Effects of immature coconut water on hyperprolactine-induced oxidative stress in female Sprague-Dawleys Rats

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Abstract

Objective: The aim of this study was to determine the effects of immature coconut water on metoclopramide-induce oxidative stress in female Sprague-Dawley rats Materials and Methods: The experiment was divided into 6 experimental study groups (I- VI). In experiment I, 5 rats received metoclopramide at 0.2mg/100g body weight/day and withdrawn for 8, 16 and 28days after induction. Experiment II, post-treated with 5ml/100gbw and 10ml/100gbw of immature coconut water. Experiment III rats were co-administered with 0.2mg/100g body weight of metoclopramide and 5ml and 10ml/100gbw of immature coconut water. In experimental group IV, the rats were pre-treated with 5ml and 10ml/100gbw of immature coconut water. Experiment V of 5 rats received immature coconut water only and Experiment VI, the control group received distilled water only. Results: There were statistical significant decrease in the concentration of MDA and increase in the concentrations of SOD, CAT and GSH when compared with the control. Conclusion: Green coconut water posses antioxidant properties in hyperprolactine rats.

Keywords: Immature coconut water, Metoclopramide, Oxidative stress, Anti-oxidant

INTRODUCTION

Reproductive cells and tissues remain stable when free radical production and the scavenging antioxidants remain balance. Oxidative stress is a confounding factor in infertility as free oxygen radicals may react with cellular components of the reproductive tract to produce destructive effects. The role of reactive oxygen species in various diseases of the female reproductive system have been established to affect a variety of physiological functions in the tract. It has been reported that free oxygen radicals may react with any of the cellular macromolecules to produce destructive effects. There are cumulative evidence which suggests that oxidative stress is involved in conditions such as abortions, preeclampsia, hydatidiform mole, fetal embryopathies, preterm labor and intrauterine growth retardation, all of which lead to an immense burden of maternal and fetal mortality **(**Van, 2009**).** Prolactin is a major stress-induced hormone, and its secretion follows psychological, environmental or physical stress. High prolactin evokes oxidative stress in the arcuate nucleus of the hypothalamus and causes nitration of tyrosine hydroxylase, the rate limiting enzyme in the synthesis of dopamine (DA). This results in a significant decrease in DA and consequently hyperprolactinemia. A positive correlation among serum prolactin and nitrite suggested that hyperprolactinemia could contribute to infertility by inducing oxidative damage as implicated by high Lipid peroxidation (Franci et al., 1992; Mohan et al., 2011).

For thousands of years coconut products have held a respected and valuable place in local folk medicine. Coconut water has a host of yet scientifically unproven but traditional uses in cultures all over the world. From ancient times in Africa, reports support the position that about 85% of the world's population rely on coconut fruit in traditional medicine. It is use to conquer irregular or painful menstruation and also taken during pregnancy to

give the unborn babies strength and vitality. It is also use to boost semen quality and induce libido (Sofowora, 1993). Coconut water contains numerous antioxidant compounds that have the ability to scavenge free radicals in the body (Fonseca et al., 2009; Yong et al., 2006). Furthermore, micronutrients such as inorganic ions present in immature coconut water play a vital role in aiding the human body antioxidant system. Kinetin was shown to act as a strong antioxidant both under in vitro and in vivo conditions. A study done by Olsen et al. demonstrated that kinetin protected DNA from oxidative damage mediated by the Fenton reaction. Kinetin inhibited the formation of 8-oxo-2'deoxyguanosine, which is a common marker of oxidative damage in DNA. The anti-oxidative properties of kinetin suggested that it may also prevent the oxidative damage of unsaturated fatty acids located within the cell membranes (Olsen et al., 1999; Verbeke et al., 2006**).**

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**.** 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.

Experimental Procedure

Fifty adult cyclic female Sprague-Dawley rats were used. The experiment was divided into 6 experimental study groups (I- VI). The estrous cycles of the rats were studied for the first 16 days to establish cyclicity in all experimental groups. In experiment I, 5 rats received metoclopramide at 0.2mg/100g body weight /day through oral route for 28days to experimentally induce hyperprolactinemia and withdrawn for 8, 16 and 28days after induction. Experiment II was made up of 10 rats subdivided in IIa and IIb of 5 rats each post-treated with 5ml/100gbw and 10ml/100gbw of green coconut water respectively. In experiment III, 10 rats were subdivided divided into IIIa and IIIb of 5 rats each, IIIa rats were co-administered with 0.2mg/100g body weight of metoclopramide and 5ml/100gbw of green coconut water and IIIb rats were co-administered with 0.2mg/100g body weight of metoclopramide and 10ml/100gbw of green coconut water . In experimental group IV, 10 rats were subdivided in IVa and IVb of 5 rats each pre-treated with 5ml/100gbw and 10ml/100gbw of green coconut water respectively. Experiment V, 5 rats received green coconut water only and Experiment VI, the control group 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 (American Physiological Society, 2002) were approved by the Departmental Committee on the Use and Care of Animals.

