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Cardioprotective and renoprotective effects of *Cocos nucifera* water in offspring of high fat diet fed Wistar rat dams

Olufadekemi Tolulope Kunle-Alabi*, Opeyemi Oreofe Akindele, Yinusa Raji

Laboratory for Reproductive Physiology and Developmental Programming, Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Nigeria

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ABSTRACT

Objective: To evaluate the effects of *Cocos nucifera* (*C. nucifera*) water on the cardiovascular and renal functions of offspring from rat dams fed high fat diet during gestation.
Methods: Four groups of pregnant Wistar rats were treated from gestation day 1 to 21; namely, control (1 mL/100 g distilled water), *C. nucifera* water (1 mL/100 g *C. nucifera* water), high fat diet (1 mL/100 g distilled water + 30% butter: 70% standard rodent diet) and high fat diet + *C. nucifera* water (1 mL/100 g *C. nucifera* water + 30% butter: 70% standard rodent diet). All dams received standard rodent diet from gestation day 22, and offspring were weaned to standard rodent diet on postnatal day 28. On postnatal day 120, serum and cardiac levels of malondialdehyde, interleukin-1β and high sensitivity C-reactive protein were determined in offspring. Serum creatinine and urea levels as well as histology of heart and kidney tissue were assessed. Data were analyzed using One-way ANOVA and $P < 0.05$ was considered statistically significant.
Results: Male high fat diet offspring showed significantly increased ($P < 0.05$) serum interleukin-1β compared with *C. nucifera* water offspring. The increase in serum high sensitivity C-reactive protein observed in female high fat diet offspring was not present in high fat diet + *C. nucifera* water offspring.Heart tissues from high fat diet offspring showed scanty fibers and congested myocardium with mild fibrosis. Male high fat diet offspring kidneys showed mesangial cell hyperplasia, fat infiltration and mild tubular necrosis. These were accompanied with alterations in serum urea and creatinine levels in high fat diet + *C. nucifera* water offspring.
Conclusions: *C. nucifera* water exerts cardioprotective and renoprotective effects on offspring of rat dams fed high fat diet during gestation via an anti-inflammatory mechanism.

1. Introduction

The close link between the cardiovascular and renal systems of the body translates to concurrent adverse outcomes to both systems with disruptions of either system. Cardiovascular or renal diseases are known to be transmitted genetically from one generation to the next. Interestingly, such transmission can also occur via epigenetic modifications as a result of environmental exposures such as the diet. High fat diet is a major culprit in disease proliferation, its effects being felt virtually in every physiological system. Maternal high fat diet during gestation "programs" offspring develops cardiovascular and renal dysfunction in postnatal life[1]. Renal abnormalities have been reported to include a reduction in the number of nephrons, higher risk of low glomerular filtration rate, albuminuria and may eventually lead to primary kidney disease[2]. These and the resultant hypertension[3] have made maternal high fat diet a highly topical issue[4-6].

Mechanisms of cardiovascular disease development with high fat diets include oxidative stress and inflammation[7]. The hypolipidemic, antioxidant, anti-inflammatory, cardioprotective and renoprotective effects of *Cocos nucifera* (*C. nucifera*) (coconut) water have been reported[8-10]. We have also reported that *C. nucifera* water prevents maternal high fat diet induced

*Corresponding author: Olufadekemi Tolulope Kunle-Alabi, Laboratory for Reproductive Physiology and Developmental Programming, Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Nigeria.
Tel: +2348033790310
E-mail: mrskalabi@yahoo.com

All experimental procedures involving animals were conducted in accordance to the guiding principles for research involving animals as recommended by the guidelines for laboratory animal care of the National Institute of Health (National Institute of Health publication No. 85–23, revised 1996) and approved by the Departmental Ethics Committee.

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hormonal and pup biometric changes during pregnancy[11]. There is however paucity of information on the fetal programming effects of *C. nucifera* water, and the possible effects on the health of offspring in adult life. This study therefore explored the effects of *C. nucifera* water on the cardiovascular and renal functions of offspring exposed to maternal high fat diet during gestation.

