



## POSITIVE INFLUENCE OF *COCOS NUCIFERA* (COCONUT) WATER ON FEMALE RAT'S ESTROUS CYCLE

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Article Received on 23/01/2018

Article Revised on 13/02/2018

Article Accepted on 05/03/2018

### ABSTRACT

*Cocos nucifera* (coconut) is frequently consumed by many for nutritional and medicinal purposes. This study evaluated the influence of coconut water on irregular estrous cycle in female Wistar rats. Thirty rats with irregular estrous cycles were divided into 3 groups (n=10) and administered orally with coconut water (1 or 2 ml) or bromocriptine (0.04 mg/kg) once daily for 14 days (3½ cycles for 4-day cycle). Estrous cycles of treated rats were determined via daily examination of vaginal smears from the fourth day of administration for 10 days. Estrous cycles of 80% of the rats that received 1 ml coconut water returned to normal, whereas 50% of the rats that were administered 2 ml coconut water or bromocriptine showed regular estrous cycles at the end of the treatment period. The result suggests that coconut water has high potency and efficacy to normalize irregular estrous cycle in female rats.

**KEYWORDS:** Coconut, estrous cycle, infertility, vaginal smear.

### INTRODUCTION

*Cocos nucifera* is a dominant type of tree belonging to the Family Arecaceae (Palm). The common name of *Cocos nucifera* is coconut or coconut palm. Coconut is believed to have its origin in Southeast Asia (Malaysia, Indonesia and the Philippines) and the islands between the Indian and Pacific Oceans, but it has spread throughout the tropics.<sup>[1]</sup> There are two varieties of coconut that have been identified; the tall and dwarf. The tall varieties grow slowly and bear fruits 6 to 10 years after planting, while the dwarf varieties are fast growing and bear fruits within 4 to 5 years.<sup>[2]</sup> The plant has fruits that consist of a thin hard exocarp or skin, a thicker layer of fibrous mesocarp (husk), a hard endocarp (shell), a white endosperm (kernel), and a large cavity filled with watery liquid (coconut water or milk). The endosperm is soft and jellylike when immature but becomes firm with maturity. Coconut water is abundant in the unripe fruit but is gradually absorbed as it ripens. The fruits are green at first and turn brownish at maturity.<sup>[2,3]</sup> The plant has a great capacity for natural dispersal as the nut can survive as long as 120 days floating in sea water and germinate when it gets to the soil. This facilitates the wide spreading of the plant to regions that are distant from its origin without human assistance.<sup>[2]</sup>

*Cocos nucifera* is referred to as the 'tree of life' or 'tree of heaven', because every part of the plant is useful. The different parts of the plant provide food, water, oil, fuel,

timber and medicine for many. Several parts of the fruits of *Cocos nucifera* are used for the promotion of human health in treatment or protection of diseases.<sup>[4]</sup> The kernel and tender coconut water have been shown to have antimicrobial, antioxidant, hypoglycemic, hepatoprotective, antidiarrheal, laxative, diuretic, immunostimulant, anticancer, and nutritional properties.<sup>[4-6]</sup> Coconut water has particularly been shown to contain essential bioactive components that make it a refreshing beverage, and useful to the food and biotechnology industries.<sup>[4]</sup> A phytochemical review of it has revealed that antioxidants predominated in the constituents of the endocarp and coconut water.<sup>[6]</sup> Aside from the rich nutritional and antioxidant constituents, coconut juice is believed to contain phytoestrogen and other sex hormone-like substances.<sup>[7,8]</sup> Fertility and reproductive health are influenced by the levels of serum sex hormones which regulate growth and function of reproductive organs. It is thus likely that the plant may have influence on female reproductive activity.

The focus of this study was to investigate the influence of coconut water on estrous cycle pattern using female Wistar albino rats with irregular estrous cycles.

### MATERIALS AND METHODS

#### Drug

Bromocriptine tablets (Lek Pharmaceutical & Chemical Company, Slovenia) were purchased from the Pharmacy

Department of the University of Port Harcourt Teaching Hospital, Nigeria.

