats per group. Group E served as control while the other groups (A, B, C, and D) served as experimental groups. They were treated with graded oral dosages of 800mg/kg, 600mg/kg, 400mg/kg and 200mg/kg extract and distilled water for groups A, B, C, D and E respectively.

#### Vaginal Cytology Preparation

This was done on the female rats daily for two weeks before treatment with extract to establish an oestrous cycle pattern. This was repeated daily for another 2 weeks simultaneously with administration of the extract. This procedure was done by inserting vaginal swab into the vaginal to make vaginal smear on a clean glass slide, fixed with

methanol for 10 minutes and then stained with Giemsa for 15 minutes. This was then washed, air dried and viewed under light microscope at x100 magnification for vaginal epithelial cell types.

#### Mating and Pregnancy Diagnosis

A proven male was introduced to each group after 3 weeks of extract administration. 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.

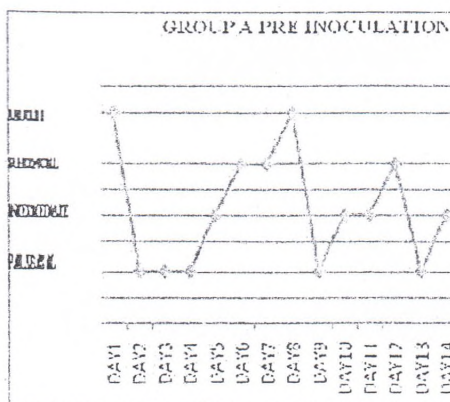
Pregnancy diagnosis was done through observation of vaginal plug and abdominal palpation.

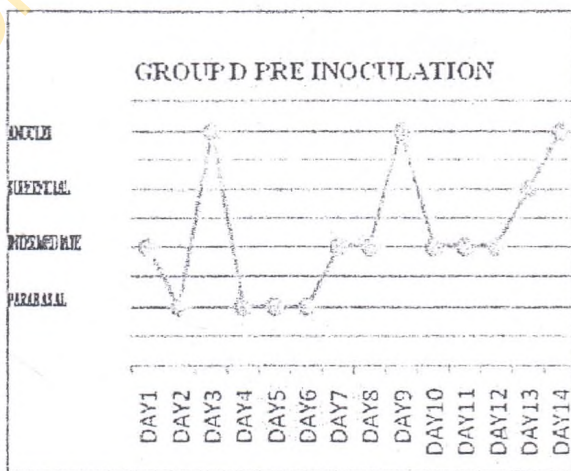
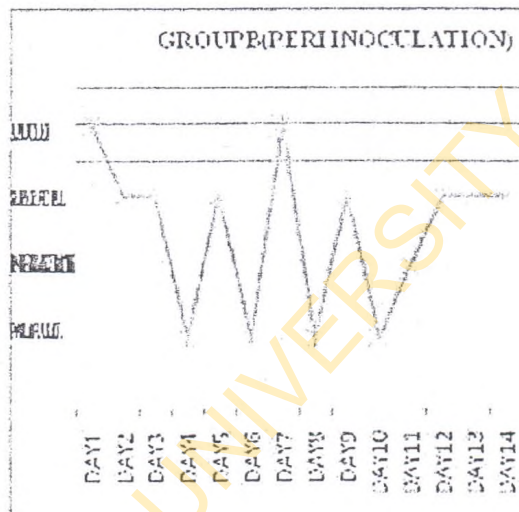
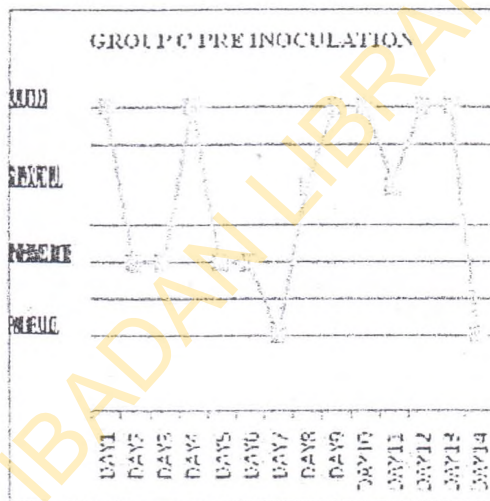
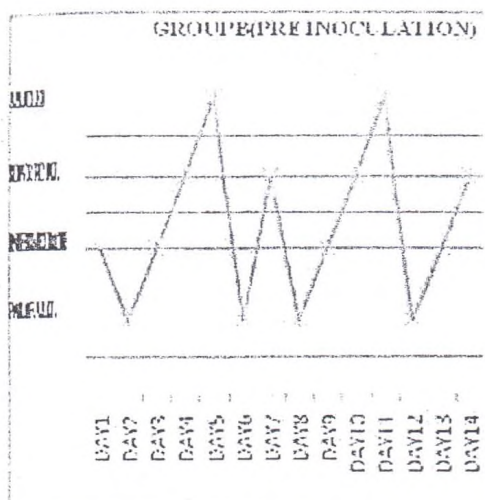
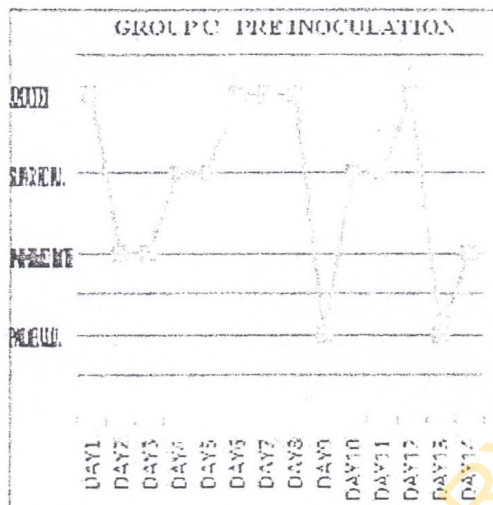
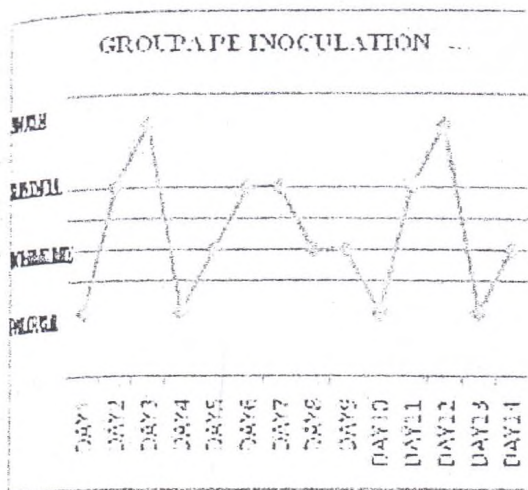
#### Determination of Birth Parameter

