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Green Coconut (*Cocosnucifera*) Water Increases serum level of Some Reproductive Hormones in Hyperprolactin-infertile Female Sprague-Dawley Rats.

[1]Bakare AA, [2]Medubi OO, [3]Afodun AM

[1]Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Nigeria.

[2]Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Nigeria.

[3]Department of Anatomy, Kampala International University, Western Campus, Uganda.

Corresponding Author: Bakare AA
E-mail: aabakare@unilag.edu.ng

ABSTRACT

Hyperprolactinaemia affects about one-third of infertile women worldwide. The major hormonal implication seen during hyperprolactinaemia is low production of estrogen as prolactin is reported to inhibit follicular estradiol production in the ovary. The water from an immature green coconut fruit contains sterol which is known to be involved in the synthesis of steroid hormones *in vivo.* Thus, this study was carried out to evaluate the serum level of some reproductive hormones in hyperprolactin-induced infertile rats treated with green coconut water. One hundred and twenty-five cyclic female Sprague-Dawley rats were used for this study. The experiment was divided into 6 experimental study groups (I- VI). In experimental study group I, animals were induced with hyperprolactinaemia and withdrawn to check for natural recovery. Experimental study group II animals were post-treated with green coconut water and group III animals were the co-administered group. In experimental group IV, animals were pre-treated prior to the induction of infertility and group V animals received green coconut water only. Experiment VI is the control group and the animals received distilled water only. The result showed that green coconut water causes significant decrease in serum prolactin level and increases in serum estrogen, luteinizing hormone and follicle stimulating hormone levels in the post-treated group. The findings from this study depict clearly that green coconut water is a promising substance in regulating endocrine secretion of reproductive hormones in hyperprolactin female Sprague-Dawley rat.

Keywords: Hyperprolactinaemia, Prolactin, Estrogen, Follicle stimulating hormone, Luteinizing hormone

INTRODUCTION

Hyperprolactinaemia remains one of the leading causes of infertility especially among women of reproductive age [1]. It is defined as a consistently high serum level of prolactin (PRL) in a non-pregnant and non-lactating individual [2]. The symptoms of hyperprolactinaemia in the reproductive system are due to both direct action of prolactin on target tissues and indirect effects mediated by the decrease in gonadotropin pulsatile secretion which leads to gonadal dysfunction [3]. High serum prolactin (PRL) level has been associated with the inhibition of the hypothalamic gonadotropin releasing hormone, suppression of preovulatory gonadotropin surge and consequent inhibition of gonadal function [3]. Thus when PRL is increased, there is the suppression of other reproductive hormones. PRL interferes with the release of gonadotropin-releasing hormone which leads to decrease in the release of gonadotropins which are follicle stimulating hormones and luteinizing hormone [4]. This eventually causes a reduction in granulosa cell proliferation in the ovary with a consequently low granulosa estrogen production.

The green coconut is an immature coconut fruit with green exocarp. It is also referred to as a young or tender coconut because it is harvested before maturity at about 6 months of age[5]. The *green coconut* water (GCW) is the water in the immature coconut fruit. It has been shown to aid the maintenance of pregnancy in mice [6,7]. It has also been shown to improve sexual vitality, boost sperm count and enhance sperm motility [8]. It was reported previously to have antioxidant property in hyperprolactin-induced oxidative stress [9]. GCW demonstrated estrogen-like properties when administered in postmenopausal rats with estrogen levels comparable to rats that still had their ovaries [10]. β-sitosterol, stigmasterol, fucosterol and α- spinasterol are plant sterols present in green coconut water and are known to be involved in the synthesis of steroid hormones *invivo*[11]. Hence this study was designed with the aim of evaluating the endocrine regulating property of GCW in hyperprolactinaemia.

