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Research Article

Estrous cycle study on green coconut water in experimentally induced hyperprolactine in female Sprague-Dawleys rats

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Abstract: The objective of present study is to determine the effect of green coconut water on estrous cycle in metoclopramide-induce hyperprolactinemia in female Sprague-Dawley rats. Fifty adult cyclic female Sprague-Dawley rats were used for this study. The animals were treated with green coconut water (GCW) before, after and concurrently with metoclopramide to induce hyperprolactinemia. The post- treated groups demonstrated a statistical comparable result in the number of completed cycles and pattern of cycling while the pre-treated and co-administered groups show significant reduction in the number of completed cycle due to delay on the diestrous phase of the cycle. There was earlier reversal of estrous cyclicity in the green coconut water post-treated rats while the pre-treated and co-administered group did not show reversal of estrous cyclicity. Findings of the present study indicate GCW has fertility enhancing properties by regulating estrous cycle pattern.
Keywords: Estrous cycle, Green coconut water, Metoclopramide

INTRODUCTION

Hyperprolactinemia is a common endocrine cause of reproductive dysfunction affecting about one-third of infertile women [1]. The clinical suppression of high prolactin in hyperprolactinemia is primarily with dopamine agonist [DA] drugs and has been reported to present a lot of side effects which affect 20-78% of patients undergoing treatments. These effects include dizziness, nausea, postural and orthostatic hypotension and vomiting through stimulation of the brainstem vomiting centre. Also vasospasms with serious consequences such as myocardial infarction, stroke and exacerbating acute psychosis [2, 3, 4]. However the major drawbacks of the DA drugs is that they are expensive and there is the need for long-term therapy and as a matter of fact, treatment with bromocriptine and some other DA drugs may lead to recurrence of hyperprolactinemia and pituitary tumor regrowth in most patients after their discontinuation [5]. The option of treatment with radiation has been reported with the risk of hypopituitarism and data of its success are limited which makes treatment less common [**6**]. Transsphenoidal surgery which is another treatment procedure for prolactinomas has been reported with a recurrence rate of 21% usually within the first year following surgery. And complications such as cerebrospinal fluid rhinorrhea and transient diabetes insipidus may also occur [**7**].

Natural products have remained the cornerstone of health care including fertility management. In Africa, natural approaches to infertility have been enormously successful [8]. The green coconut fruit is a young or immature coconut fruit about the six month of age. Nearly one third of the world population depends on coconut as a source of food and medicine. Each nut may contain between 200 to 1000 ml of water depending on the age and size of the fruit. The fruit is harvested at an immature green stage mainly because of the high volume of water and most healing properties as more nutrients are still contained in it. The nutrient travels to other part of the fruit as the fruit ages [9]. It has been reported to treat many disease conditions as this is justified by its unique chemical composition. It is a rich source of nutrients (Sugars, Minerals, Proteins, Vitamins, Fat and fibers) and Photochemicals (Phytohormones, Nitrogenous compounds, Organic acids and Enzymes) [10, 11, 12].

It was reported that green coconut water (GCW) demonstrated estrogen-like properties when administered in several groups of postmenopausal rats. The rats demonstrated estrogen levels comparable to rats that still had their ovaries [13]**.** GCW has been reported to aid the maintenance of pregnancy as the number delivered at the end of the gestation period corresponded to the number of implantation sites counted on day10 of pregnancy in mice. It can therefore be used in women with threatened or habitual abortion [14, 15]. GCW have been reported to aid the elimination of toxins and enhances weight loss in a completely natural form [16]. This present study was carried out to investigate the effects of green coconut water on hyperprolactine induced estrous acyclicty in female Sprague-Dawley rats exploring it fertility enhancing potential that is affordable and widely available in all most every part of the world.

MATERIALS AND METHODS

Green coconut fruit

The immature coconut fruits were purchased from a coconut farm in Ajara, Topa, Badagry, Lagos. The average weight of the fruit was 1.55kg. The fruit was authenticated in the forest herbarium, Ibadan. The plant's ascension number is No FHI 109665.

Extraction of the green coconut water

The unripe coconut fruits were washed and dehusked. The extraction of the water was done through the germinal pore, poured directly into an airtight bottle and kept refrigerated for three weeks.

Animal Material

A total of fifty adult female SD- rats weighing 145-170g between 6-8 weeks old were obtained from the Nigerian Institute of Medical Research, Yaba, Lagos and were authenticated by a taxonomist in the department of Zoology of the University of Lagos. The animals were kept in standard plastic cages in the animal house of the Department of Anatomy and allowed to acclimatize for two weeks under standard laboratory conditions of room temperature 27ºC with a photoperiodicity of twelve hours light alternating with twelve hours of darkness. The animals had free access to clean tap water and pellets.