2. Materials and methods

2.1. Plant material

C. nucifera (coconut) fruits were obtained from a *C. nucifera* plantation in Oyo State, Nigeria and verified by a botanist from the Department of Botany, University of Ibadan, Ibadan, Nigeria. *C. nucifera* water was obtained by piercing the soft "eye" of the *C. nucifera* (the germination pore) with a sterile screw driver and decanting the water into a large sterile container. Fresh *C. nucifera* water was administered via oral gavage at a daily dosage of 1 mL/100 g body weight using blunt-tipped oral cannulas attached to 2 mL syringes. Administration of *C. nucifera* water was done between 8:00–9:00 a.m. daily.

2.2. High fat diet

High fat diet was consisted of 70% standard rodent diet and 30% butter (with 82% milk fat) and was given *ad libitum*.

2.3. 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 (National Institute of Health publication No. 85–23, revised 1996) and were approved by the Departmental Ethics Committee. Virgin female rats weighing 140–150 g obtained from the Central Animal House, University of Ibadan, Nigeria were mated with proven breeder male rats from the Laboratory for Reproductive Physiology and Developmental Programming, Department of Physiology, University of Ibadan, Nigeria. Pregnancy was confirmed by the presence of spermatozoa in vaginal smears and the day of observation of spermatozoa was taken as gestation day 1 for each female. Twenty-four pregnant rats were then randomly divided into four groups (n = 6), namely, control, *C. nucifera* water, high fat diet and high fat diet plus *C. nucifera* water.

2.4. Experimental design

From gestation day 1 to 21, high fat diet and high fat diet plus *C. nucifera* water dams received high fat diet, while control and *C. nucifera* water dams were fed standard rodent diet (Ladokun Feeds, Ibadan, Nigeria). All rats were fed with standard rodent diet before mating and after parturition. The animals had access to feed and drinking water *ad libitum* throughout the experimental period. All dams were allowed to litter naturally and were allowed to nurse their young which were weaned on postnatal day 28. On postnatal day 28, male and female offsprings from each dam were housed in separate cages and had access to standard rodent feed and drinking water *ad libitum* until postnatal day 120.

2.5. Sample collection

On postnatal day 120, adult offsprings were weighed and anaesthetized with 50 mg/kg body weight thiopentone sodium (Samarth Life Sciences Pvt. Ltd., Mumbai, India) intraperitoneally[12]. Blood was collected via cardiac puncture to obtain serum, while the hearts and kidneys were also harvested.

2.6. Organ analysis

The heart and kidneys of each animal were weighed. The relative organ weight was calculated for each organ as the percentage of the ratio of the organ weight to the rat's body weight. One heart and one kidney from a male and a female from each group were fixed in 10% formal saline for histological assessment. The hearts from one randomly selected male and female offspring from each dam were homogenized in 4 mL of ice-cold 100 mmol/L phosphate buffer (pH 7.4) per gram of tissue. The supernatant was obtained after centrifuging at 3 000 r/min for 15 min and used for the determination of malondialdehyde (Oxford Biomedical Research, Michigan, USA) and high sensitivity C-reactive protein (Cloud-Clone Corp., Houston, USA) using kits according to the manufacturer's instructions.

2.7. Biochemical assays

Serum collected was used to assay for interleukin-1β (IL-1β) (RayBiotech, Georgia, USA), high sensitivity C-reactive protein (Cloud-Clone Corp., Houston, USA), creatinine and urea (Fortress Diagnostics Limited, Antrim, UK) using kits according to the manufacturer's instructions.

2.8. Statistical analysis

Data were expressed as mean ± SEM. Significance of difference of means was analyzed using One-way ANOVA followed by least significant difference *post-hoc* analysis where necessary. Statistical significance was taken as $P < 0.05$.