MATERIALS AND METHODS

Plant material

The immature coconut fruits were harvested at six months of age from Chief William Kudofoke Coconut Farm, Ajara-Topa, Badagry, Lagos. The average

weight of the fruit was 1.55 Kg. The fruit was authenticated at the Federal Institute of Forestry Research in Ibadan with plant's accession No. FHI 109665.

Extraction of the green coconut water
The immature coconut fruits were washed and dehusked. A sterile iron rod was used to open the germinal pore. The extraction of the coconut water was done through the pore, poured into a clean airtight bottle and kept in the refrigerator at 4°C. The GCW was replaced every three weeks during the course of the experiment[12].

Experimental Animals
One hundred and twenty-five adult female Sprague-Dawley rats weighing 145 g -170 g (7-9 weeks old) were obtained from the Nigerian Institute of Medical Research, Yaba, Lagos. The animals were kept in standard plastic cages in the Animal House of the Department of Anatomy, College of Medicine, University of Lagos and allowed free access to food and water ad libitum. The animals were kept in accordance to conditions stipulated by the U.S National Institute of Health involving a cycle of 12 hours light and dark. The animals were processed in accordance with guidelines and regulations of the Institutional Animal and Care and Use Committee for the use of animals. The animals were allowed to acclimatize for two weeks in the animal house of the Department of Anatomy, University of Lagos before the commencement of the study.

Ethical approval
All authors hereby declare that "Principles of laboratory animal care" according to NIH publication, revised in 1985 were followed. All experiments were examined and approved by the College of Medicine University of Lagos Health Research Ethics Committee and were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Induction of Hyperprolactinaemia
Metoclopramide hydrochloride (MCH) was used to induce hyperprolactinaemia [13]. The drug was purchased from Pfizer pharmaceutical, Ikeja Lagos, Nigeria. MCH at a dose of 0.2 mg/100 gram body weight (g. b. w.) was administered daily for 28 days [13, 14]. The drug was dissolved in water and dose was calculated by simple fraction estimation based on the animal's weight and the administration was done via oral route with the use of an Oropharyngeal canula.

Animal grouping and Administration of GCW
The animals were randomly divided into six (6) major experimental groups I to VI. In experimental study group I, 20 rats were subdivided into four groups Ia, Ib, Ic and Id of 5 rats each. MCH at 0.2mg/100 g. b. w. was administered daily for 28days in Ia and this was used to induce hyperprolactinaemia. The administration of MCH was withdrawn for 8, 16 and 28days in Ib, Ic and Id respectively to check for natural recovery. Experimental study group II was made up of 30 rats subdivided into six groups; IIa, IIb,IIc, IId, IIe and IIf of 5 rats each. The animals were post-treated with 5 ml/100 g. b. w. and 10ml/100 g. b. w. of GCW following the administration of MCH for 8, 16 and 28days in (IIa, IIb and IIc) and (IId, IIe and IIf) respectively. In experiment III, 10 rats were subdivided divided into IIIa and IIIb of 5 rats each; IIIa animals were co-administered with 0.2mg/100g g. b. w. of MCH and 5ml/100 g. b. w. of GCW and IIIb were co-administered with 0.2mg/100g g. b. w. of MCH and 10ml/100 g. b. w. of GCW. In experimental group IV, 30 rats were subdivided into six groups; IVa, IVb, IVc, IVd, IVe and IVf of 5 rats each. The animals were pre-treated with 5ml/100 g. b. w. of GCW before the administration of MCH for 8, 16 and 28days in IVa, IVb and IVc and 10ml/100 g. b. w. of GCW for 8, 16 and 28days in IVd, IVe and IVf. Experiment V was made up of 30 rats subdivided into six groups; Va, Vb, Vc, Vd, Ve and Vf of 5 rats each. The animals received 5 ml/100 g. b. w. of GCW only for 8, 16 and 28days in (Va, Vb and Vc) and 10ml/100 g. b. w. of GCW only (Vd, Ve and Vf) and Experiment VI, the control group received distilled water only.

