



*Original Article*

## Comparative effects of coconut water and N-Acetyl cysteine on the hypothalamo-pituitary-gonadal axis of male rats

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### Abstract

Coconut water (CW) contains cysteine, an amino acid which influences glutathione metabolism and promotes reproductive functions. The effects of CW and N-acetyl cysteine (NAC) were compared on the hypothalamo-pituitary-gonadal-axis of danazol-(a GnRH antagonist)-treated rats. Seven groups of male rats were treated orally for six weeks as follows; 1: 20 mL/kg distilled water (Control); 2: 20 mL/kg Corn oil; 3: 20 mL/kg CW; 4: 100 mg/kg NAC; 5: 200 mg/kg Danazol; 6: Danazol+CW; 7: Danazol+NAC. Serum gonadotropins and testosterone; sperm indices; testicular malondialdehyde and glutathione were determined. Sperm quality increased in groups 3, 4, 6 and 7, and reduced in group 5 compared with groups 1 and 2. Gonadotropins and testosterone increased in groups 6 and 7 compared with group 5. Malondialdehyde reduced, while glutathione increased, in groups 3 and 4 compared with group 1. These similarities suggest that cysteine contributes to the effects of coconut water along the hypothalamo-pituitary-gonadal-axis.

**Keywords:** coconut water, cysteine, danazol, fertility

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### 1. Introduction

Coconut (*Cocos nucifera* L.) water is a pleasant tasting drink, rich in amino acids, enzymes, minerals, sugars, cytokinins, auxins and other substances (Balasubramanian & Boopathy, 2013; Nair & Rajamohan, 2014; Yong *et al.*, 2009). Coconut water has immense health benefits on virtually all physiological systems and alleviates various pathological conditions (DebMandal & Mandal, 2011). Coconut water also has positive effects on reproductive functions, even in conditions which do not favor reproduction; such as when danazol is administered (Kunle-Alabi *et al.*, 2014; 2015). Danazol is a synthetic steroid which suppresses gonadotropin releasing hormone (GnRH) and inhibits the release of

pituitary gonadotropins. Danazol also directly inhibits systemic and gonadal steroidogenesis in rodents (Barbieri *et al.*, 1977; Dmowski, 1979; Potts, *et al.*, 1974).

Some components of coconut water such as ascorbic acid and arginine, have been reported to have positive effects on reproductive function (Nair & Rajamohan, 2014; Nunes & Salgueiro, 2011; Radenahmad *et al.*, 2006). Cysteine is another component of coconut water which seems to have been largely overlooked (Lin *et al.*, 2004; Prades *et al.*, 2012). Coconut water contains 3.7% carbohydrate, 0.2% fat and 0.7% protein, its major components being water, vitamins and minerals (U.S. Department of Agriculture [USDA], 2010). Cysteine makes up about 34.7% of the protein fraction in coconut water. Cysteine improves male reproductive functions by improving the glutathione antioxidant mechanism which is essential for optimal sperm functions (Kumar *et al.*, 2013; Takemura *et al.*, 2014). N-acetyl cysteine is a prodrug for cysteine (Atkuri *et al.*, 2007; Samuni *et al.*, 2013). Thus, this study was carried out to compare the effects of coconut water

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and N-acetyl cysteine on the reproductive functions of danazol-treated male Wistar rats.

## 2. Materials and Methods

### 2.1 Danazol

The contents of 200 mg capsules of Danazol (West Coast Pharmaceutical Works, India) were dissolved in corn oil to obtain a homogenous stock solution and then administered orally via gavage at a dose of 200 mg/kg body weight once every 96 hours (Castracane *et al.*, 1994) for a period of six weeks.

### 2.2 Coconut water

The coconut (*Cocos nucifera* L.) fruits used were obtained from a local farm in Ibadan, Oyo State, and authenticated by a botanist from the Department of Botany, University of Ibadan. The coconut husks were peeled off prior to administration, the shell was cracked and the coconut water poured into a sterile container. Fresh coconut water was administered orally at a dose of 20 mL/kg body weight per day for a period of six weeks (Kunle-Alabi *et al.*, 2014).

### 2.3 N-acetyl cysteine

600 mg tablets of N-acetyl cysteine (Finlab Pharmaceuticals, USA) were dissolved in distilled water and administered orally at a daily dose of 100 mg/kg body weight (Elgerbed, 2010; Maheswari *et al.*, 2014) for a period of six weeks.

### 2.4 Experimental animals

Thirty-five male Wistar rats weighing about 150 g obtained from the Central Animal House, College of Medicine, University of Ibadan, Nigeria, were used for this study. All procedures 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) and were approved by the departmental ethics committee.