2.9. Theory

Concurrent administration of *C. nucifera* water to pregnant dams exposed to a high fat diet may protect their offspring from being adversely programmed for cardiovascular and renal diseases.

3. Results

3.1. Effects of maternal high fat diet and C. nucifera water administration during gestation on heart and kidney weight and structure of offspring

The weights of the hearts of adult female offspring from high fat diet plus *C. nucifera* water dams were significantly higher than those of female offspring of control dams (Table 1). The hearts of adult male offspring (Table 1) and the kidneys from both male and female offspring (Table 2) did not show any significant difference in weight among all the groups.

Table 1
Effects of maternal high fat diet and *C. nucifera* water administration during gestation day 1–21 on relative weight of offspring's hearts (%).

Group	Male heart	Female heart
Control	0.33 ± 0.01	0.33 ± 0.01
C. nucifera water	0.33 ± 0.00	0.35 ± 0.02
High fat diet	0.33 ± 0.01	0.35 ± 0.01
High fat diet + *C. nucifera* water	0.33 ± 0.02	0.38 ± 0.02[a]

[a]: $P < 0.05$ compared with control group.

Table 2
Effects of maternal high fat diet and *C. nucifera* water administration during gestation day 1–21 on relative weights of offspring's kidneys (%).

Group	Male kidney	Female kidney
Control	0.56 ± 0.01	0.57 ± 0.02
C. nucifera water	0.54 ± 0.01	0.53 ± 0.03
High fat diet	0.53 ± 0.02	0.55 ± 0.02
High fat diet + *C. nucifera* water	0.53 ± 0.01	0.57 ± 0.02

The hearts showed congested myocardia with scanty fibers (blue arrows), widened interstitial spaces (black arrows) and mild fibrosis (red arrow) in both male (Figure 1C) and female (Figure 2C) offspring of high fat diet dams. Kidneys from male offspring of high fat diet dams (Figure 3C) showed normal glomerulus (white arrow) and renal tubules (black arrows) with mesangial cell hyperplasia (blue arrow) and focal area of fat infiltration (red arrow). However female offspring of high fat diet dams (Figure 4C) showed poor architecture of the renal glomeruli (white arrow) and mild tubular necrosis (black arrow). These changes were present with reduced severity in the high fat diet plus *C. nucifera* water offspring (Figures 3D and 4D) and completely absent in the control and *C. nucifera* water offspring (Figures 3 and 4A and B respectively) which showed normal architecture.

3.2. Effects of maternal high fat diet and C. nucifera water administration during gestation on cardiac inflammation and malondialdehyde levels of offspring

Serum levels of IL-1β were significantly increased in male offspring of high fat diet dams when compared with those of male offspring of *C. nucifera* water dams (Table 3). Co-administration of *C. nucifera* water to high fat diet dams in high fat diet plus *C. nucifera* water group reduced serum IL-1β levels of male offspring to control values (Table 3).

Table 3
Effects of maternal high fat diet and *C. nucifera* water administration during gestation day 1–21 on serum IL-1β levels of offspring (pg/mL).

Group	Male	Female
Control	1706.67 ± 275.36	1453.33 ± 134.00
C. nucifera water	1226.67 ± 118.51	1520.00 ± 116.24
High fat diet	2221.33 ± 220.12[b]	1706.67 ± 299.33
High fat diet + *C. nucifera* water	1493.33 ± 134.33[c]	1650.00 ± 63.10

[b]: $P < 0.05$ compared with *C. nucifera* water group; [c]: $P < 0.05$ compared with high fat diet group.

High sensitivity C-reactive protein levels in the heart were not significantly different among the groups (Table 4). The female offspring of high fat diet fed dams showed a significant reduction in serum high sensitivity C-reactive protein levels when compared with all the other groups (Table 4).

