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VITAL REPRODUCTIVE INDICES IN NON GRAVID FEMALE RABBITS TREATED WITH CRUDE ETHANOL EXTRACT OF *SPONDIAS MOMBIN*

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ABSTRACT

Ten female Chinchilla breed of rabbits with a mean weight of 1.65 ± 0.06 kg, were randomly divided equally into a treatment and a control group. The treatment group received orally 800 mg.kg^{-1} of an ethanol extract of *Spondias mombin* for fourteen days. The control group received only distilled water utilizing the same method of administration. The females were kept separately away from males. Assays of the reproductive hormones before and after treatment with the extract in the treatment group were done. Both groups were sacrificed after fourteen days of oral administration and their reproductive tracts carefully examined. The studies revealed the normal morphology of ovaries, oviduct, uterine horns, and uterine body in both groups. The histological sections of different reproductive segments were normal. Left and right ovaries appeared to show marked oogenesis in the treated group going by the larger number of follicles observed compared to the control. The body weight adjusted mean paired ovarian diameter of the control group (0.51 ± 0.02 cm) was significantly (P ≤ 0.05) different from the treated animals (0.41 ± 0.39 cm) but this did not translate into a significant difference in the paired ovarian weights. The body weight adjusted lengths, widths and weights of other parts of the reproductive tracts did not differ significantly comparing the treated and control groups, except for the length of the right uterine horn and the weight of the right oviduct. The studies showed that all of the hormones did not change significantly, comparing the pre and post treatment in the treated group. It was concluded that 800 mg.kg^{-1} of ethanol extract of *Spondias mombin* appeared to favour folliculogenesis in non-gravid rabbits and resulted in no observed pathological effects on the entire reproductive tract of the rabbit.

Key words: ethanol; rabbit; reproduction; *Spondias mombin*

INTRODUCTION

Interest in the effects of various natural products on reproductive parameters has been on the increase in recent times [1], [2], [12], [17], [18]. This interest in natural products emanate from their advantage over synthetic drugs [16]. Also, productivity in livestock is based upon the ability of animals to reproduce, hence the need to ensure fertility. Fertility remains a key determinant of life time performance [10] and to achieve this, optimization of litter size, good fecundity and treatment and prevention of pathological reproductive conditions are significant [9]. In the rabbit, the normal reproductive tract consists of the ovaries, oviducts, uterine horns joining to continue as a body and then forms the cervix and vagina. Under favourable conditions, does will remain in oestrus for long periods during which time ovarian follicles are continually developing and regressing at more or less the same rate. The active life of a follicle is around 12—16 days [11]. *Spondias mombin*, a tropical fructiferous plant, used for abortifacient purposes, among other uses, by traditional folks, has been termed an antifertility agents by a few authors based on its oxytocic effect [12]. However, its effect on non gravid animals is important to assess for possible positive effects.

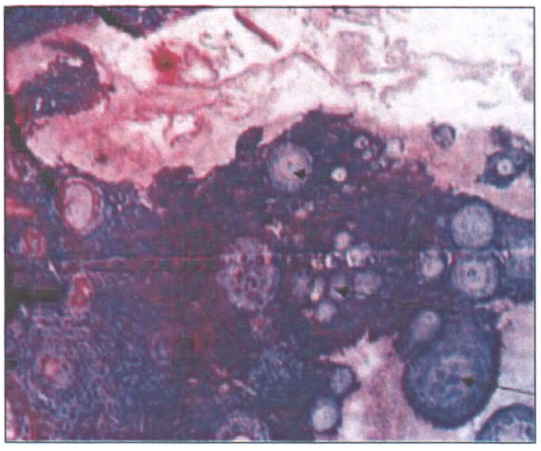

Fig. 1. Histological section of the right ovary of the treatment group with arrows showing follicles at different stages of development.
Periodic acid-Schiff (PAS) stain. Magn. ×100