### Plant material

Coconut fruits were obtained from a local fruit market (Kaduna Street, Port Harcourt, Nigeria) and identified by Dr. E. N. L. Wosu, a botanist of the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. The outer coverings were removed, the kernel was carefully broken and the liquid content (coconut water) was collected and filtered through a Whatman filter paper and stored in a refrigerator at  $-4^{\circ}\text{C}$ .

### Animals

Female Wistar albino rats with an average weight of  $200 \pm 20$  g were obtained from the Animal House of Department of Pharmacology, University of Port Harcourt, Nigeria. The animals were housed in standard cages and fed daily with rodent feeds and allowed free access to tap water. The animals were kept in a well-ventilated room with a 12 h light/dark cycle at room temperature. Animal Ethics and proper handling methods were strictly adhered to.<sup>[9]</sup>

### Experimental protocol

#### Determination of rats with irregular estrous cycles

Estrous cycles of rats were determined through vaginal smear examination before treatment with coconut water or bromocriptine. Vaginal smears were obtained daily between 9.00 am and 10.00 am from the animals for the examination. Briefly, normal saline (Juhel Nigeria Ltd., Nigeria) was drawn into the tip of the pipette and inserted about 2 mm deep into the vaginal canal of the rats, and few drops were emptied. The mixture of vaginal fluid and normal saline was then suctioned into the tip of the pipette. Few drops of this was used to prepare smear on a glass slide, covered with a cover slip and viewed under a light microscope (Olympus Microscope, Japan) using 100x and 400x magnifications. The number of occurrences of proestrous, estrous, metestrus and diestrus phases were determined. The phases are characterized by nucleated epithelial cells, cornified needle-like cells, combination of epithelial, cornified, and leucocyte cells, and mostly leucocyte cells, respectively.<sup>[10]</sup> Vaginal smear was analyzed daily for 21 days to establishment cyclicity.

Regular estrous cycle of the rats was defined by the presence of a four- or five-day cycle, typically having a regular sequence of proestrus, estrus, metestrus, and diestrus (P-E-M-D) phases. Irregular estrous cycle show deviation from this typical P-E-M-D pattern, and absence of distinct four- or five-day cycle.<sup>[10]</sup> Rats with irregular cycles were selected for the study.

#### Administration of experimental agents

Thirty rats with irregular estrous cycles were randomly divided into 3 groups ( $n=10$  per group). The rats were administered coconut water (1 or 2 ml) or bromocriptine

(0.04 mg/kg). Both agents were administered orally using an oral cannula once daily for 14 days. The dose of bromocriptine used corresponded to its average dose for treatment of infertility in females.<sup>[11]</sup> Estrous cycles of animals were studied from fifth day of administration and continued daily for 10 days and compared with irregular cycles that were present before treatment.

### Statistical analysis

Where necessary, data were analyzed using student's t-test using GraphPad Prism 5 software. Values were considered significant at  $p < 0.05$ .

## RESULTS

### Stages (or phases) of estrous cycle

Rat's vaginal smear showed different cell types which were described as proestrus, estrus, metestrus, and diestrus phases (Figures 1A-D). The proestrus phase showed presence of rounded and nucleated epithelial cells (Figure 1a). The estrus phase showed presence of cornified cells (Figure 1B). The metestrus phase was characterized by presence of a combination of leucocyte, cornified and rounded epithelial cells (Figure 1C). Leucocytes were mostly seen in the diestrus phase (Figure 1D). Animals with regular estrous cycle had estrous phase sequence of proestrus-estrus-metestrus-diestrus per cycle which was absent in rats with irregular estrous cycles.