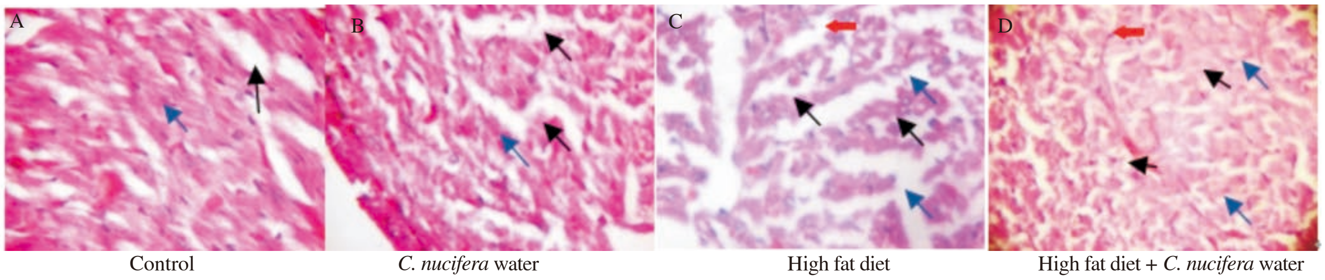


Figure 1. Photomicrographs of heart sections from male offsprings of dams treated with high fat diet and/or *C. nucifera* water from gestation day 1–21 stained by Haematoxylin and Eosinmagnification ×400.
Blue arrow: Myocardium; Black arrow: Interstitium; Red arrow: Mild fibrosis.

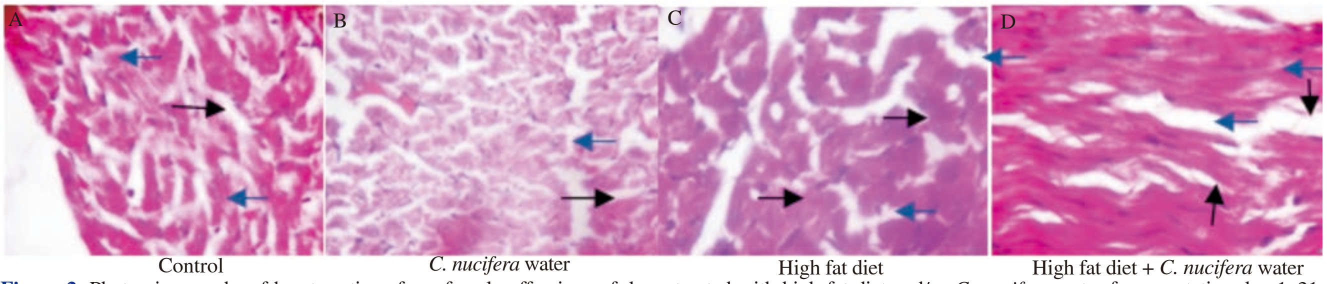
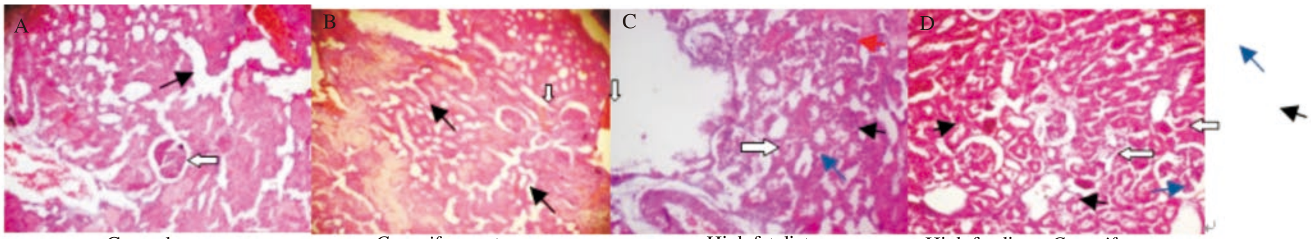
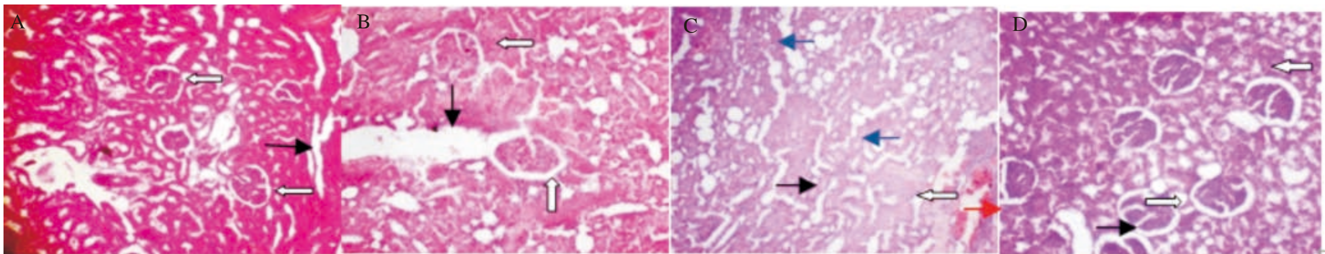