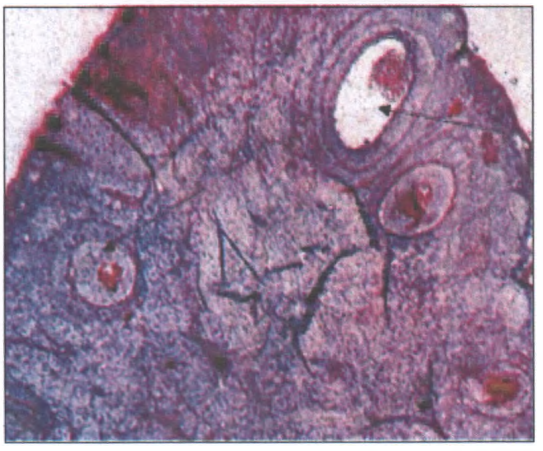

Fig. 2. Histological section of the right ovary of the control group with arrows showing follicles at different stages of development.
Periodic acid-Schiff (PAS) stain. Magn. ×100

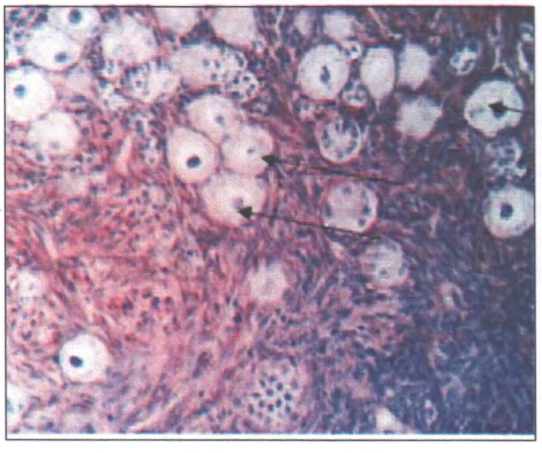

Fig. 3. Histological section of the right ovary of the treatment group. Arrows showing primary follicles.
Periodic acid-Schiff (PAS) stain. Magn. ×400

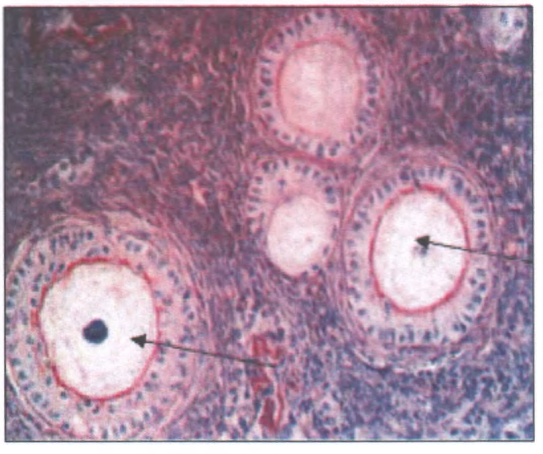

Fig. 4. Histological section of the right ovary of the treatment group. Arrows showing secondary follicles.
Periodic acid-Schiff (PAS) stain. Magn. ×400

This work was aimed at studying the effects of 800 mg.kg^{-1} crude ethanolic extract of *Spondias mombin* on the morphology of the reproductive tract and reproductive hormonal profile of non gravid mature does.

MATERIALS AND METHODS

Spondias mombin leaves were collected, identified and prepared into an extract at the University of Ibadan. Pulverized leaves weighing 3.62 kg were soaked in hexane for three hours in order to remove the fat content. The mixture was decanted and the residue was air dried. The residue was then soaked in ethanol for 3 days, after which, it was filtered. The resultant filtrate was then concentrated *in vacuo* using a rota-evaporator at low temperatures. Ethanol and the residual jelly like dark brownish paste were recovered. This paste was kept in a fume hood for efflorescence of the residual ethanol. A yield of 80 g of dried extract was obtained from which a

