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Cocos nucifera L. water improves reproductive indices in Wistar Rats

OT Kunle-Alabi, OO Akindele, MO Oyovwi, MA Duro-Ladipo and Y Raji
Laboratory for Reproductive Physiology and Developmental Programming.
Department of Physiology, College of Medicine,
University of Ibadan, Ibadan, Nigeria

Abstract

Background: This study explored the effects of *Cocos nucifera* L. water (CW) on the hypothalamo-pituitary-gonadal axis (HPG) and fertility in Wistar rats.

Methods: Adult male and female Wistar rats were treated orally as follows; Study 1: Group 1: control (distilled water), group 2: 20 ml/kg corn oil (danazol vehicle), group 3: 20 ml/kg CW, group 4: 40 ml/kg CW, group 5: danazol, group 6: danazol + 20 ml/kg CW and group 7: danazol + 40 ml/kg CW. 200 mg/kg danazol was administered. Serum levels of LH, FSH, estradiol and testosterone; gonadal weights and sperm indices were assessed. Study 2: Group 1: control (distilled water), group 2: 20 ml/kg CW, group 3: 40 ml/kg CW for 6 and 2 weeks prior to mating in male and female rats respectively.

Results: Significant ($p<0.05$) increases in estradiol concentration were observed in groups 3, 4, 6 and 7. Significant reductions in LH, FSH, estradiol and testosterone levels were observed in group 5 which were ameliorated in groups 6 and 7. Males showed significant increases in sperm count and motility in groups 3, 4, 6 and 7, and reductions in these variables along with viability in group 5. CW pre-treatment increased fecundity index and proportion of female pups from dams, while the pups from sires showed higher birth weights.

Conclusions: CW acts on the HPG to positively influence reproductive function in both males and females and may aid in maternal preconception sex selection of female offspring.

Keywords: *Cocos nucifera, fertility, reproductive hormones, rats*

Résumé

Contexte: Cette étude a exploré les effets du *Cocos nucifera* L. eau (CW) sur l'axe hypothalamus-hypophyse-gonadique (HPG) et la fertilité chez des rats Wistar.

Méthodes: Rats Wistar adulte mâles et femelles ont été traités par voie orale comme suit; Etude 1: Groupe 1: contrôle (eau distillée), groupe 2: 20 ml / kg d'huile de maïs (véhicule de danazol), groupe 3: 20 ml / kg CW, groupe 4: 40 ml / kg CW, groupe 5: le danazol, groupe 6: danazol + 20 ml / kg CW et groupe 7: danazol + 40 ml / kg CW. 200 mg / kg de danazol a été administrée. Les niveaux de sérum du LH, FSH, œstradiol et de la testostérone; poids des gonades et les indices de sperme ont été évalués. Étude 2: Groupe 1: le contrôle (de l'eau distillée), groupe 2: 20 ml / kg CW, groupe 3: 40 ml / kg CW pour 6 et 2 semaines avant l'accouplement des rats mâles et femelles respectivement.

Résultats: Des incréments significatives ($p<0,05$) dans la concentration d'œstradiol ont été observées dans les groupes 3, 4, 6 et 7. Des réductions significatives de LH, FSH, les niveaux d'œstradiol et de testostérone ont été observés dans le groupe 5 qui étaient amélioré dans les groupes 6 et 7. Les mâles ont montré une augmentation significative du nombre de spermatozoïdes et de la motilité dans les groupes 3, 4, 6 et 7, et des réductions dans ces variables ainsi que la viabilité dans le groupe 5. Prétraitement avec CW a augmenté l'indice de fécondité et la proportion de jeunes femelles des barrages, tandis que les souriceaux des sires ont montré un poids de naissance plus élevés.

Conclusions: CW acte sur l'HPG pour influencer positivement la fonction reproductive chez les mâles et les femelles et peut aider dans la préconception maternelle de la sélection du sexe de progéniture femelle.