Establishment of estrous cyclicity

The rats then went through a recruitment phase of establishing estrous cyclicity determined from the cytology of vaginal smears obtained daily between 8.00 a.m. and 10.00 a.m. briefly, normal saline was drawn into the tip of the pipette, which was inserted 2mm deep into the vaginal canal and 2 drops emptied into the vaginal cavity. The mixture of vaginal fluid and normal saline was then suctioned into the tip of the pipette. The smear was placed on glass slide and examined under the light microscope immediately before drying up. The first day of the estrous cycle designated as metestrus, leukocytes amidst remnants of large squamous cells. The second day, diestrus phase showed predominance of leukocytes and a few large nucleated cells. The third day showed large nucleated cells with the leukocytes, designated as the proestrus phase. The fourth day designated the estrous phase showed large flakes of squamous cells.

Treatment protocol

The animals with established estrous cyclicity of 4days were randomly divided into six (6) major experimental groups I to VI. Experiment I was designed to induce hyperprolactinemia treated with metoclopramide at 0.2mg/100g body weight/day through oral route for 28days and withdrew for 8, 16 and 28days after induction. In experiment II, the post-treated group, rats were randomly subdivided into 2 subgroups (IIa and IIb) with 5 rats in each treated with 5ml/100gbw and 10ml/100gbw of green coconut water respectively after induction. Experiment III was the co-administered group subdivided into 2 subgroups (IIIa and IIIb) with 5 rats in each. The subgroup IIIa was treated with 0.2mg/100g body weight/day and 5ml/100gbw concurrently and IIIb received 0.2mg/100g body weight/day and 10ml/100gbw of green coconut water concurrently. The experiment IV was the pre-treated group and rats in this group were randomly divided into 2 groups of 5rats each pre-treated with 5ml/100gbw and 10ml/100gbw of green coconut water respectively before induction. Experiment V was the GCW treated group subdivided into 2 subgroups (Va and Vb) with 5 rats in each treated with 5ml/100gbw and 10ml/100gbw of green coconut water respectively. In experiment VI, the control group, 5rats received distilled water only. All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals [17] and were approved by the Departmental Committee on the Use and Care of Animals.

Statistical analysis

Results were expressed as means ± standard deviation (SD) and subjected to statistical analysis using one-way analysis of variance (ANOVA) and the Scheffe's post-hoc test. The significance level considered was $p < 0.05$.

RESULTS

Number of completed estrous cycles

The control group animals completed a cycle every 4 days (Table1a, b and c). The animals in the induced group spent more days in completing a single cycle with a statistically significant decrease in the number of cycles completed when compared with the control (Table 1c). MCH withdrew for 8, 16 and 28days demonstrated a statistically significant decrease in the number of completed cycles in the respective time of withdrawal when compared with the control (Table1a, b and c). The Post-treated groups for 8 and 16 days at both doses demonstrated a statistically significant decrease in the number of completed cycles and when compared with the control (Table 1a and b). However, post-treatment with GCW for 28 days demonstrated number of cycles that was statistically comparable with the respective control group. This shows that at 28days of treatment with GCW, a reversal of normal cyclic pattern was re-established when compared with the group withdrew for 28 days (Table 1c). The pretreated groups and co-administered groups demonstrated a comparable number of completed cycles with the induced group with no statistical significant differences (Table 1c). The administration of GCW only for 8, 16 and 28 at both low and high doses demonstrated result that was statistically comparable with the control. The animals completed a cycle in a normal duration of 4 days (Table 1a, b and c).

Pattern of estrous cycling

The rats in the control group exhibited 4days of cycling with phases changing daily from metestrus, diestrus, and proestrus to estrus leading to an equal number of days on each phase of the estrous cycle (Table 2a, b and c). The administration of MCH for 28 demonstrated a statistically significance difference in the number of days of estrous phases and when compared with the control. There was a statistical significant increase in the number of days spent on diestrus while statistical significant decrease in the number of days was spent on metestrus, proestrus and estrus (Table2c). MCH withdrew for 8, 16 and 28 days demonstrated statistically significant decrease in the number of days spent on Metestrus, proestrus and estrous while a statistically significant increase in the number of days was spent on the diestrous phase (Table 2a, b and c). Post-treated with GCW for 8 and 16 days demonstrated statistically significant decrease in the number of days spent on metestrus, proestrus and estrus and statistically significant increase in the number spent on the diestrous phase (Table 2a and b). The post-treated with GCW for 28 days demonstrated a statistically comparable result in the number days spent on each phase with the control group (Table 2c). The number of days spent on each phase in the pretreated groups for 8, 16 and 28 days at both low and high doses demonstrated a comparable result with induced group (Table 2c). The administration of GCW for 8, 16 and 28 at both low and high doses demonstrated result that was statistically comparable with the control. The rats spent equal number of days on each phase of estrous cycle (Table 2a, b and c).