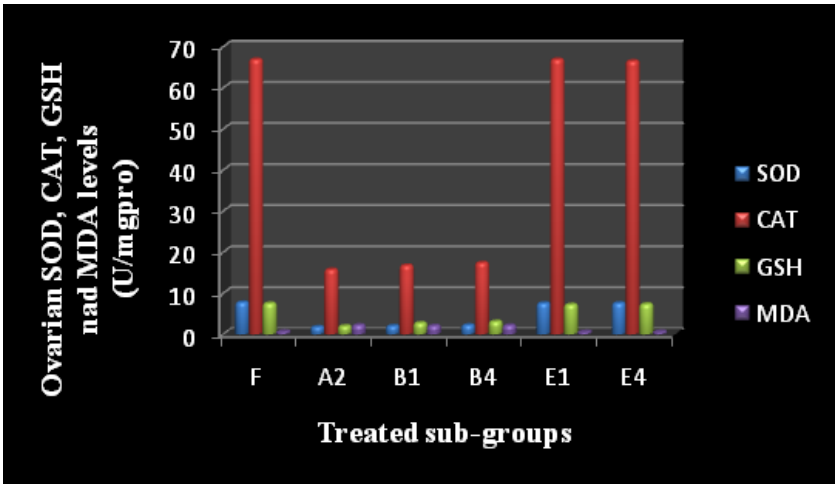
Statistics

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

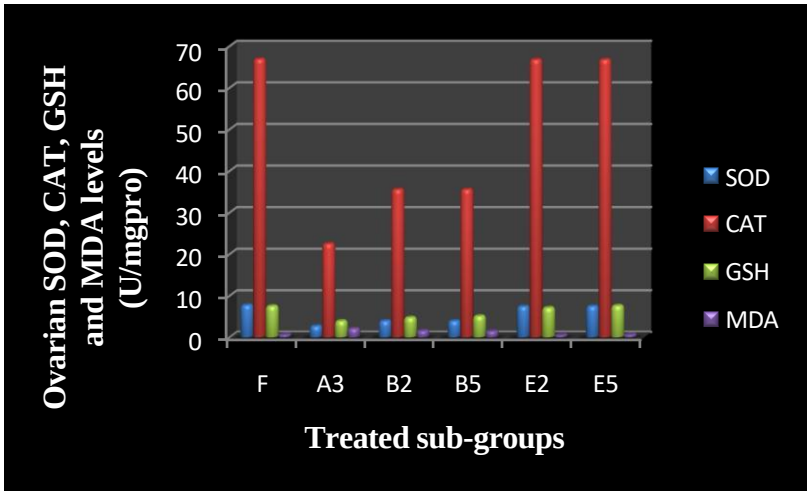
The control group: There were high levels of SOD, CAT and GSH in the ovarian tissues. The stress marker level of MDA was low. The induced group demonstrated statistically significant lower levels of SOD, CAT and GSH when compared with the control. The MDA level rose higher than the control with a statistically significant difference (Figure 1a, b and c). In the withdrawal groups, there were increases in the concentrations of SOD, CAT and GSH with increase in the days of withdrawal which were not statistically significant when compared with the control (Figure 1a,b and c). The stress marker level of MDA decreases but was not statistically significant (Figure 1a, b and c).The post-treated groups show insignificant increase in the concentrations of SOD, CAT and GSH in 8 and 16 days of post-treatment (Figure 1a and b) while statistically significant increase in the concentrations of SOD, CAT and GSH were demonstrated in 28days post-treated period that were statistically comparable with the control (Figure 1c). There was insignificant decrease in the concentration of MDA in 8 and 16 days of post-treatment (Figure 1a and b) while statistical significant decrease in the concentration of MDA and

Figure1a: SOD, CAT, GSH AND MDA CONCENTRATIONS IN THE OVARIES OF EXPERIMENTAL AND CONTROL 8DAYS PERIOD IN SPRAGUE-DAWLEY RATS