Mots-clés: *Cocos nucifera, fertilité, hormones reproductive, rats*

Introduction

The numerous health benefits of *Cocos nucifera* L. (Coconut) water in several disease states have been recognized [1,2]. *Cocos nucifera* L. water has several biologically active constituents such as L-arginine, ascorbic acid, minerals and electrolytes which contribute to its hypoglycemic, antioxidant, hypolipidemic and anti-inflammatory actions [3–5]. The mechanisms through which *Cocos nucifera* L.

Correspondence: Dr. Olufadekemi T. Kunle-Alabi, Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Nigeria. E-mail: mrskalabi @ yahoo.com.

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water performs its actions are yet to be fully elucidated, but recent research findings from *Cocos nucifera* L. water hold promise of breakthrough ethnopharmacological solutions to common systemic diseases.

Cocos nucifera L. water has been used in folkloric medicine to treat disorders associated with the male and female reproductive systems such as menstruation, pregnancy, infertility and libido although there is paucity of information for the scientific basis of such uses. *Cocos nucifera* L. water has been used extensively and successfully as a semen extender for *in vitro* insemination of animal models [6,7]. *In vivo* studies report that *Cocos nucifera* L. water alleviates estrogen withdrawal symptoms in ovariectomized rats [8] and testosterone withdrawal symptoms in orchidectomized rats [9].These suggest that *Cocos nucifera* L. water either possesses sex hormone-like actions or that it promotes extragonadal steroidogenesis. This study was therefore carried out to determine the effects of immature *Cocos nucifera* L. water on (1) the hypothalamo-pituitary gonadal axis (HPG) using Danazol, a synthetic gonadotropin releasing hormone (GnRH) antagonist and (2) fertility indices of Wistar rats.

Materials and methods

Animals

All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the guidelines for laboratory animal care of the National Institute of Health (NIH publication no. 85-23, revised 1996). Adult male and female Wistar rats were obtained from the Central Animal House, College of Medicine, University of Ibadan, where the experiment was carried out.

Study 1

The animals were randomly divided into fourteen groups; seven male and seven female groups. The male and female groups were given the same treatments as follows; Group 1: (Control) 20 ml/kg body weight distilled water, Group 2: 20 ml/kg body weight corn oil (vehicle for danazol), Group 3: 20 ml/kg body weight *Cocos nucifera* L. water, Group 4: 40 ml/kg body weight *Cocos nucifera* L. water, Group 5: 200 mg/kg Danazol, Group 6: 200 mg/kg Danazol + 20 ml/kg body weight *Cocos nucifera* L. water and Group 7: 200 mg/kg Danazol + 40 ml/kg body weight *Cocos nucifera* L. water. The male groups were administered this treatment for six weeks while the female groups were treated for two weeks.

Study 2

The animals were randomly divided into six groups comprising three male and three female groups. The male groups were treated for six weeks while the female groups were treated for two weeks as follows: Group 1: (Control) 20 ml/kg body weight distilled water, Group 2: 20 ml/kg body weight *Cocos nucifera* L. water, Group 3: 40 ml/kg body weight *Cocos nucifera* L. water. After the administration period, the animals were mated with proven breeders (i.e. male rats which had previously mated successfully and female rats which had previously littered successfully) obtained from the Laboratory for Reproductive Physiology and Developmental Programming, Department of Physiology, University of Ibadan. Mating ratio was 1 male to 2 females. Mating was confirmed by the presence of spermatozoa in vaginal smears, and the day of detection of spermatozoa was taken as gestation day 1 (GD1). *Cocos nucifera* L. water administration was stopped on GD1 for both male and female animals. Pregnant rats in all six groups were allowed to litter naturally.

In both studies, each group was housed separately in a large well ventilated plastic cage lined with wood shavings which were changed daily. The animals had access to standard rodent pellets (Ladokun Feed Mills, Ibadan, Nigeria) and drinking water *ad libitum* throughout the period of the experiment.