Figure 2. Photomicrographs of heart sections from female offsprings of dams treated with high fat diet and/or *C. nucifera* water from gestation day 1–21 stained by Haematoxylin and Eosin magnification ×400.
Blue arrow: Myocardium; Black arrow: Interstitium.



Control C. *nucifera* water High fat diet High fat diet + *C. nucifera* water

Figure 3. Photomicrographs of kidney sections from male offsprings of dams treated with high fat diet and/or *C. nucifera* water from gestation day 1–21 stained by Haematoxylin and Eosin ×100.
White arrow: Glomerulus; Black arrow: Tubule; Blue arrow: Mesangial cells; Red arrow: Fat infiltration.



Control *C. nucifera* water High fat diet High fat diet + *C. nucifera* water

Figure 4. Photomicrographs of kidney sections from female offsprings of dams treated with high fat diet and/or *C. nucifera* water from gestation day 1-21 stained by Haematoxylin and Eosin ×100.
White arrow: Glomerulus; Black arrow: Tubule; Blue arrow: Mesangial cells; Red arrow: Fat infiltration.

Table 4
Effects of maternal high fat diet and *C. nucifera* water administration during gestation day 1–21 on serum and cardiac high sensitivity C-reactive protein levels of offspring (pg/mL × 10^5).

Group	Male serum	Male heart	Female serum	Female heart
Control	893.33 ± 211.97	4326.67 ± 836.49	883.33 ± 52.55	8160.00 ± 737.65
CW	550.00 ± 165.19	6766.67 ± 430.48	906.67 ± 39.23	6733.33 ± 1089.30
HFD	1146.67 ± 407.59	5710.00 ± 569.79	470.00 ± 190.54^{ab}	7983.33 ± 1106.64
HFD + CW	893.33 ± 290.19	5163.33 ± 1877.18	1120.00 ± 175.58^{c}	8242.22 ± 1531.83

^{a}: $P < 0.05$ compared with control group; ^{b}: $P < 0.05$ compared with *C. nucifera* water group; ^{c}: $P < 0.05$ compared with high fat diet group. CW: *C. nucifera* water; HFD: High fat diet; HFD + CW: High fat diet + *C. nucifera* water.

Malondialdehyde levels in heart homogenates were significantly lower in males from high fat diet plus *C. nucifera* water dams when compared with male offspring of *C. nucifera* water dams (Table 5).

Table 5
Effects of maternal high fat diet and *C. nucifera* water administration during gestation day 1–21 on cardiac malondialdehyde levels of offspring (µm).

Group	Male	Female
Control	67.82 ± 9.10	73.39 ± 4.95
C. nucifera water	80.90 ± 2.07	76.26 ± 7.86
High fat diet	66.90 ± 5.26	64.21 ± 9.90
High fat diet + *C. nucifera* water	58.36 ± 7.91^{b}	62.93 ± 1.08

^{b}: $P < 0.05$ compared with *C. nucifera* water group.

