Fertility Assessment of Green Coconut Water in Experimentally Induced Hyperprolactinaemia in Female Sprague-Dawley Rats

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ABSTRACT
Background: The majority of the causes of female infertility have been associated with imbalances in reproductive hormones particularly hyperprolactinaemia. Green coconut water has been shown to demonstrate estrogenic effect.
Objective: To determine the effects of green coconut water on fertility rate in hyperprolactin-induced infertility in female Sprague-Dawley rats.
Methods: One hundred and twenty-five cyclic female Sprague-Dawley rats were used for this study. The rats were divided into 6 experimental study groups (I-VI). In experimental study Group I, animals were induced with hyperprolactinaemia and withdrawn to check for recovery.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 and Group V animals received green coconut water only. Experiment Group VI was the control group where animals received distilled water only.
Results: All the animals in the control group were pregnant and carried their individual pregnancy to full term with typical number of foetuses. There was no foetus seen in the uterus of the induced animals after 20 days of assumed gestation after mating was confirmed. The number of foetuses in the hyperprolactin animals increased following treatment with green coconut water.
Conclusion: The results from this investigation have demonstrated that green coconut water is an effective pro-fertility agent in the management of hyperprolactin-induced infertility.

Keywords: Green coconut water, metoclopramide, hyperprolactinaemia, fertility, foetus.

INTRODUCTION
Fertility refers to biological ability to achieve and carry a live pregnancy to full term (1). It is a common assumption that the inability of couples to reproduce is a female inaccuracy (2). This is because the female reproductive system is a highly dynamic system which undergoes numerous sequential morphological changes than those of the male. Hence it is more vulnerable to anatomical and physiological factors that could induce infertility (2, 3). In Africa, women are often blamed for infertility while men are assumed innocent. In some culture, it is an abomination to declare a man infertile while women are often charged with the consequences of infertility (4). Furthermore, several studies have shown that females are responsible for more than half of the causes of infertility in Nigeria; 51.8% female, 26.8% male and 21.4% combined (5, 6).

The processes involved in procreation, starting with sexual intimacy to parturition occur due to hormonal influence (7). Ovulation, conception and maintenance of pregnancy are complicated processes that depend on the fact that the reproductive organs must be active and there must be balances in the endocrine system. In female infertility, hyperprolactinaemia has been implicated in anovulation, endometriosis, polycystic ovarian syndrome and galactorrhea (8, 9). Hyperprolactinaemia is a consistently high serum level of prolactin (PRL) in a non-pregnant and non-lactating individual (10). High serum prolactin has been associated with the inhibition of hypothalamic gonadotropin releasing hormone (GnRH), suppression of preovulatory gonadotropin surge and consequent inhibition of gonadal function with decrease in the growth of ovarian follicles (9).

Cocos nucifera is the botanical name of coconut fruit (11). Green coconut water (GCW) is the water from an immature coconut fruit. Nearly one third of the world population depends on coconut as a source of food and medicine (12). It has been reported that green coconut water has been used in the treatment of many diseases and this is justified by its unique chemical composition. It is a rich source of nutrients (sugars, minerals, proteins, vitamins, fat and fibres) and phytochemicals (phytohormones, nitrogenous compounds, organic acids and enzymes) (13, 14, 15). GCW has been reported to aid the maintenance of pregnancy as the number of pups delivered at the end of the gestation period corresponded to the number of implantation sites counted on day 10 of pregnancy in mice (16, 17). More so, GCW contains numerous antioxidant compounds that have the ability to scavenge free radicals in the body. It was suggested that the naturally occurring vitamin C in GCW along with other vitamins and amino acids, may be responsible for its antioxidant effects (18). The phytoestrogenic property...
was confirmed when oestradiol levels in menopausal rats increased to the same level in ovulating rats when GCW was administered (19). Therefore, this present study was carried out to investigate the effect of green coconut water on fertility rate in hyperprolactin-induced infertility in female Sprague-Dawley rats.