Plant material

Cocos nucifera L. is a native plant of the tropics. It is known as "coconut" in English. *Cocos nucifera* L. fruits of 5-6 months old were obtained from the coconut plantation of the Nigerian Institute for Oil and Palm Research (NIFOR), Badagry, Lagos, Nigeria. The *Cocos nucifera* husks were peeled off, the shell was cracked and the water poured into a sterile container. The *Còcos nucifera* L. water was administered to the rats in two daily divided doses between 8.00am and 6.00 pm using an orogastric tube for a period of two weeks in the females and six weeks in the males. The two weeks administration in the females was to enable them complete an average of four estrus cycles [10], while the six weeks for the males was to cover the duration for spermatogenesis in rats [11,12]. A fresh *Cocos nucifera* fruit was cracked for each administration session.

Drug

The contents of 200mg capsules of Danazol (West Coast Company limited, US) were dissolved in corn oil to obtain a homogenous stock solution and then administered orally using an orogastric tube at a dose of 200 mg/kg body weight [13] once every three days

for a period of two weeks in the females and six weeks in the males.

Hormone assay

Twenty-four hours after the last administration, the animals were weighed and sacrificed by cervical dislocation. Prior to sacrifice, blood was collected via the retro orbital sinus into serum tubes. The tubes along with its contents were centrifuged at 3000rpm for 15 minutes to obtain serum for ELISA of follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol and testosterone using kits according to the manufacturer's instructions (Monobind Inc. Lake Forest, CA, USA). All samples were collected and assayed on the same day to avoid inter-assay variations.

Sperm analysis

The cauda epididymal fluid was immediately collected for sperm analysis. Motility, morphology, viability and counts were carried out according to the methods of Zemjanis [14] and Oyeyemi *et al.* [15].

Organ morphometry

The ovary, uterus (female), testis, seminal vesicle and prostate gland (male) were harvested, freed from adherent tissue and weighed on an electronic weighing balance (Lisay, China). Relative organ weight was calculated as the percentage of the ratio of the organ weight to the body weight for each animal.

Fertility index

The fecundity index (which is also known as the pregnancy index in females) was calculated for each group according to the Development and Reproductive Toxicology (DART) guidelines [16];

Fecundity index for dams=

$$\frac{\text{Number of females pregnant}}{\text{Number of females mated}} \times 100$$

Fecundity index for sires=

$$\frac{\text{Number of males siring a litter}}{\text{Number of males mated}} \times 100$$

Pup morphometry

Litter size was recorded for each dam and each pup was weighed on an electronic weighing balance (Lisay, China) within twenty-four hours after parturition i.e. on post-natal day 1 (PND1). Anogenital distance (AGD) of each pup was measured from the anus to the genital tubercle [17] using a digital Vernier caliper (Mitutoyo, Japan) and the Anogenital Distance Index (AGDi) was calculated as the ratio of AGD to the cube root of body weight [18]. The AGD was used to differentiate between male and female pups [17] and the sex ratio was recorded.

Statistical analysis

Data obtained were analysed using Student's *t* test or one way analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) test for multiple comparisons of means. Statistical analysis was done using the GraphPad Prism version 5.0 statistical software package (GraphPad Software, San Diego, CA, USA). The criterion for statistical significance was set at $p<0.05$. All results are expressed as mean ± standard error of mean (SEM).

Results

Body and organ weights; *Cocos nucifera* L. water significantly reduced body weight gain when compared to the control, corn oil and danazol groups in the females and to the danazol group in the males (Figure 1). Danazol administration caused a significant increase in body weight gain when compared with the control, corn oil and coconut water treated groups (Fig. 1). *Cocos nucifera* L. water significantly increased the relative weight of the uterus at 40 ml/kg when compared with the six other groups (Figure 2B). Danazol significantly reduced the relative weights of the ovaries (Figure 2B), testes, seminal vesicles and prostate glands (Figure 2A), while *Cocos nucifera* L. water restored the relative weights of these organs to control values.