The rats were randomly divided into seven groups (of 5 rats each) and treated daily as follows for six weeks:

- |                       |  |
|-----------------------|--|
| Group 1: CONTROL:     | 20 mL/kg Distilled water,                              |
| Group 2: CORNOIL:     | 20 mL/kg Corn oil<br>(vehicle for danazol),            |
| Group 3: CW:          | 20 mL/kg Coconut water,                                |
| Group 4: NAC:         | 100 mg/kg N-acetyl<br>cysteine,                        |
| Group 5: DANAZOL:     | 200 mg/kg Danazol,                                     |
| Group 6: DANAZOL+CW:  | 200 mg/kg Danazol +<br>20 mL/kg Coconut water,         |
| Group 7: DANAZOL+NAC: | 200 mg/kg Danazol +<br>100 mg/kg N-acetyl<br>cysteine. |

### 2.5 Serum hormones

After sacrifice, serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone were measured using ELISA kits (Fortress Diagnostics Limited, Unit 2C Antrim Technology Park, Antrim, BT41 1QS, United Kingdom) according to the manufacturer's instructions.

### 2.6 Sperm indices

The epididymis was dissected out and its volume was determined by fluid displacement in warm normal saline. Epididymal fluid was obtained from the caudal part for determination of sperm motility, viability and count according to the methods of (Oyeyemi *et al.*, 2000; Zemjanis, 1970).

### 2.7 Testicular histology

Testes were weighed and the right testis was fixed in 10% formalin, dehydrated stepwise in graded ethanol, cleared in xylene and then embedded in paraffin wax. A section of 5  $\mu$ m thickness paraffin section was taken from the mid portion of each testis and stained with Haematoxylin and Eosin (H&E), then examined under a light microscope at X400 magnification. Tissues from which slides were taken were randomly selected.

### 2.8 Testicular oxidative stress biomarkers

The left testis was homogenized in ice-cold 100 mM phosphate buffer (pH 7.4), centrifuged at 3000 rpm for 15 minutes and the supernatant was used for malondialdehyde (Caetano *et al.*, 2013; Ohkawa *et al.*, 1979), total protein (Lowry *et al.*, 1951) and reduced glutathione (Jollow *et al.*, 1974) analysis.

### 2.9 Statistical analysis

Data were expressed as mean  $\pm$  standard error of mean (SEM) and compared using one way analysis of variance (ANOVA) on the SPSS software version 20. Differences in means were considered statistically significant at  $p < 0.05$ .

## 3. Results

### 3.1 Sperm indices

There was a significant increase in sperm motility and count in the coconut water (CW) and N-acetyl cysteine (NAC) groups when compared with the control and corn oil (vehicle for danazol) groups (Table 1). The danazol group showed a significant decrease in sperm motility, viability and count when compared with the control, corn oil, CW and NAC groups. There was also a significant increase in sperm motility, viability and count in danazol+CW and danazol+NAC groups when compared with the danazol group (Table 1).

Table 1. Effects of Coconut water and N-Acetyl cysteine on sperm characteristics.

Groups	Sperm Count (million/ml)	Sperm Motility (%)	Sperm Viability (%)
Control	118.33 ± 30.56	66.83 ± 4.37	96.50 ± 0.50
Corn oil	116.33 ± 17.78	70.50 ± 5.10	90.00 ± 6.02
CW	162.00 ± 28.44 <sup>abc</sup>	93.33 ± 1.05 <sup>abc</sup>	95.50 ± 1.23 <sup>c</sup>
NAC	141.87 ± 25.92 <sup>abc</sup>	93.33 ± 1.05 <sup>abc</sup>	96.33 ± 0.62 <sup>c</sup>
Danazol	49.96 ± 12.48 <sup>ab</sup>	46.33 ± 8.41 <sup>ab</sup>	64.17 ± 4.17 <sup>ab</sup>
Danazol + CW	143.67 ± 11.39 <sup>abc</sup>	91.67 ± 1.67 <sup>abc</sup>	94.00 ± 1.32 <sup>c</sup>
Danazol + NAC	122.58 ± 19.62 <sup>abc</sup>	76.67 ± 9.10 <sup>abc</sup>	83.33 ± 7.90 <sup>c</sup>

CW = Coconut water, NAC = N-Acetyl cysteine. n = 5. <sup>a</sup>p ≤ 0.05 compared with control; <sup>b</sup>p ≤ 0.05 compared with corn oil; <sup>c</sup>p ≤ 0.05 compared with danazol.