Table 1: The Number Of Completed Number Of Estrous Cycles In The Experimental And Control Sprague-Dawley Rats.

Table 1a: 8days duration

Sub-Group Detail	No Of Completed Estrous Cycle
$DSTL_{8days}$	2.00± .00
$MCH_{28\ days}$-$WD_{\ 8\ days}$	0.00± .00
$MCH_{28\ days}$-$GCW_{8daysM/d}$	0.00± .00
$MCH_{28\ days}$-$GCW_{\ 8days\ H/d}$	0.00± .00
$GCW_{\ 8days\ M/d}$	2.00± .00
$GCW_{\ 8days\ H/d}$	2.00± .00

Table 1b: 16days duration

Sub-Group Detail	No Of Completed Estrous Cycle
$DSTL_{16days}$	4.00± .00
$MCH_{28\ days}$-$WD_{\ 16\ days}$	0.00± .00
$MCH_{28\ days}$- $GCW_{16days\ M/d}$	1.80± .45 [a]
$MCH_{28\ days}$- $GCW_{16days\ H/d}$	2.00±.00 [a]
$GCW_{16days\ M/d}$	4.00± .00
$GCW_{16days\ H/d}$	3.80± .45

Table 1c: 28days duration

Sub-Group Detail	No Of Completed Estrous Cycle
$DSTL_{28days}$	7.00± .00
$MCH_{\ 28\ days}$	3.20± .45[b]
$MCH_{28\ days}$-$WD_{\ 28\ days}$	4.40± .55[a]
$MCH_{28\ days}$- $GCW_{28days\ M/d}$	6.00± .00
$MCH_{28\ days}$- $GCW_{28days\ H/d}$	6.40± .55 [a]
$MCH_{28\ days}$+ $GCW_{28days\ M/d}$	5.60 ±.55 [a]
$MCH_{28\ days}$+ $GCW_{28days\ H/d}$	4.80±.45 [a]
$GCW_{\ 8days\ M/d}$-$MCH_{\ 28days}$	5.40 ±.55 [a]
$GCW_{8daysH/d}$-$MCH_{\ 28days}$	5.00±.00 [a]
$GCW_{16days\ M/d}$-$MCH_{\ 28days}$	5.40 ±.55 [a]
$GCW_{16days\ H/d}$ -$MCH_{\ 28days}$	5.20±.45 [a]
$GCW_{\ 28days\ M/d}$ -$MCH_{\ 28days}$	4.40±.55 [a]
$GCW_{\ 28days\ H/d}$ -MCH_{28days}	5.20±.45 [a]
$GCW_{\ 28days\ M/d}$	7.00± .00
$GCW_{\ 28days\ H/d}$	7.00± .00

Keys; DSTL: Distilled water; MCH: Metoclopramide; GCW: Green coconut water . All values are expressed as mean ± standard deviation, [a]Significant differences; $p < 0.05$, [b]Significant differences; < 0.001

Table 2: The Number Of Days Of Estrous Phases In The Experimental And Control Sprague-Dawley Rats

Table 2a: 8days duration

Sub-Group Detail	Metaestrous	Diestrous	Proestrous	Estrous
DSL_{8days}	2.00±.00	2.00± .00	2.00± .00	2.00± .00
$MCL_{28\ days}$-$WD_{8\ days}$	0.00 ±.00	8.00 ± .00 [b]	0.00± .00	0.00± .00
$MCL_{28\ days}$-$GCW_{8daysM/d}$	0.00± .00	8.00 ±.00 [b]	0.00± .00	0.00±.00
$MCL_{28\ days}$-$GCW_{8days\ H/d}$	0.00± .00	7.40± .55 [b]	0.60± .55 [a]	0.00± .00
$GCW_{8days\ M/d}$	1.80±.55	2.00±.00	2.20±.55	2.00±.00
$GCW_{8days\ H/d}$	1.80±.55	2.00±.00	2.20±.55	2.00±.00

Table 2b: 16days duration

Sub-Group Detail	Metaestrous	Diestrous	Proestrous	Estrous
DSL_{16days}	4.00± .00	4.00± .00	4.00± .00	4.00± .00
$MCH_{28\ days}$-$WD_{16\ days}$	0.00± .00	15.40±.55 [b]	0.60± .55 [b]	0.00±.00
$MCH_{28\ days}$-$GCW_{16days\ M/d}$	1.00±.00*	11.40± .89 [b]	2.20± .45 [a]	1.20±.45 [a]
$MCH_{28\ days}$-$GCW_{16days\ H/d}$	1.80± .45 [a]	10.00± 1.22 [b]	2.40± .55 [a]	1.80±.45 [a]
$GCW_{16days\ M/d}$	3.80±.45	4.00± .00	4.20 ± .45	4.00± .00
$GCW_{16days\ H/d}$	3.70±.84	4.40 ±.55	4.10±.55	3.80±.45