### Influence of coconut water and bromocriptine on irregular estrous cycle

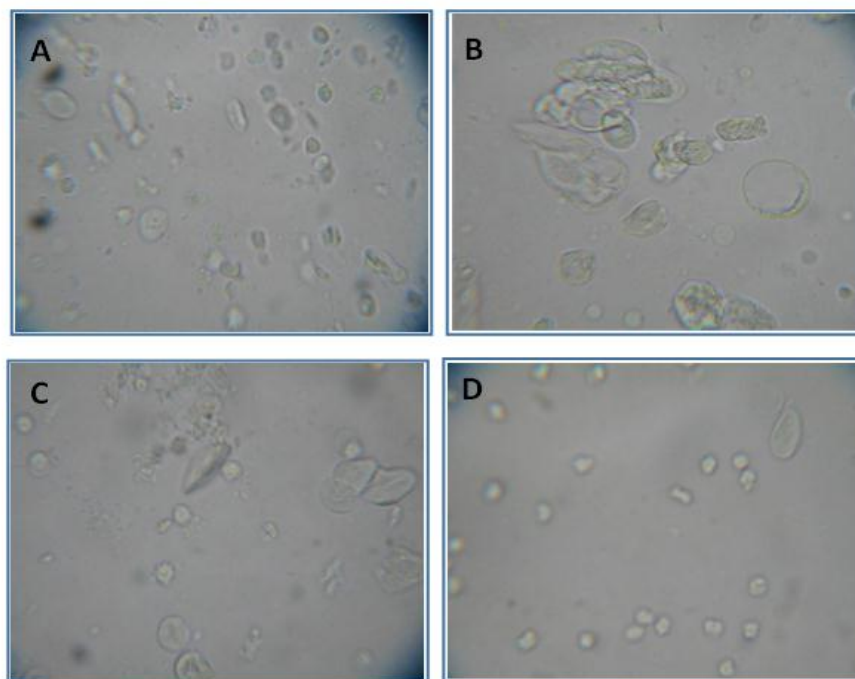
At the end of the treatment period, eight (80%) of the rats that were administered coconut water (1 ml), and five (50%) of rats that received coconut water (2 ml) showed regular estrous cycles, whereas the rest showed estrous phase patterns that were irregular. Bromocriptine induced regular estrous cycles in five rats (50%), the rest showed irregular cycles (Table 1).

The mean number of occurrences of proestrus, estrus, metestrus and diestrus stages obtained during each estrous cycle before and after coconut water and bromocriptine treatment over the study period are presented in Figure 2. Proestrus or estrus occurred at higher rates ( $p < 0.001$ ) than the other stages in all rats before they received coconut water or bromocriptine (Figure 2). The length of the stages in rats that received coconut water or bromocriptine were not significantly different from each other after treatment (Figure 2).

**Table 1: Effects of coconut water and bromocriptine on irregular estrous cycle in Wistar female rats.**

Treatment group	No. of rats with irregular estrous cycle pattern of PEMD		% of animals with irregular estrous cycle pattern after treatment
	Before Treatment	After Treatment	
Coconut water (1 ml)	10	2	20
Coconut water (2 ml)	10	5	50
Bromocriptine (0.04 mg/kg)	10	5	50

P: Proestrus, E: Estrus, M: Metestrus, D: Diestrus

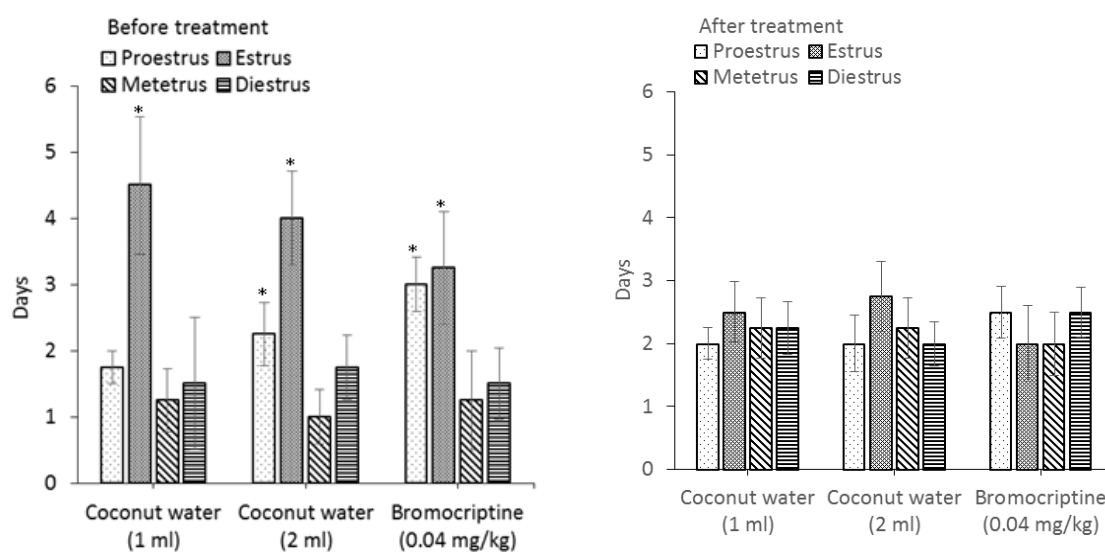
**Figure 1: Photomicrographs of unstained vaginal smear from female rats (400x)**