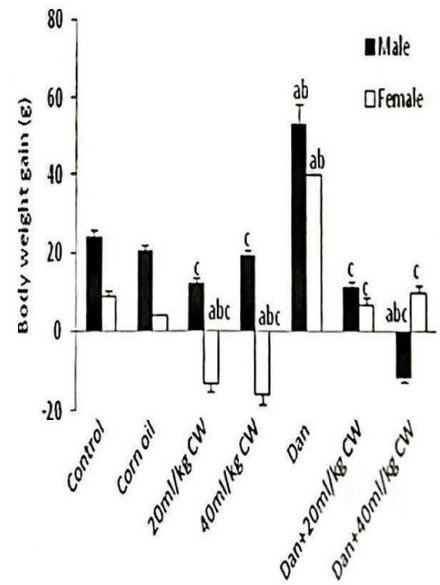
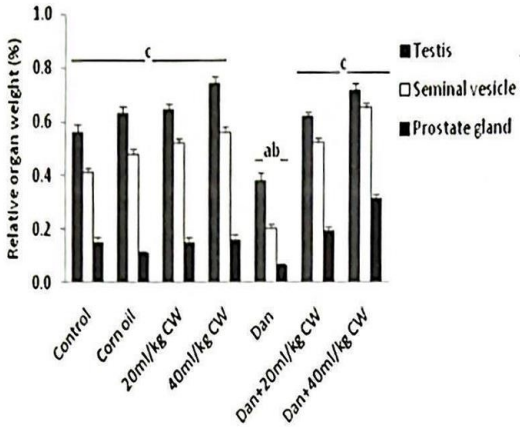
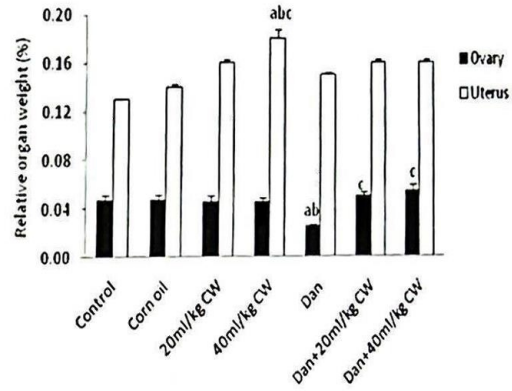


Fig.1: Effect of *Cocos nucifera* L. water (CW) on body weight gain in danazol (Dan) treated Wistar rats. Columns represent mean ± standard error of the difference between final (after administration) and initial (before administration) body weights (n=6 animals per group). $p<0.05$ compared with control[a], corn oil[b] and danazol[c] groups.