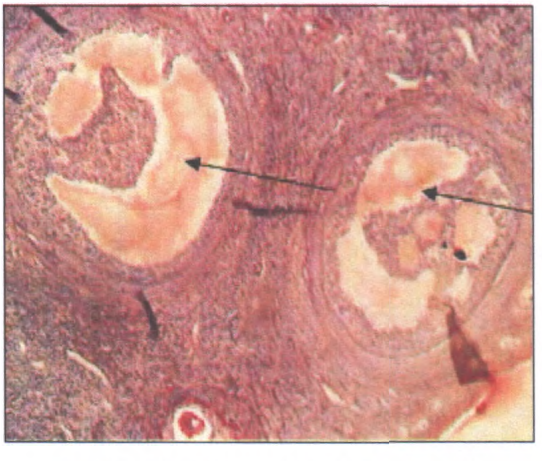

Fig. 5. Histological section of the right ovary of the treatment group. Arrows showing tertiary follicles (antral follicles).
Periodic acid-Schiff (PAS) stain. Magn. ×400

stock solution of 80 g of extract in 100 ml of propylene glycol was constituted. The dose was calculated using the formula:

$$\text{Dose} = \frac{\text{Weight} \times \text{Dosage}}{\text{Concentration}}$$

Experimental animals

Ten Chinchilla breed of rabbits weighing on average 1.65 ± 0.06 kg were utilized. They were pubertal primiparous rabbits. They were kept in standard hutches and fed formulated feed. Water was given *ad libitum*. Fourteen days of acclimatisation was observed during which prophylactic treatment against endo and ecto parasites using avermectin at a dosage rate of 1ml per 50 kg body weight was administered.

Experimental design

The ten rabbits were divided into two groups with five rabbits each. This constituted the extract-treated group and the control group. Ethanol leaf extract was administered orally at a dosage rate of 800 mg.kg^{-1} [15] once daily for fourteen days to the treated group, while the control animals received distilled water during the same period and for the same length of time. Blood samples for hormonal assays were collected carefully from the ear vein of the treated group before the commencement of extract administration and a day after the last administration. ELISA was utilised for assays using hormonal kits. The hormones assayed from the sera were; follicle stimulating hormone (FSH), luteinising hormone (LH), estrogen, progesterone and prolactin. The kits utilised were from Biorex diagnostics (ISO 13485). Batches were; BXEO86OA (Estradiol), BXEO671A (Prolactin), BXEO661A (Progesterone), BXEO631A (FSH), and BXE0651A (LH).

At the cessation of the treatment period, the does from both groups were sacrificed using the guidelines on euthanasia, set by the Committee for the purpose of control and supervision on experiments on animals (CPCSEA, 600 041 Tamil Nadu, India). Afterward, the reproductive tracts were carefully exteriorized. The different segments; the ovaries, the oviduct, the uterine horn, and the uterine body were carefully separated and examined for lesions. Each segment was evaluated for length, width, and weight.

Histopathology and morphometric analysis of the ovaries

All of the extracted ovaries were routinely fixed in 10 % neutral buffered formalin, then dehydrated in graded levels of alcohol, embedded in paraffin wax, sectioned at 5 µm and stained with *Periodic acid-Schiff (PAS)*. Histological alterations and the number of different follicles were counted at 5 different foci per field of the histological slides of the ovary at ×100 and ×400 magnification with the aid of an Olympus light microscope.

Statistical analysis

The data obtained were collated and analysed into descriptive statistics using GraghPad Prism 5 (Version 5.04). Means, with Standard Error of the Means (SEM) were calculated. Means were compared using one sample *T*-test. A value of $P \leq 0.05$ was considered significant.