Gestation length, Litter size, Live birth weights using a sensitive electronic weighing device were taken

#### 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 deviation.





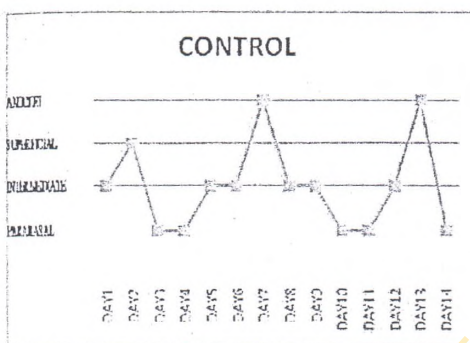
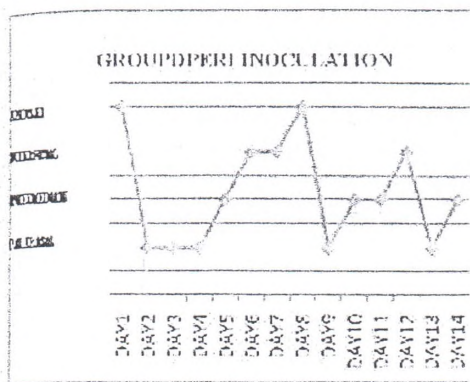


Figure 1; Shows predominant vaginal epithelial cell of non treated and treated period in the groups

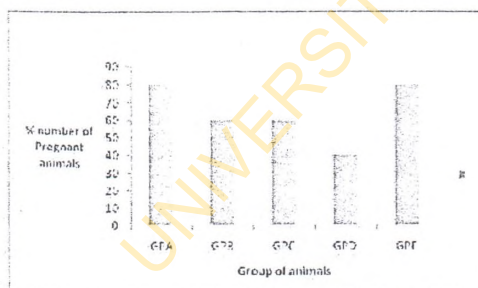


Figure 2: Shows the various percentage of pregnant after treatment with Ethanolic extract of *Spondias mombin* of different dosage

**Result**

The exfoliative vaginal cytology of female rat revealed that all the female rats cycled normally from one phase of oestrous

cycle to another. This consistent cycle pattern was observed for both pre treatment cytology and cytology during treatment. (Fig 1) (Plates 1-4)

Pregnancy and Birth rate were 80% for groups A and E (control), 60% for groups B and C and dismal 40% for group D. (Fig 2)

Average litter sizes in all the groups were not significantly different (Table 1). The average live birth weight of the neonates measured was observed to be highest for Group A with average live birth weight of 6.27g, followed by Groups B and the control with average live birth weight of 5.83g and 5.50g. The Groups C and D had lower average live birth weights of 4.01g and 4.74g respectively. (Table 1). The group A average weight was significantly high compared with other treatment groups and control ( $p < 0.05$ ).

**Discussion and Conclusion**

The experiment showed no effect of the extract on oestrous cycle pattern comparing pre treatment with treatment. It has been reported that during preclinical investigations into the safety of drugs and chemicals, many are found to interfere with reproductive function in the female rat. This interference is commonly expressed as a change in normal morphology of the reproductive tract or a disturbance in the duration of particular phases of the estrous cycle. (Westwood, 2008). There seems not to be any interference with the cycle of the rats during treatment going by the wave-like consistent pattern of oestrous cycle from the proestrus phase to the diestrus phase .

The average birth weight of 5.49g and 5.83g for Group E (Control) and Group B (600mg/kg) respectively was in line with 5-6g reported by National laboratory animal centre (2010), however Group A's higher average birth weight of 6.27g even with the sizeable average number of pups. Litter sizes of all groups fell slightly below 9-11 pups reported by National laboratory animal centre (2010) the same with average Gestation lengths which fell slightly outside the range of 19-22 days reported. The profertility indications at 800mg/kg using oral dosages of ethanol extract of *spondias mombin* is against the finding of Chukwuka and Thomas (2008) working with aqueous ethanol

leaf extract of *Spondias mombin* administered intraperitoneally. The 800mg/kg dosage was concluded as possessing anticonceptive activity. The difference in these findings could be as a result of difference in the route of administration. The choice of oral route in this work was informed by the fact that dosing of livestock using *spondias leaves'* extract are more often through the oral route.

It can be concluded that Ethanolic extract of *Spondia Mombin* at 800mg/kg fed orally before copulation has no anti fertility effect on female wistar rat rather it appears to potentiate reproduction. The same cannot be said for dosages as low as 200mg/kg.

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