A: Proestrus stage, characterized by predominance of nucleated epithelial cells.

B: Estrus stage, showed presence of mostly cornified cells.

C: Metestrus smear, characterized by combination of epithelial, cornified, and leucocyte cells.

D: Diestrus smear, primarily consisted of leucocyte cells.

**Figure 2: Estrous phases before and after 14 days treatment with coconut water and bromocriptine in rats with irregular estrous cycles**

Values are expressed as mean $\pm$ SEM, n=10.

\* Significant at  $p < 0.05$ , compared to metestrus or diestrus

## DISCUSSION

This study reports the ability of coconut water to normalize irregular estrous cycle in rats. Rats with predetermined estrous cycles having irregular estrous cycles were treated with coconut water (1 or 2 ml) over a period of 14 days (3½ estrous cycles). Bromocriptine, which is a semisynthetic ergot alkaloid and effectively used in treatment of women with abnormal menstrual cycles was used as reference drug.

Rats have short estrous cycle which lasts for four to five days. The rat's estrous cycle consists of proestrus, estrus, metestrus and diestrus stages or phases. The 4 stages are detected through vaginal lavage or vaginal smear, noting characteristic cell types that are visible during each stage, in addition to differences in cell density.<sup>[12,13]</sup> In the present study, in nearly all the rats that had irregular cycles, proestrus or diestrus or both phases were prolonged; while metestrus and diestrus were shortened or missed in some animals. But coconut water was able to normalize the estrous cycles of about 80% rats that were treated. The rats exhibited regular proestrus, estrus, metestrus and diestrus sequence with a four-day cycle after receiving coconut treatment. Surprisingly, this high outcome or efficacy was produced by the lower dose (1 ml) of coconut water that was used in the experiment, while the higher dose (2 ml) produced similar effect only in 50% of the animals. Bromocriptine equally regularized estrous cycles but this occurred only in 50% of the rats that were treated, similar to what was obtained after treatment with 2 ml coconut water. This demonstrates that coconut water is very potent and appears to be more effective in restoring estrous cycle irregularity than bromocriptine over the period of exposure in this study.

Furthermore, the study showed that long term treatment may be required to obtain beneficial effects as normal estrous phase patterns were observed in animals only towards the end of the study period for both agents. This finding is not strange, however, as it is reported that the effect of bromocriptine during infertility treatment is seen only after about 6-8 weeks of administration in humans.<sup>[14]</sup>

Menstrual irregularities is an identified cause of infertility among females, and hyperprolactinemia has been established as a major cause.<sup>[15,16]</sup> High levels of prolactin inhibit production of FSH and LH which interferes with ovulation and menstruation. Bromocriptine suppresses high prolactin levels via D<sub>2</sub>-receptor activation in hyperprolactinemia and normalizes ovarian function.<sup>[16]</sup> Similarly, coconut water has been reported to decrease blood levels of prolactin and increases FSH and LH.<sup>[17]</sup> In view of the above, we suggest that bromocriptine and coconut water may act via mechanisms that involve reduction of prolactin levels to regularize estrous cycle of the rats.

## CONCLUSION

*Cocos nucifera* (coconut) water can cause regularity of the estrous cycle of female rats with irregular estrous cycle pattern.

## CONFLICTS OF INTEREST

None.

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