RESULTS

Does from both the test group and control group had normal ovaries, oviduct, uterine tubes, and uterine body as determined grossly. The uneven surfaces of the ovaries of all of the does suggested the presence of follicles at different stages of development. There were no evidences of corpus lutea or cystic conditions in any of the ovaries. No defective parts were observed throughout the tracts in any of the animals. Histological sections of the oviduct, uterine tubes, uterine body revealed normal epithelial cells. Left and right ovaries showed marked oogenesis in the treated group, both having a larger number of follicles, compared to the controls (Fig. 1 and 2). Views of different fields of the histological slides of the treated group revealed Primary, Secondary and Tertiary follicles (Fig. 3, 4 and 5). The body weight adjusted mean paired ovarian diameter of the control group was 0.51 ± 0.02 cm (Table 1). This was significantly different from the treated group which had 0.41 ± 0.39 cm ($P \leq 0.05$); however this did not translate to a significant difference in the paired ovarian weights (Table 1). The weight of the right oviduct and the length of the right uterine horn were significantly different ($P \leq 0.05$) in the control group than the treatment group (Table 2 and 3). There was no significant difference between the means of the control and treatment groups in: the length, width and weight of the uterine body; the left

Table 1. Mean (± SEM) diameter and weight of paired ovaries

	Treatment group	Control group	P value
Diameter [cm]	0.41 ± 0.04	0.51 ± 0.02	0.04
Weight [g]	0.07 ± 0.01	0.09 ± 0.008	0.06

$P \leq 0.05$ was considered significant

Table 2. Mean weight in g (x ± SEM) of the oviduct, uterine horn and uterine body in the treated and control groups

	Treatment group	Control group	P value
Right oviduct	0.07 ± 0.03	0.17 ± 0.03	0.05
Left oviduct	0.16 ± 0.05	0.13 ± 0.02	0.52
Right uterine horn	0.18 ± 0.03	0.2 ± 0.06	0.77
Left uterine horn	0.21 ± 0.06	0.22 ± 0.07	0.89
Uterine body	0.28 ± 0.09	0.19 ± 0.04	0.39

$P \leq 0.05$ was considered significant

Table 3. Mean length in cm (x ± SEM) of the oviduct, uterine horn and uterine body in the treated and control groups

	Treatment group	Control group	P value
Right oviduct	4.84 ± 0.58	5.58 ± 0.71	0.45
Left oviduct	4.69 ± 0.27	5.87 ± 0.75	0.18
Right uterine horn	3.47 ± 0.22	4.19 ± 0.12	0.02
Left uterine horn	3.47 ± 0.24	4.08 ± 0.15	0.06
Uterine body	0.3 ± 0.03	0.25 ± 0.06	0.45

P ≤ 0.05 was considered significant

Table 4. Mean width in cm (x ± SEM) of the oviduct, uterine horn and uterine body in the treated and control groups

	Treatment group	Control group	P value
Right oviduct	0.08 ± 0.01	0.11 ± 0.02	0.19
Left oviduct	0.09 ± 0.01	0.11 ± 0.02	0.34
Right uterine horn	0.16 ± 0.01	0.31 ± 0.15	0.37
Left uterine horn	0.17 ± 0.02	0.16 ± 0.02	0.826
Uterine body	0.43 ± 0.01	0.42 ± 0.03	0.53

P ≤ 0.05 was considered significant

Table 5. Mean hormonal concentrations in treatment group (x ± SEM)

Hormones	Pre-treatment	Post-t reatment	P value
Progesterone [ng.ml^{-1}]	0.11 ± 0.09	2.99 ± 2.49	0.28
Estrogen [ng.ml^{-1}]	48.24 ± 28.03	13.26 ± 9.69	0.27
FSH [mIU.ml^{-1}]	1.71 ± 0.31	0.97 ± 0.19	0.08
LH [mIU.ml^{-1}]	55.14 ±18.44	32.76 ± 20.93	0.45
Prolactin [ng.ml^{-1}]	13.96 ± 3.04	23.48 ± 13.27	0.50

P ≤ 0.05 was considered significant

oviduct and uterine horn; and width of the right oviduct and the uterine horn (Tables 2, 3 and 4). All the hormones did not change significantly comparing the pre and post treatment in the treated group (Table 5).