Hormone assays
Blood samples were obtained from the orbital venous sinus with microhematocrit tube inserted into the medial canthus of the eye between 7- 8 a.m. and immediately spurned at 3,000 rpm for 10 minutes. The serum was separated and frozen at - 20°C until the assays were carried out. The assays employed competitive inhibition enzyme immunoassay technique using enzyme immunoassay ELISA kits.

Prolactine assay: This was carried out using the enzyme immunoassay ELISA kit, catalogue number: 4226Z. The double-antibody immunoassay assay utilized PRL antibody for solid phase immobilization and mouse monoclonal antibody enzyme (horseradish peroxidase) conjugate solution. The test sample was allowed to react simultaneously with the antibodies, resulting in the molecules being sandwiched between the solid phases. After incubation for 60minutes at room temperature, the wells were washed with water to remove unbound labelled antibodies. A solution of tetramethylbenzidine (TMB) was added and incubated for 20minutes resulting in the development of a blue colour. The colour development was stopped with the addition of hydrochloric acid and the colour was changed to yellow and measured at 450nm. The mean values for each specimen was determined with the corresponding concentration of PRL in mIU/ml from the standard curve.

Oestrogen assay: This assay employed the competitive inhibition enzyme immunoassay technique using enzyme immunoassay ELISA kit of catalogue number: ab108667. Absorbance was measured with an automatic spectrophotometer at 450nm. A standard curve was obtained by plotting the

concentration of the standard versus the absorbance and the concentration of estrogen was read from the standard curve.

Follicle stimulating hormone assay: This was carried out using the enzyme immunoassay ELISA kit, catalogue number: 4224Z. The assay utilized a polyclonal FSH antibody for solid phase and mouse monoclonal antibody enzyme conjugate solution. The test sample was allowed to react simultaneously with the antibodies, resulting in the FSH molecules being sandwiched between the solid phases. After incubation for 60minutes at room temperature, the wells were washed with water to remove unbound labelled antibodies. A solution of TMB was added and incubated for 20minutes resulting in the development of a blue colour. The colour development was stopped with the addition of hydrochloric acid and the colour was changed to yellow and measured spectrometrically at 450nm. The concentration of FSH is directly proportional to the colour intensity of the test sample. The value for each set of reference standards, specimens, controls and samples were calculated by constructing a standard curve. The mean absorbance values obtained from each reference standard was plotted against its concentration in mIU/ml on graph paper with absorbance values on vertical or Y-axis and concentration on horizontal or X-axis. The mean values for each specimen was determined with the corresponding concentration of FSH in mIU/ml from the standard curve.

Luteinising hormone assay: This was carried out using the enzyme immunoassay ELISA kit, catalogue number: 4225Z. The assay utilized LH antibody for solid phase and mouse monoclonal antibody enzyme conjugate solution. The test sample was allowed to react simultaneously with the antibodies, resulting in the LH molecules being sandwiched between the solid phase and enzyme linked antibodies. After 60minutes incubations at room temperature, the wells were washed with water to remove unbound labelled antibodies. A solution of TMB was added and incubated for 20minutes resulting in the development of a blue colour. The colour development was stopped with the addition of hydrochloric acid and the colour was changed to yellow and measured at 450nm. The concentration of LH is directly proportional to the colour intensity of the test sample. The value for each set of reference standards, specimens, controls and samples were calculated by constructing a standard curve. The mean absorbance values obtained from each reference standard was plotted against its concentration in mIU/ml on graph paper with absorbance values on vertical or Y-axis and concentration on horizontal or X-axis. The mean values for each specimen was determined with the corresponding concentration of LH in mIU/ml from the standard curve.

Statistics

Data were analysed using SPSS 16.0 software package (SPSS Inc; Chicago U.S.A). The results were expressed as mean ± standard error. Statistical comparisons were made using analysis of variance (ANOVA) with Scheffe's post hoc test for within group and between groups comparison. The level of significance was considered at $p < 0.05$.