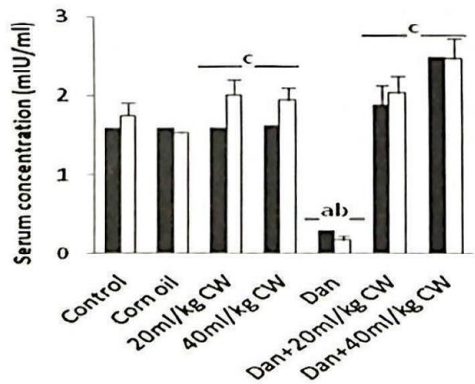


2A Male reproductive organs

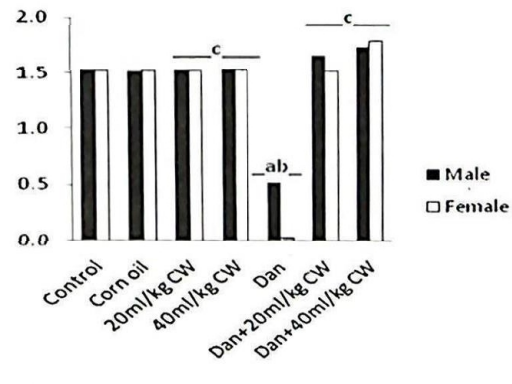


2B Female reproductive organs

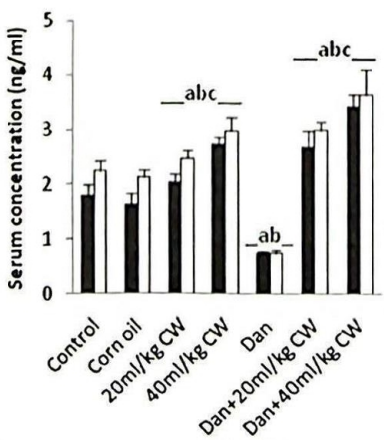
Fig.2: Effect of *Cocos nucifera* L. water (CW) on relative weights of the testis, seminal vesicle and prostate gland in the male (A) and the relative weights of the ovary and uterus in the female (B) danazol (Dan) treated Wistar rats. Columns represent mean ± standard error of the relative weights of the reproductive organs (n=6 animals per group). $p<0.05$ compared with control[a], corn oil[b] and danazol[c] groups.



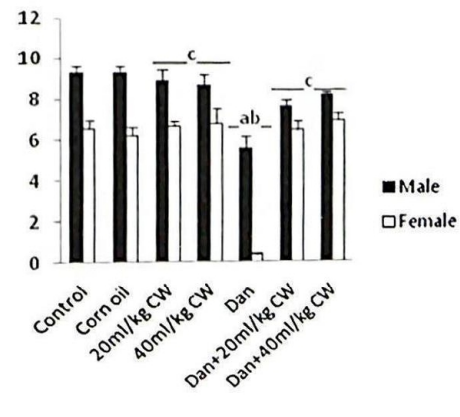
3A LH



3B FSH



3C Estradiol



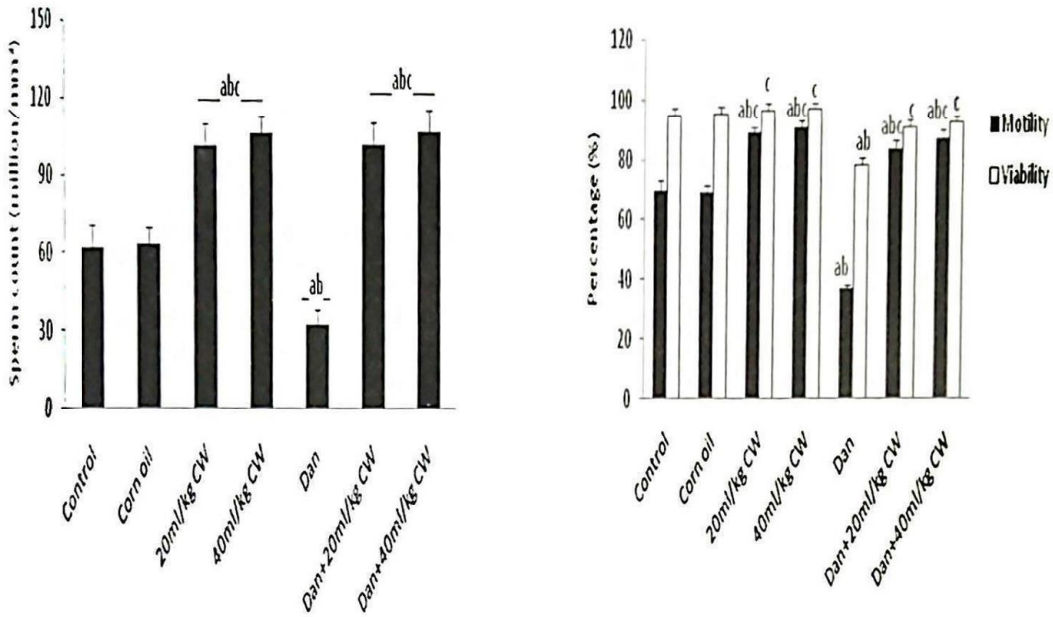
3D Testosterone

Fig.3: Effect of *Cocos nucifera* L. water (CW) on serum concentrations of LH (A), FSH (B), estradiol (C) and testosterone (D) in danazol (Dan) treated Wistar rats. Columns represent mean ± standard error of the means (n=6 animals per group). $p<0.05$ compared with control[a], corn oil[b] and danazol[c] groups.