MATERIALS AND METHODS

Collection of Green Coconut Fruit

The immature coconut fruits were harvested from a coconut farm in Ajara, Topa, Badagry, Lagos State, Nigeria between November 2011 and February 2012. The fruit was authenticated in the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria by Dr. (Mrs.) AO Ugbo. The plant’s ascension number is No FHI 109665. The average weight of the fruit was 1.55 kg. The unripe coconut fruits were washed with water and dehusked. The extraction of the water was done with a syringe through the germinial pore and the GCW was poured directly into an airtight bottle and kept in the refrigerator.

Animals

A total of one hundred and twenty-five adult female Sprague-Dawley rats weighing 145–170 g between 6–8 weeks old were obtained from the Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria. The animals 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, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Lagos, Nigeria, and allowed to acclimatize for two weeks under standard laboratory conditions with a photoperiodicity of 12 h light alternating with 12 h darkness. The animals had free access to clean tap water and rat chow.

Infertility-Inducing Agent

Metoclopramide hydrochloride (MCH) is a white crystalline, odourless substance that is soluble in water. It is well established that metoclopramide causes hyperprolactinaemia. The data available indicate that metoclopramide stimulates pituitary PRL secretion by dopamine antagonistic properties in rat (20). Metoclopramide hydrochloride (Pfizer Pharmaceuticals, Ikeja, Lagos, Nigeria) was used to induce hyperprolactinaemia. Metoclopramide hydrochloride at a dose of 0.2 mg/100 g b.w. was administered daily for 28 days (21). The drug was dissolved in water and dose was calculated by simple proportion based on animal weight and administered via oral route with the use of a metal oropharyngeal cannula.

Determination of the Phases of Oestrous Cycle in the Animals

The phases of oestrous cycle were studied to determine the time for possible mating. The phases of estrous cycle were established by daily cytological examination of fresh vaginal smear in the morning between 8:00 am and 10:00 am. Approximately 0.2 ml of normal saline was drawn into the suction pipette. The tip of the pipette was pushed gently into the entrance of the vagina to a depth of 5 mm and the fluid was flushed into the vagina and back up into the pipette two or three times by gently squeezing and releasing the bulb of the pipette. The smear was gently dropped onto the glass slide and viewed under a light microscope with 40× objective lens. The first day of the estrous cycle was designated as metestrus; the vagina smear histology showed leukocytes amidst few squamous cells. The second day was the diestrus phase and this showed predominantly leukocytes. The third day showed large nucleated cells and this was designated as the proestrus phase. The fourth day was designated as the estrus phase and the vaginal cytology showed large flakes of squamous cells (22).

Experimental Procedure

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. Metoclopramide hydrochloride at 0.2 mg/100 g b.w. per day was administered for 28 days through oral route. This was used to experimentally induce hyperprolactinaemia in Ia and this was withdrawn for 8, 16 and 28 days in Ib, Ic and Id respectively to check for 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. for 8, 16 and 28 days following the administration of MCH in (IIa, IIb and IIc) and 10 ml/100 g b.w. of GCW in IID, IIE and IIF. In experiment study Group III, 10 rats were subdivided divided into IIIa and IIIb of 5 rats each. Group IIIa animals were co-administered with 0.2 mg/100 g b.w. of MCH and 5 ml/100 g b.w. of GCW and IIIb were co-administered with 0.2 mg/100 g b.w. of MCH and 10 ml/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 5 ml/100 g b.w. of GCW before the administration of MCH for 8, 16 and 28 days in IVa, IVb and IVc and 10 ml/100 g b.w. of GCW for 8, 16 and 28 days in IVd, IVe and IVf. Experimental Group 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 28 days in Va, Vb and Vc, and 10 ml/100 g b.w. of GCW only in Vd, Ve and Vf. Experiment Group VI (control group) received distilled water only.

The animals were subjected to mating at the end of experimental duration and after the gestation period, fertility assessment was done by evaluating the number of pregnancies in the group and the number of litters in each animal. 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 (23).

Fertility Assessment

The Male Group

This group comprised of male rats of proven fertility. The animals were kept in separate cages strictly for mating. In all, they were made up of 30 rats. The fertility of the male animals was established with pregnancies recorded after coupling with the female rats.
Mating

At the end of the administrations, animals were mated with sexually matured male S-D rats of proven fertility on the estrus day of the cycle. The coupling was done in female to male ratio of 2:1.