RESULTS

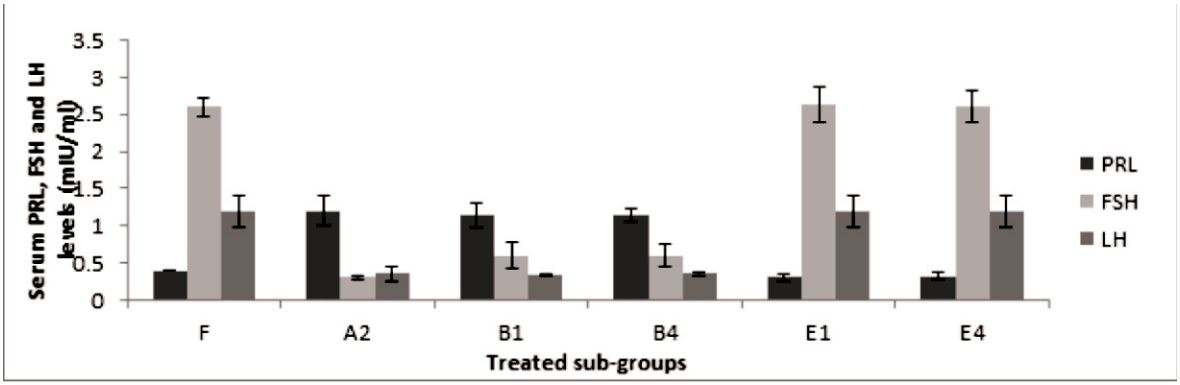

Figure a: Serum Prolactin, Luteinizing and Follicle Stimulating Hormone Levels in the 8 Days Experimental Groups

Table a: The Serum Levels of Estrogen in the 8 Days Experimental Groups

GROUP		ESTRG (mg/ml)
Control	(F1)	28.76 ± 0.51
Withdrawal	(A2)	6.67 ± 0.42**
Post-treated	(B1)	8.96 ± 0.92**
	(B4)	10.06 ±0.16**
GCW treated	(E1)	29.21 ± 4.31
	(E4)	29.12 ± 0.26

F – Control, A2 - MCH $_{28\ days}$-$WD_{8\ days}$, B1 - $MCH_{28\ days}$-$GCW_{L8\ days}$, B4 - $MCH_{28\ days}$-$GCW_{H8\ days}$, E1 - $GCW_{L8\ days}$, E4 - $GCW_{H8\ days}$. **Key for Figure a**: DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; GCW_L:5 ml/100 g. b. w. of green coconut water; GCW_H: 10 ml/100 g. b. w. of green coconut water; –: treated followed by.

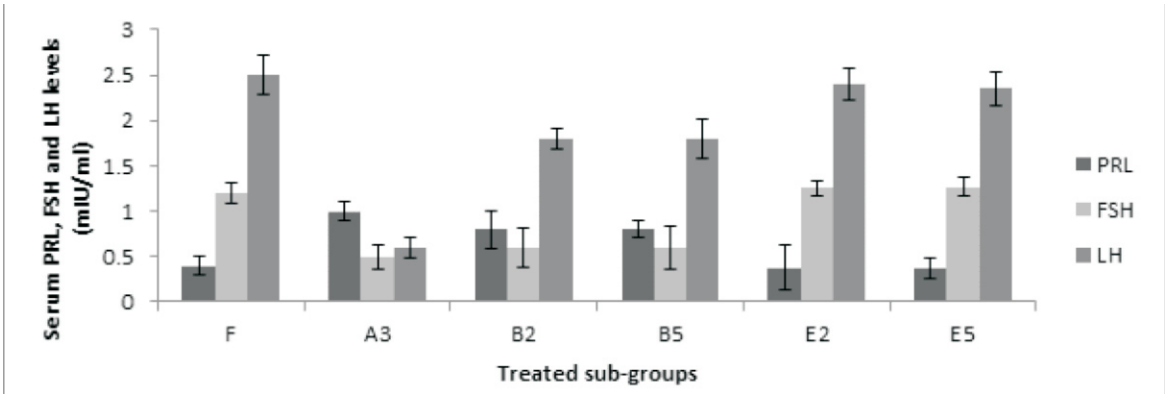

Figure b: Serum Prolactin, Luteinizing and Follicle Stimulating Hormone Levels in the16 Days Experimental Groups