Reproductive hormones; Danazol significantly reduced the serum concentrations of LH, FSH, estradiol and testosterone in both males and females (Fig. 3A-D). Co-administration of danazol and *Cocos nucifera* L. water restored the serum concentrations of these hormones to control values. *Cocos nucifera* L. water administration also caused significant increases in serum estradiol concentrations in both males and females (Fig. 3C).

Sperm indices; *Cocos nucifera* L. water significantly increased sperm count, motility and viability and also reversed the danazol induced reduction of these variables (Fig. 4A and 4B).

Fertility study; *Cocos nucifera* L. water at a dose of 40 ml/kg resulted in 100% mating of dams which all littered successfully (Fig. 5). Both sires and dams treated with *Cocos nucifera* L. water prior to mating produced significantly larger litters (Figure 6B), with



4A Sperm count 4B Sperm indices

Fig.4: Effects of *Cocos nucifera* L. water (CW) on sperm count (A), motility and viability (B) in danazol (Dan) treated Wistar rats. Columns represent mean ± standard error of the means (n=6 animals per group). $p<0.05$ compared with control[a], corn oil[b] and danazol[c] groups.

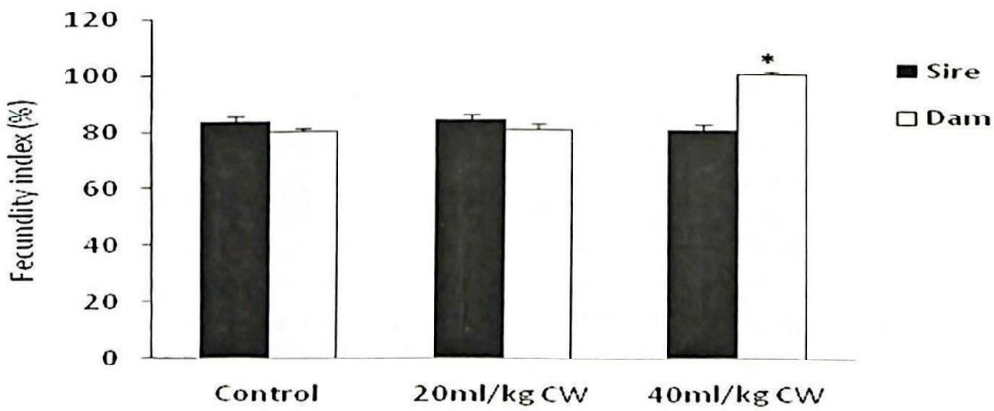
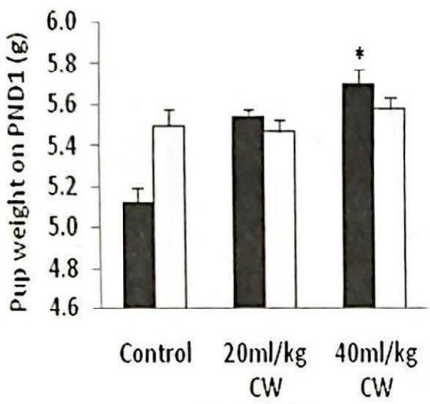


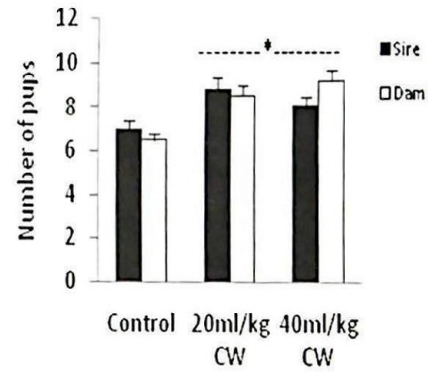
Fig.5: Effect of *Cocos nucifera* L. water (CW) on fecundity/pregnancy index in male and female Wistar rats. Columns represent mean ± standard error of the means (n=10 animals per group). *$p<0.05$. (Unpaired Student's t- test).