3.3. Effects of maternal high fat diet and C. nucifera water administration during gestation on serum urea and creatinine levels of offspring

Male offspring of high fat diet plus *C. nucifera* water dams showed significant reductions in serum urea concentrations when compared with male offspring from the other groups (Table 6). Serum creatinine levels in offspring of high fat diet plus *C. nucifera* water dams showed significant increments in the males and reductions in the females (Table 7).

Table 6
Effects of high fat diet and *C. nucifera* water administration during gestation day 1–21 on serum urea concentration of offspring (mg/dL).

Groups	Male	Female
Control	54.58 ± 8.48	56.81 ± 4.44
C. nucifera water	75.28 ± 5.64	62.64 ± 7.94
High fat diet	59.26 ± 9.41	63.89 ± 9.31
High fat diet + *C. nucifera* water	32.30 ± 3.51^{a,b,c}	63.61 ± 9.18

^{a}: $P < 0.05$ compared with control group; ^{b}: $P < 0.05$ compared with *C. nucifera* water group; ^{c}: $P < 0.05$ compared with high fat diet group.

Table 7
Effects of high fat diet and *C. nucifera* water administration during gestation day 1–21 on serum creatinine concentration of offspring (mg/dL).

Groups	Male	Female
Control	0.21 ± 0.01	0.29 ± 0.05
C. nucifera water	0.25 ± 0.03	0.21 ± 0.03
High fat diet	0.22 ± 0.03	0.23 ± 0.04
High fat diet + *C. nucifera* water	0.28 ± 0.01^{a,c}	0.18 ± 0.03^{a}

^{a}: $P < 0.05$ compared with control group; ^{c}: $P < 0.05$ compared with high fat diet group.

4. Discussion

Maternal high fat diet consumption can adversely program offspring to develop a preference for fatty foods and obesity during childhood or adulthood[13-15]. Such effects predispose offspring not only to the development of metabolic disorders, but also place them at a higher risk of cardiovascular and renal diseases[3,4]. This study explored if the anti-inflammatory, antioxidant and hypolipidemic activities of *C. nucifera* water[8-10], could reverse this fetal programming and thus protect the offspring from disease development in adult life.

Though inflammation is one of the major hallmarks of cardiovascular disease in the presence of excess body fat and inflammatory cytokines can be used as a yardstick for inflammatory status[16], the levels of IL-1β and high sensitivity C-reactive protein did not suggest any significant inflammation within the cardiovascular and systemic milieu. This however does not rule out the presence of inflammation which was observed on histological examination of the heart tissues from high fat diet offspring. The cardioprotective, and more so, the renoprotective effects of *C. nucifera* water in offspring of dams fed high fat diet during gestation were observed in the histological slides of high fat diet plus *C. nucifera* water offspring.

High fat diets predispose to oxidative stress[17], and oxidative stress has been implicated in the etiopathogenesis of cardiovascular and renal diseases[18,19]. Thiobarbituric acid reactive substances, specifically malondialdehyde, can be used to determine the level of oxidative stress status in tissues[20]. The reduced malondialdehyde levels observed in the heart of male high fat diet plus *C. nucifera* water offspring suggested that the antioxidant action of *C. nucifera* water[8] protects these offspring against lipid peroxidation in the cardiac tissue and may thus protect them against cardiovascular disease. The renal tissue appeared significantly improved in both male and female offspring of high fat diet plus *C. nucifera* water dams when compared with high fat diet offspring. The significantly lower levels of serum urea and creatinine in these (high fat diet plus *C. nucifera* water) offspring further suggested that their cardiorenal function has been programmed to compensate for the potentially destructive effects of maternal high fat diet during gestation.

C. nucifera water holds promise of cardiovascular and renal protection in offspring of dams fed high fat diet during gestation and these ameliorative effects are more pronounced in male offspring.

Conflict of interest statement

We declare that we have no conflict of interest.

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