DISCUSSION

The treatment seemed to favour folliculogenesis going by the number of follicles observed in the treated rabbits compared to the control. The mean diameter of the ovaries was significantly higher in the control, but this did not translate into a significant higher weight as expected [8], [4]. The diameter was indeed smaller in treated animals, but they had a greater number of developing follicles. The follicles potentially were available for ovulation, hence more follicles means more possible kids after successful fertilization [11]. The mechanism for favoured folliculogenesis may likely be explained by a follicular dynamic postulation that a higher level of embryo yield occurs in rabbits pretreated with progesterone before being superovulated by gonadotropin [3]. In this study, the progesterone increase (though not significantly) that was recorded after treatment with *Spondias* crude extract might have provided the platform for the observed increase in folliculogenesis, in the presence of FSH. Igwe *et al.* [7] in their study, observed a significant progesterone elevation and higher values than obtained in this study, after intraperitoneal treatment with 750 mg.kg^{-1} body weight of *Spondias* crude extract, lending credence to the progestogenic property of *Spondias*. A postulation of interference with signalling pathways of the Kit Ligand and c-Kit system in the regulation of oogenesis and folliculogenesis could be of consideration also [5].

There was no significant change in the levels of LH, FSH, estrogen and prolactin, comparing the pre-treatment and post treatment results in the treatment group, probably signifying non-interference with the oestrous cycle. The insignificant effect on estrogen is in agreement with Igwe *et al.* [7], who reported that the 7 and 14-day intraperitoneal treatment of rabbits with 750 mg.kg^{-1} body weight of crude extract of *Spondias* had no significant effect on the serum level of estrogen. This is also in agreement with the report of Oloye *et al.* [15] after rats were treated orally with 800 mg.kg^{-1} body weight and Uchendu *et al.* [17] when rats were treated subcutaneously with 500 mg.kg^{-1} body weight. The estrogen concentration of 1.87 ± 1.60 pg.ml^{-1} after 14 days of treatment, was lower in Igwe *et al.* [7] work, compared to 13.26 ± 9.69 pg.ml^{-1} in this study.

The uterine body weight was not significantly different comparing the treated group and control group of non-gravid rabbits. This also correlates with the non-significant difference in estrogen concentration. Uterine weight increase in rodents has been used as a bioindicator of the presence of estrogens [6].

The weight of the paired ovaries of the treated and control were higher than 0.017 ± 0.003 g recorded by Bitto *et al.* [4] for intact non gravid rabbits. Paired uterine horn of 0.043 ± 0.014 g recorded by Bitto *et al.* [4], was lower than

that recorded for the right and left treated and control in this work and the paired oviduct of 0.008 ± 0.002 g was lower than that recorded for the right and left treated and control in this work. Comparing lengths and widths, Bitto *et al.* [4] record of 8.07 ± 0.409 cm and Ogbuewu *et al.* [13] 8.85 cm for the length of the uterine horn which were higher than the non-derived length measured for the right and left uterine horns in the treatment and control groups in this work. The 6.00 ± 0.794 cm recorded for the length of the oviduct by Bitto *et al.* [4], was however, lower than the non-derived length recorded for the right and left oviducts for the treated and control groups in this work. The 0.933 ± 0.054 cm value recorded by Bitto *et al.* [4] for the width of the uterine horn was higher than the non-derived measurements (measured parts are in proportion with the animal's weight) recorded for any of the right or left uterine horns in the treated and control groups. According to Ogbuewu *et al.* [13] 0.06 g weight for paired uterine horn was quite lower than that recorded for the derived left and right uterine horns (measured reproductive parts were not justified by the overall weight of the animal) of treated and control groups, while the 0.49 g weight of the paired oviducts was higher than that recorded in this work comparing it with the derived right and left oviduct of the treated and control groups.

CONCLUSION

The 800 mg.kg^{-1} ethanol extract of *Spondias mombin* appeared to favour folliculogenesis in non-gravid rabbits and had no pathological effects on the entire reproductive tract of the rabbits.

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