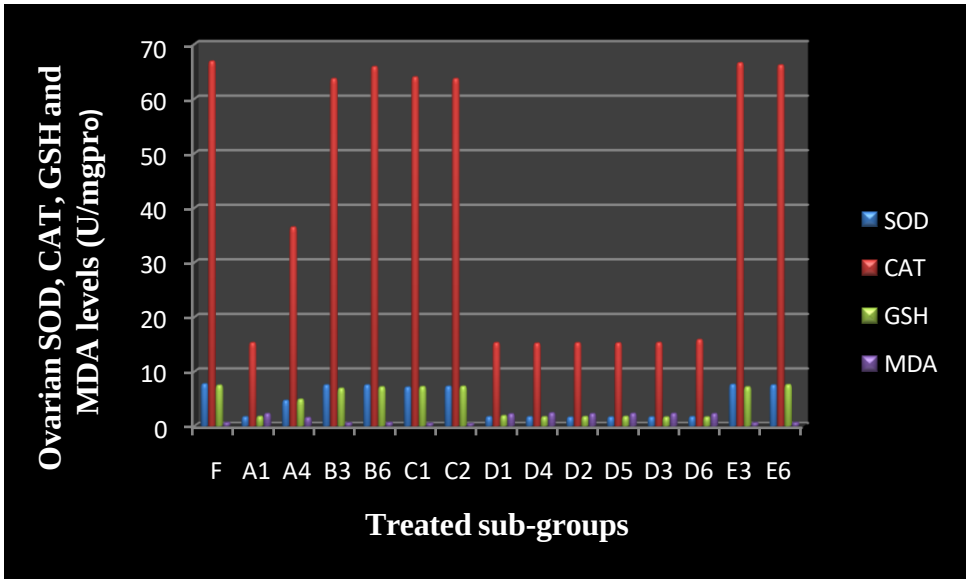
Key for Figure 1a
F- Control (Distilled water)
A2 - MCH_{28days}-$WD_{8\ days}$ (0.2 mg/100gbw metoclopramide hydrochloride for 28 days then withdrew for 8days)
B1 - MCH_{28days}-$GCW_{M/d\ 8\ days}$ (0.2 mg/100gbw metoclopramide hydrochloride for 28 days then 5ml/100gbw of green coconut water for 8days)
B4 - MCH_{28days}-$GCW_{H/d\ 8\ days}$ (0.2 mg/100gbw metoclopramide hydrochloride for 28 days then 10ml/100gbw of green coconut water for 8days)
E1 - $GCW_{M/d\ 8days}$ (5ml/100gbw of green coconut water for 8days)
E4 - $GCW_{H/d\ 8days}$ (10ml/100gbw of green coconut water for 8days)

Figure 1b: SOD, CAT, GSH AND MDA CONCENTRATIONDS IN THE OVARIES OF EXPERIMENTAL AND CONTROL 16DAYS PERIOD IN SPRAGUE-DAWLEY RATS.



Key for Figure 1b
F- Control (Distilled water)
A3 - $MCH_{28\ days}$-WD_{16days} (0.2 mg/100gbw metoclopramide hydrochloride for 28 days then withdrew for 16days)
B2 - $MCH_{28\ days}$-$GCW_{M/d16days}$ (0.2 mg/100gbw metoclopramide hydrochloride for 28 days then 5ml/100gbw of green coconut water for 16days)
B5 - $MCH_{28\ days}$-$GCW_{H/d16\ days}$ (0.2 mg/100gbw metoclopramide hydrochloride for 28 days then 10ml/100gbw of green coconut water for 16days)
E2 - $GCW_{M/d16\ days}$ (5ml/100gbw of green coconut water for 16days)
E5 - $GCW_{H/d16\ days}$ (10ml/100gbw of green coconut water for 16days)

Figure 1c: SOD, CAT, GSH AND MDA CONCENTRATIONDS IN THE OVARIES OF EXPERIMENTAL AND CONTROL 28DAYS PERIOD IN SPRAGUE-DAWLEY RATS



Key for Figure 1c
F- Control (Distilled water)