### 3.2 Serum hormones

Danazol group showed significantly reduced serum LH, FSH and testosterone levels when compared with control and corn oil groups (Table 2). CW, NAC, Danazol + CW and Danazol + NAC groups showed a significant increase in the serum LH, FSH and testosterone levels when compared with the Danazol group.

### 3.3 Testicular oxidative stress biomarkers

All groups showed a significant increase in reduced glutathione, total protein and a significant reduction in malondialdehyde in testicular homogenates when compared with the control and corn oil groups.

Table 2. Effects of Coconut water and N-Acetyl cysteine on serum gonadotropin and testosterone levels.

Groups	Follicle Stimulating Hormone (mIU/mL)	Luteinizing Hormone (mIU/mL)	Testosterone (ng/mL)
Control	33.78 ± 0.11	7.21 ± 0.13	31.17 ± 0.96
Corn oil	33.73 ± 0.06	7.46 ± 0.31	31.80 ± 0.43
CW	33.78 ± 0.07 <sup>c</sup>	7.27 ± 0.17 <sup>c</sup>	31.97 ± 0.72 <sup>c</sup>
NAC	33.73 ± 0.06 <sup>c</sup>	6.85 ± 0.32 <sup>c</sup>	32.16 ± 0.27 <sup>c</sup>
Danazol	18.91 ± 2.28 <sup>ab</sup>	4.18 ± 0.33 <sup>ab</sup>	25.26 ± 1.40 <sup>ab</sup>
Danazol + CW	33.84 ± 0.11 <sup>c</sup>	7.12 ± 0.16 <sup>c</sup>	30.83 ± 1.74 <sup>c</sup>
Danazol + NAC	33.73 ± 0.06 <sup>c</sup>	7.15 ± 0.23 <sup>c</sup>	29.25 ± 1.32 <sup>c</sup>

CW = Coconut water, NAC = N-Acetyl cysteine. n = 5. <sup>a</sup>p ≤ 0.05 compared with control; <sup>b</sup>p ≤ 0.05 compared with corn oil; <sup>c</sup>p ≤ 0.05 compared with danazol.

Table 3. Effects of Coconut water and N-Acetyl cysteine on testis oxidative stress biomarkers.

Groups	Malondialdehyde (nmol/mg protein)	Glutathione (nmol/mg protein)	Protein (mg)
Control	8.03 ± 0.35	0.03 ± 0.01	4271.19 ± 178.60
Corn oil	8.10 ± 0.35	0.03 ± 0.01	4330.24 ± 174.93
CW	5.87 ± 0.69 <sup>ab</sup>	0.05 ± 0.00 <sup>ab</sup>	4652.85 ± 133.18 <sup>ab</sup>
NAC	5.69 ± 0.81 <sup>ab</sup>	0.05 ± 0.00 <sup>ab</sup>	4598.45 ± 98.89 <sup>ab</sup>
Danazol	5.98 ± 0.89 <sup>ab</sup>	0.06 ± 0.00 <sup>ab</sup>	4699.80 ± 119.71 <sup>ab</sup>
Danazol + NAC	5.81 ± 0.54 <sup>ab</sup>	0.07 ± 0.00 <sup>ab</sup>	4717.62 ± 110.02 <sup>ab</sup>
Danazol + CW	5.75 ± 0.25 <sup>ab</sup>	0.07 ± 0.00 <sup>ab</sup>	4769.88 ± 122.00 <sup>ab</sup>

CW = Coconut water, NAC = N-Acetyl cysteine. n = 5. <sup>a</sup>p ≤ 0.05 compared with control; <sup>b</sup>p ≤ 0.05 compared with corn oil.