the *Cocos nucifera* L. water pre-treated dams having a significantly higher proportion of female pups (Figure 6C). Pups from *Cocos nucifera* L. water treated sires had significantly higher birth weights than the controls (Figure 6A). There was no significant difference between the anogenital distance indexes of either the male or female pups from the *Cocos nucifera* L. water pre-treated sires and dams (Figure 6D).

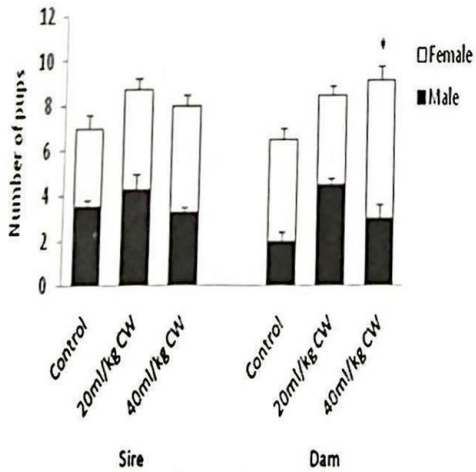
and improve semen quality. Though these claims are yet to be proven scientifically, related studies suggest that *Cocos nucifera* L.water may indeed play a role in reproductive function. Nair and Rajamohan [19] reported that *Cocos nucifera* L.water counters nicotine-induced testosterone reduction possibly through its L-arginine content. L-arginine stimulates the production of nitric oxide which in turn stimulates the release of gonadotropins.



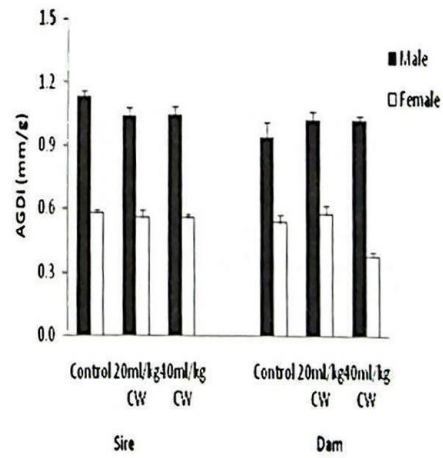
6A Birth weight



6B Litter size



6C Sex ratio



6D Anogenital distance index

Fig. 6: Effect of administration of *Cocos nucifera* L. water (CW) prior to mating on size (A), pup litter weight (B), sex ratio (C) and AGDi (D) of offspring. Columns represent mean ± standard error the mean of the variables recorded on PND 1 (n=52-80 pups per group). *p<0.05.

Discussion and conclusions

Cocos nucifera L.water has in recent times taken on more noteworthy uses in addition to its age-long use as a nutritious source of rehydration. Folkloric medicine in several parts of the tropics and anecdotal reports claim that *Cocos nucifera* L.water is able to reduce perinatal mortality, improve galactopoiesis

Gonadotropins are essential to the normal functioning of both the male and female reproductive systems. Hence, the present work was carried out to investigate the effect of *Cocos nucifera* L. water on the hypothalamo-pituitary-gonadal axis using danazol a gonadotropin releasing hormone (GnRH) antagonist. Danazol (17-alpha-Pregn-4-en-20-

yno(23-d)-isoxazol-17-ol) is a synthetic steroid derivative of 17 alpha-ethinyltestosterone which suppresses GnRH, pituitary gonadotropins and directly inhibits systemic and gonadal steroidogenesis in rodents [13,20,21]. Danazol also binds to steroid receptors with the exception of estrogen receptors [22]. Danazol was used in this study to determine if *Cocos nucifera* L. water has steroidal actions on the gonads and if it performs these probable actions directly or by stimulating gonadotropin activity. The gonadotropins; luteinizing hormone (LH) and follicle stimulating hormone (FSH), are released from the anterior pituitary gland under the influence of hypothalamic GnRH to produce their tropic effects on the gonads. The ability of *Cocos nucifera* L. water to restore the serum levels of LH and FSH to control values in the danazol treated rats suggests that *Cocos nucifera* L. water stimulates the gonadotropic activity of the pituitary gland. It is not sure however, if this stimulus emanated from the hypothalamic axis or directly within the pituitary gland.