A1 – MCH $_{28days}$ (0.2 mg/100gbw metoclopramide hydrochloride for 28 days)
A4 - MCH $_{28days}$- WD$_{28days}$ (0.2 mg/100gbw metoclopramide hydrochloride for 28 days then withdrew for 28days)
B3 - MCH $_{28days}$-GCW $_{M/d28\ days}$ (0.2 mg/100gbw metoclopramide hydrochloride for 28 days then 5ml/100gbw of green coconut water for 28
B6 - MCH $_{28days}$-GCW $_{H/d16\ days}$ (0.2 mg/100gbw metoclopramide hydrochloride for 28 days then 10ml/100gbw of green coconut water for 28days)
C1- MCH$_{28day}$ + GCWM/d$_{28day}$ (0.2 mg/100gbw metoclopramide hydrochloride and 5ml/100gbw of green coconut water concurrently administered for 28days)
C2- MCH$_{28day}$ + GCWH/d$_{28day}$ (0.2 mg/100gbw metoclopramide hydrochloride and 10ml/100gbw of green coconut water concurrently administered for 28days)
D1- GCW$_{8days\ M/d}$ - MCH $_{28days}$ (5ml/100gbw of green coconut water for 8days then 0.2 mg/100gbw metoclopramide hydrochloride for 28 days)
D4- GCW$_{8days\ H/d}$- MCH $_{28days}$ (10ml/100gbw of green coconut water for 8days then 0.2 mg/100gbw metoclopramide hydrochloride for 28 days)
D2- GCW$_{16days\ M/d}$ - MCH $_{28days}$ (5ml/100gbw of green coconut water for 16days then 0.2 mg/100gbw metoclopramide hydrochloride for 28 days)
D5- GCW$_{16days\ H/d}$ - MCH $_{28days}$ (10ml/100gbw of green coconut water for 16days then 0.2 mg/100gbw metoclopramide hydrochloride for 28 day
D3- GCW$_{28days\ M/d}$ - MCH$_{28days}$ (5ml/100gbw of green coconut water for 28days then 0.2 mg/100gbw metoclopramide hydrochloride for 28 days)
D6- GCW$_{28days\ H/d}$ - MCH $_{28days}$ (10ml/100gbw of green coconut water for 28days then 0.2 mg/100gbw metoclopramide hydrochloride for 28 days)
E3 - GCW $_{M/d28\ days}$ (5ml/100gbw of green coconut water for 28days)
E6 - GCW $_{H/d28\ days}$ (10ml/100gbw of green coconut water for 28days)

increase in the concentrations of SOD, CAT and GSH were demonstrated in 28days post-treatment duration when compared with the control (Figure 1c). In the Co-administered groups concentrations of SOD, CAT, GSH and MDA were statistically comparable with the control group with high levels of SOD, CAT and GSH and low MDA concentration (Figure 1c). The pretreated groups demonstrated concentrations of SOD, CAT, GSH and MDA which were comparable with the induced group with statistically significant lower levels of SOD, CAT and GSH, and higher MDA level when compared with the control (Figure 1c).The GCW treated groups demonstrated concentrations of SOD, CAT, GSH and MDA which were comparable with the control (Figure 1a, b and c).

DISCUSSION

Oxidative stress markers such as superoxide dismutase (SOD), glutathione peroxidase (GSH) and Catalase (CAT) have been identified to be low while malondialdehyde (MDA) concentration is high within the

and the peritoneal fluid of oxidative stressed women inflicting significant damage to reproductive cells structures **(**Veena et al., 2008**).** This study demonstrated that immature coconut water demonstrated anti-oxidant properties by significantly decreasing ovarian MDA and increasing SOD, CAT and GSH concentrations shown in both post-treated and co-administered groups. The therapeutic effects of tender coconut water on oxidative stress in fructose fed insulin resistant hypertensive rats demonstrated activities of antioxidant enzymes regulated significantly by inhibition of lipid peroxidation and upregulation of antioxidant status **(**Bhagya et al., 2012**).** Studies have suggested that GCW antioxidant activity in laboratory tests is attributed to naturally occurring vitamin C in coconut water, along with its other vitamins and amino acids (Fonseca et al., 2009; Yong et al., 2009). Also possible protective effects of coconut oil on alcohol-induced oxidative stress and serum lipid values in male rats have been reported. The administration of virgin coconut oil improved the antioxidant status by decreasing the levels of MDA and lipid profile to normal. Also sperm count and motility and serum testosterone levels were also significantly increased when compared with the alcohol treated groups (Dosumu et al., 2012).

CONCLUSION

The results of this investigation have demonstrated that immature coconut water effectively reduces hyperprolactin induced oxidative stress by decreasing ovarian MDA and increasing SOD, CAT and GSH concentrations shown in both ameliorative and modulating groups.

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