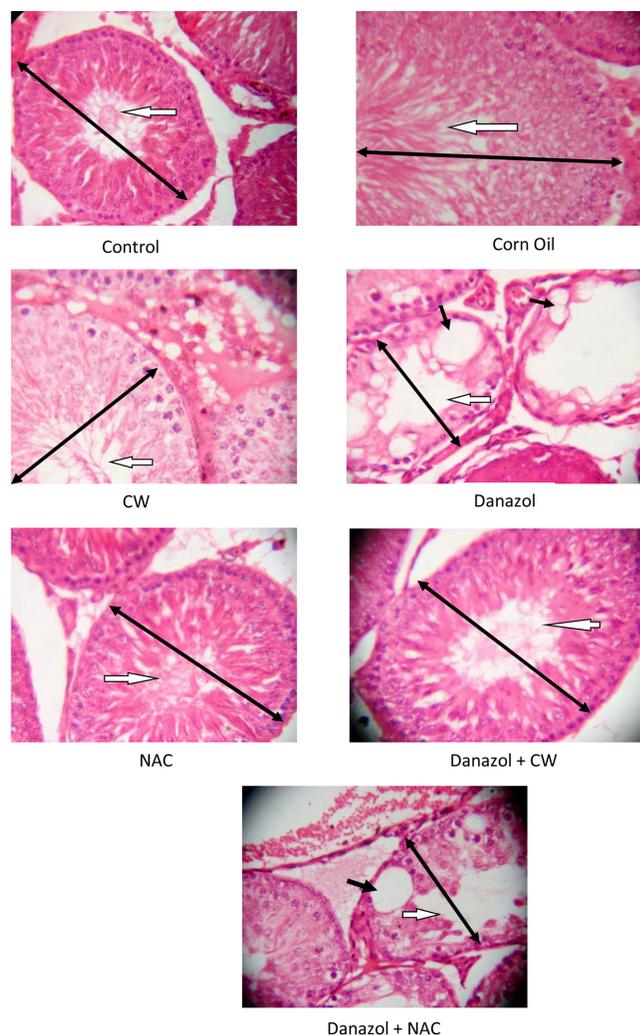


Figure 1. Photomicrographs of testicular sections showing the effects of Coconut water (CW) and N-Acetyl cysteine (NAC) on Danazol-treated male Wistar rats. H&E. Magnification X400. Seminiferous tubules (spanned), their lumen (white arrows) and defective germinal epithelium (black arrows) are shown.

### 3.4 Testicular histology

Primary maturation arrest and absence of spermatozoa is observed in testicular sections from the danazol group (Plate 1). Testicular sections from the danazol+NAC group show moderate similarities to the danazol group (Plate 1). Testicular sections from control, corn oil, CW, NAC and danazol+CW groups show normal architecture (Plate 1).

## 4. Discussion

Danazol disrupts several enzymatic steps along the steroidogenic pathway (Barbieri *et al.*, 1977) and coconut water successfully counteracts these effects (Kunle-Alabi *et al.*, 2014). Coconut water is rich in amino acids and peptides

(Cooper, 1986; Gopikrishna *et al.*, 2008; Mandal *et al.*, 2009). These may contribute to the enzymatic activity of coconut water in countering danazol activity. We explored the role of the amino acid cysteine in coconut water's anti-danazol activity.

N-acetyl cysteine is a prodrug for the amino acid cysteine (Burgunder *et al.*, 1989). Both N-Acetylcysteine and cysteine have been reported to act as antioxidants and improve male reproductive functions especially through the production of glutathione (Safarinejad & Safarinejad, 2009). Comparison between the effects of coconut water and n-acetyl cysteine revealed notable similarities in their actions on improving sperm characteristics and male sex hormone levels. This suggests that the cysteine content of coconut water may play a significant role in the actions of coconut water on the reproductive system.

Both coconut water and n-acetyl cysteine reduced the oxidative stress biomarker – malondialdehyde, while increasing glutathione levels in the testis. Oxidative stress has been implicated in the pathogenesis of several conditions related to male infertility (Agarwal *et al.*, 2014; Walczak-Jedrzejowska *et al.*, 2013). Cysteine is a sulfhydryl compound which plays a substantial role in *in vivo* redox reactions (Giles *et al.*, 2003). This does not however seem to be its sole mechanism of action in male reproductive functions, as danazol did not result in significant lipid peroxidation in this study and has not previously been reported to do so. Indeed, these results suggest that danazol protects against oxidative stress, supporting what has previously been suggested (Ngô *et al.*, 2009; Welder *et al.*, 1995). This study therefore supports previous reports that the amino acid, cysteine, acts via the regulation of reproductive hormone levels and activity (Dufau, 1998; Forgács *et al.*, 2001; Simoni *et al.*, 1997). Considering the striking similarity between its effects and those of coconut water on male reproductive functions, cysteine may well be the major element in the actions of coconut water on reproductive endocrinology.

This also suggests a synergistic role for the other constituents of coconut water with cysteine. Some of these constituents such as ascorbic acid, arginine and flavonoids, have also been reported to improve male reproductive functions (Husein *et al.*, 2011; Luck, 1995; Mazzi *et al.*, 2012). Normal testicular architecture is essential for the maintenance of optimal reproductive function. The inability of n-acetyl cysteine to completely prevent danazol-induced testicular damage supports the hypothesis that other components of coconut water are also important in its fertility-enhancing actions.

## 5. Conclusions

It was therefore concluded that the cysteine component of coconut water is essential for its actions on male reproductive functions along the hypothalamo-pituitary-gonadal-axis.

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