Table 2c: 28days duration

Sub-Group Detail	Metaestrous	Diestrous	Proestrous	Estrous
DSL_{28days}	7.00± .00	7.00± .00	7.00± .00	7.00± .00
$MCH_{28\ days}$	4.80± .84 [a]	14.40± 1.14 [b]	4.40± .55 [a]	4.40± .55 [a]
$MCH_{28\ days}$-$WD_{28\ days}$	2.40 ±.55 [a]	18.00± 1.00 [b]	3.00± .00 [a]	3.60± .55 [a]
$MCH_{28\ days}$-$GCW_{28days\ M/d}$	6.20± .45	9.60± .55	6.00± .00	6.80± .45
$MCH_{28\ days}$-$GCW_{28days\ H/d}$	6.80± .45	9.20± .45	7.00± .00	6.00± .00
$(MCH+GCW_{\ M/d})_{\ 28days}$	5.60±.89 [a]	11.40±1.67 [b]	5.40±.55 [a]	5.60±.55 [a]
$(MCL+GCW_{\ H/d})_{\ 28days}$	4.40±.54 [a]	14.40±1.14 [b]	4.60±.54 [a]	4.80±.45 [a]
$GCW_{8days\ M/d}$-$MCH_{\ 28days}$	4.40±.89 [a]	12.60±.54 [b]	5.40± .55 [a]	5.60±.54 [a]
$GCW_{\ 8days\ H/d}$-$MCH_{\ 28days}$	5.00±.00 [a]	12.60±.55 [b]	5.00±.00 [a]	5.40±.54 [a]
$GCW_{\ 16days\ M/d}$-MCH_{28days}	5.20±.84 [a]	11.80±1.09 [b]	5.40±.54 [a]	5.60±.55 [a]
$GCW_{\ 16days\ H/d}$-$MCH_{\ 28days}$	5.40± .55 [a]	12.00±1.22 [b]	5.20±.45 [a]	5.40±.55 [a]
$GCW_{\ 28days\ M/d}$-$MCH_{\ 28days}$	5.00±.00 [a]	13.20±1.30 [b]	4.60±.89 [a]	5.20±.45 [a]
$GCW_{\ 28days\ H/d}$- $MCH_{\ 28days}$	5.00±.00 [a]	12.60±.89 [b]	5.20±.45 [a]	5.20±.45 [a]
$GCW_{28days\ M/d}$	6.60± .45	7.10±.45	7.20±.84	7.10±.55
$GCW_{28days\ H/d}$	6.90±.45	6.90± .45	7.20±.55	7.00±.00

Keys; DSTL: Distilled water; MCH: Metoclopramide; GCW: Green coconut water. All values are expressed as mean ± standard deviation. [a]Significant differences; $p < 0.05$; [b]Significant differences; < 0.001

DISCUSSION

Estrous cycle is a major aspect of reproductive capacity which comprises recurring physiological changes characterize by reproductive hormones in fertile female mammals [18].The findings, from the present study depict clearly that green coconut water is a promising substance in reversing infertility induced as a result of high prolactin. It has been reported to be a source of estrogen [13**]** and may be responsible for reversing infertility. Hence may be use as hormone replacement therapy in hyperprolactine model. In the post- treated rats, estrous cycles were reversed as shown on table 1 and 2 while there was no reversal estrous cyclicity in the pre-treated and co-administered group. It is indicated in these groups that GCW did not offer it fertility enhancing properties as a prophylactic and modulating substance in hyperprolactin- acycling rat. However, in the post-treated group, estrous cycle remained conveniently reversed by 28day of post-treatment after discontinuation of metoclopramide. The cycling pattern in the post-treatment groups were comparable to the control particularly at the 28days post-treated groups suggesting that GCW has amel-iorative effect on disrupted estrous cycle induced by metoclopramide. The prolongation of diestrous phase have been attributed to infertility [19] and shown in metoclopramide treated rats. Our study demonstrated that green coconut water reverse estrous acyclicity by significantly reducing the duration of the diestrus phases as metoclopramide delayed estrous cycling in rats by spending more days on diestrous phases. The reversal occurs as a result of stimulated follicular maturation prior to inhibition by metoclopramide. Though this study was carried out in rat, it can be assumed to be possible occurrence in human since the mechanism of reproduction is quite similar in the two subjects. This study may therefore serve as a template

for further researches in hyperprolactine- induce infertility.

CONCLUSION

The results of this investigation have demonstrated that green coconut water has estrous cycling regulating properties in the animals. The GCW may therefore have fertility Potential that can further be explored.

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