Table b: The Serum Levels of Estrogen in the 8 Days Experimental Groups

GROUP		ESTRG (mg/ml)
Control	(F2)	27.15 ± 0.23
Withdrawal	(A3)	9.30 ± 0.52**
Post-treated	(B2)	22.60 ± 0.37*
	(B4)	23.04 ± 0.35*
GCW treated	(E2)	28.28 ± 0.04
	(E5)	28.08 ± 3.89

F- Control, A3 - MCH_{28days}-WD_{16days}, B2 - MCH_{28days}-$GCW_{L16\ days}$, B5 - $MCH_{28\ days}$-$GCW_{H16\ days}$, E2 - $GCW_{L16\ days}$, E5 - $GCW_{H16\ days.}$ **Key for Figure b:** DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; $GCW_{M/d}$:5 ml/100 g. b. w. of green coconut water; $GCW_{H/d}$: 10 ml/100 g. b. w. of green coconut water; –: treated followed by.

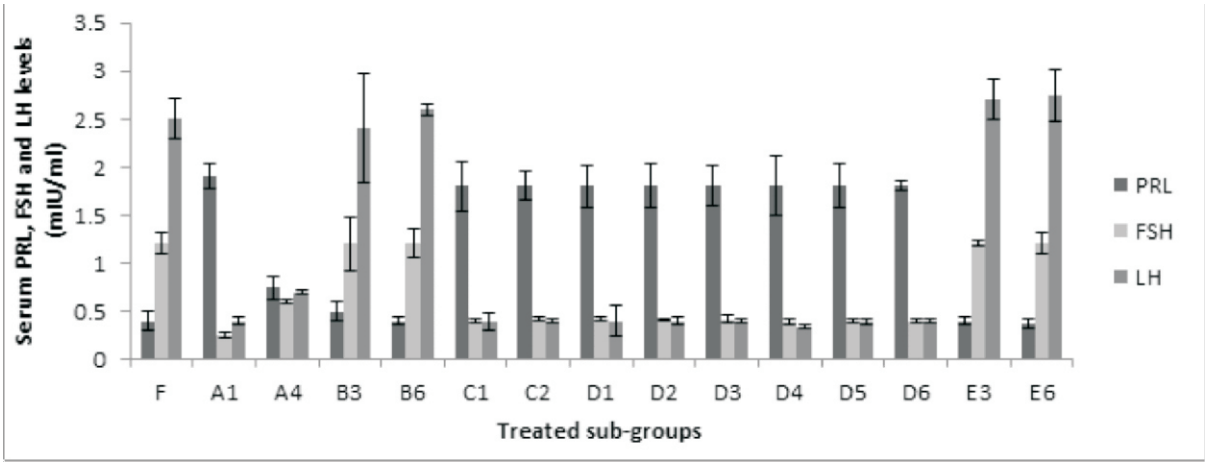

Figure c: Serum Prolactin, Luteinizing and Follicle Stimulating Hormone Levels in the28 Days Experimental Groups.