Establishment of Gestation

The female rats were smeared the next morning and presence of sperm plug was used to ascertain mating. This day was taken as the first day of pregnancy and the animals were sacrificed on 20\textsuperscript{th} day of gestation. The foetuses were removed by ventral laparotomy, counted, weighed and observed for gross congenital malformations (24).

Statistical Analysis

Results were expressed as means ± standard error of mean (S.E.M.) 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

All the rats in the Group VI (control group) were pregnant and carried their individual pregnancy to full term with typical number of foetuses; $10.24 \pm 1.41$ (Tables 1–3). There was no foetus seen in the uterus of Group Ia (induced animals) after 20 days of assumed gestation after mating was confirmed (Table 1). This implies that pregnancy did not occur in the induced group. In addition, no pregnancy occurred in Group Ib withdrawn for 8 days (Table 1). However, in Group Ic and Id withdrawn for 16 and 28 days respectively, pregnancies occurred but with a lower number of foetuses when compared with the control; $5.60 \pm 0.79$ and $5.76 \pm 0.65$ vs. $10.24 \pm 1.41$ respectively (Tables 2 and 3). There was no foetus seen in the uterus of Group IIa and IId animals post-treated for 8 days (Table 1). In Group IIb and IIc, post-treated for 16 days at both low and high doses respectively, pregnancy occurred but with a lower number of foetuses when compared with the control; $8.10 \pm 0.49$ and $8.98 \pm 0.74$ vs. $10.24 \pm 1.41$ (Table 2). However, Group IIc and IIf, post-treated with GCW for 28 days, presented a comparable values in the number of pregnancies and foetuses with that of the control group; $9.20 \pm 0.84$ and $10.60 \pm 1.67$ vs. $10.24 \pm 1.41$ (Table 3). There was no foetus seen in the uterus of the animals in Group IIIa and IIIb that were co-administered and Group IVa, IVb, IVc, IVd, IVe and IVf pre-treated with GCW (Table 3). The administration of GCW only for 8, 16 and 28 days demonstrated comparable values in the number of pregnancies and foetuses when compared with the control in Group Va, Vb, Vc, Vd, Ve and Vf (Tables 1–3).

Table 1: The Number of Pregnancies and Foetuses in 8 Days Experimental Groups in Female Sprague-Dawley Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Sub-Group Detail</th>
<th>No. of Pregnant Rats</th>
<th>Mean No. of Foetus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>VI</td>
<td>5</td>
<td>$10.24 \pm 1.41$</td>
</tr>
<tr>
<td>Withdrawal</td>
<td>Ib</td>
<td>0</td>
<td>$0.00 \pm 0.00^{**}$</td>
</tr>
<tr>
<td>Post-treated</td>
<td>IIa</td>
<td>0</td>
<td>$0.00 \pm 0.00^{**}$</td>
</tr>
<tr>
<td>GCW treated</td>
<td>Va</td>
<td>5</td>
<td>$10.31 \pm 1.33$</td>
</tr>
<tr>
<td></td>
<td>Vd</td>
<td>5</td>
<td>$9.98 \pm 1.92$</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± S.E.M. and ANOVA with Scheffe’s post-hoc test was used to check the level of significance and analysis of variance, where $n=5$. **$p<0.01$ vs. control.

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. Hyperprolactinemia has been shown to suppress the release of gonadotropin releasing hormone by the hypothalamus which consequently inhibit the secretion of follicle stimulating hormone and luteinizing hormone by the pituitary gland. The decrease in pituitary Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) through integrated central and peripheral mechanisms suppress the physiological activities in the gonad thus leading to infertility (25).

In a previous study, GCW was shown to cause increase in...
CONCLUSION

The results of this investigation have demonstrated that GCW is an effective pro-fertility agent in the management of hyperprolactin-induced infertility. GCW may therefore have fertility potential that can further be explored.

CONFLICT OF INTEREST STATEMENT

We have no conflict of interest.

REFERENCES