Several enzymes act along the steroidogenic pathway to synthesize the steroid hormones (including the sex steroids) from the precursor; cholesterol. Danazol directly inhibits several of these enzymes thereby preventing the initial and subsequent conversion of cholesterol to its steroid precursors [23]. *Cocos nucifera* L. water administration increased the serum concentrations of estrogen and testosterone to control levels in both the male and female rats treated with danazol. This suggests that *Cocos nucifera* L. water acts centrally and/or locally to affect reproduction. Gonadotropins are essential for gonadal growth and function, thus the reduction in testicular and ovarian weights and in the weights of the assessory sex organs by danazol reflects its anti-gonadotropic effect [13,21]. The significant increase in relative ovarian and testicular weight as well as cauda epididymal sperm motility and counts observed in all the groups treated with *Cocos nucifera* L.water supports the premise that Cocos *nucifera* L. water either stimulates or exerts a gonadotropic effect. The fact that *Cocos nucifera* L. water restored the weights to control values despite the body weight changes further supports its potential gonadotropic activity. The significant reductions in body weights observed in the *Cocos nucifera* L.water treated females corroborates previous reports by Sandhya and Rajamohan [24] that *Cocos nucifera* L. water causes alterations in lipid metabolism and these alterations may favour weight loss. In light of this, the increases in relative organ weights observed in this study may also be related to the reductions in body weight.

Cocos nucifera L. water did not result in any significant change in serum estrogen and testosterone concentrations in orchidectomized rats [9], it however increased estradiol levels in ovariectomized rats [8]. This result corroborates the increased serum estradiol concentration observed in this study and suggests that *Cocos nucifera* L. water has estrogenic properties. *Cocos nucifera* L. water has been reported to contain phenolic compounds, phytoestrogens, flavonoids, kinetin, L-arginine and ascorbic acid among other notable constituents [1]. Ascorbic acid, arginine, phytoestrogens and flavonoids have been reported to augment reproductive hormone levels and sperm indices [19,25,26] and may thus enable *Cocos nucifera* L. water to exhibit its reproductive hormone-like activity [8,27,28].

Preconception diets have been hypothesized to influence gender selection in offspring [29,30]. Though this concept is yet to be fully explored, the possibility of its existence should not be hastily overlooked. Increase in maternal energy intake prior to conception was associated with a tendency towards having male offspring [29]. *Cocos nucifera* L. water resulted in a significant reduction in maternal weight prior to conception and the dams subsequently produced a significantly higher proportion of female offspring. Interestingly, the anogenital distance (AGD) which serves as a hormone sensitive indicator of fertility status [18] was not affected in male and female offspring from either the *Cocos nucifera* L. water pre-treated sires or dams. *Cocos nucifera* L. water contains phytoestrogens [8] which have been reported to reduce AGD [31]. These results suggest that the effects of *Cocos nucifera* L. water on fertility are limited to the period of *Cocos nucifera* L. water consumption and its intake should therefore be dictated by the desired results.

These findings report, for the first time, that *Cocos nucifera* L. water ameliorates the anti-gonadotropic activity of danazol in both male and female Wistar rats and can aid in the preconception selection of female offspring in dams. Further study is required to elucidate the point(s) along the hypothalamo-pituitary-gonadal axis at which *Cocos nucifera* L. water acts to exert its actions and to determine the effects of phytoestrogens in preconception dietary sex selection of offspring.

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