Table c: The Serum Levels of Oestrogen in the 28 Days Experimental Groups

GROUP		ESTRG (mg/ml)
Control	(F1)	28.76 ± 0.51
	(F2)	27.15 ± 0.23
Induction	(A1)	3.91 ± 0.29**
Withdrawal	(A4)	19.30 ± 2.54*
Post-treated	(B3)	28.66 ± 0.50
	(B6)	30.96 ± 2.54
Co-administered	(C1)	4.17 ± 0.31**
	(C2)	4.17 ± 0.57**
Pre-treated	(D1)	4.19± 0.32**
	(D2)	3.93± 0.66**
	(D3)	4.09± 0.49**
	(D4)	4.25± 1.034**
	(D5)	4.13 ± 1.41**
	(D6)	4.41± 0.03**
GCW treated	(E3)	29.96 ± 5.35
	(E6)	30.04 ± 4.88

F- Control**;** A1 – $MCH_{28\ days}$; A4 - $MCH_{28\ days}$-$WD_{28\ days}$; B3 - $MCH_{28\ days}$-$GCW_{L28\ days}$; B6 - $MCH_{28\ days}$-$GCW_{H28\ days}$; C1- $MCH_{28\ day}$ + $GCW_{L28\ day}$; C2- $MCH_{28\ day}$ + $GCW_{H28\ day}$; D1- $GCW_{L8\ days}$- $MCH_{28\ days}$; D2- $GCW_{H8\ days}$ - $MCH_{28\ days}$; D3- $GCW_{L16\ days}$- $MCH_{28\ days}$; D4- $GCW_{H16\ days}$- $MCH_{28\ days}$; D5- $GCW_{L28\ days}$ - $MCH_{28\ days}$; D6- $GCW_{H28\ days}$ - $MCH_{28\ days}$; E3 - $GCW_{L28\ days}$; E6-$GCW_{H28\ days.}$ **Key for Figure c:** DSTL: Distilled water; MCH: 0.2 mg/100 g. b. w. metoclopramide hydrochloride; WD: Withdrawn; $GCW_{M/d}$:5 ml/100 g. b. w. of green coconut water; $GCW_{H/d}$: 10 ml/100 g. b. w. of green coconut water; –: treated followed by.

DISCUSSION

Many lines of evidence in human and experimental model indicate that changes in the secretion and action of prolactin are sufficient to affect the integrity of the hypothalamic-pituitary-gonadal axis. Hyperprolactinaemia has been shown to suppress the release of gonadotropin releasing hormone by the hypothalamus which consequently inhibits the secretion of follicle stimulating hormone and luteinizing hormone by the pituitary gland. The decrease in the pituitary release of FSH and LH through integrated central and peripheral mechanisms suppresses the physiological activities in the gonad thus leading to infertility [15].

FSH is transported the ovaries where it stimulates the growth of ovarian follicles while LH triggers ovulation and promote the formation of corpus luteum. Estrogen is thus produced by the developing follicle and corpus luteum. Hyperprolactinaemia affects ovarian steroidogenesis as high serum prolactin has been shown to reduce granulosa *cell* proliferation which leads to a decrease in follicular estradiol production [16,17]. In moderate amounts estrogen helps to control the levels

of GnRH, FSH and LH. Hence any factor that causes imbalance in hormonal profile disrupts reproductive processes. In hyperprolactinaemia, it has been reported that the inhibition of follicular maturation is attributed to decrease in the secretion of FSH and LH [18]. More so, this disrupts luteinizing activities of the ovarian follicles which leads to luteal phase defect that prevent preovulatory LH- surge [19]. The result from this study is in conformity with the findings of Crave where increase in serum estrogen stimulates pituitary gland release of FSH and LH. More so, the result from this present study is in line with estrogenic negative feedback on the lactotrophic cells of the anterior pituitary gland to suppress the release of prolactin [20]. It is clear that the estrogenic property of GCW is responsible for the increase in FSH and LH serum levels.

CONCLLUSION

Green coconut water causes significant decrease in serum PRL level and increases in serum estrogen, LH and FSH levels. The findings from this study suggest that green coconut water is a promising substance in reversing the hormone imbalance associated with hyperprolactinaemia. This study would serve as a preliminary template for further studies and subsequent research work on hyperprolactin-induced infertility.

Conflict of interest

The authors declare that there is no conflicts of interest**.**

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