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Protective and Ameliorative Effects of Virgin Coconut Oil on Cadmium-Damaged Wistar Rats' Testes

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Abstract

Purpose: Cadmium (Cd) is an environmental and industrial pollutant that affects the male reproductive system. Cd induces its effect by affecting tissue antioxidant enzyme systems. Coconut (Cocos nucifera) is consumed locally as food, used for cooking and production of margarine. It is commonly used as herbal medicine worldwide. The purpose of this study was to investigate the protective and ameliorative effects of Virgin Coconut Oil (VCO) against testes-damaged induced by Cd.

Methodology: Twenty-five healthy male rats were grouped into five as following: Controls (A), Cd-treated (B), VCO-treated (C), Cd+VCO (D) and VCO+Cd (E). The control group received distilled water. Cd was given at dose of 4.56 mg/kg body weight for 2 weeks duration and VCO was given at 6.7 ml/kg body weight for 7 weeks. The rats were sacrificed by cervical dislocation, the semen was collected for sperm analysis and testes were removed for microscopic and histomorphometric evaluations.

Findings: The current study showed marked morphological changes in the form of irregular tubular shape, deleterious basement membrane and irregularity of testicular shape of rats treated with Cd alone. However, the rats treated with Cd+VCO and VCO+Cd showed milder irregular tubules, wide interstitial space and minute foci of necrosis in the testes. The VCO rats showed normal histological features when compared with the control. Body weight analysis of the rats shows insignificant increase from the initial week to the final week of the experiment for all Groups. The rats of VCO showed insignificant increase of testicular weight, likewise, the rats of Cd+VCO and VCO+Cd when compared with Cd group.

Highest value of SOD was observed in VCO group followed by the control group, Cd+VCO and VCO+Cd groups, Lipid peroxidation (MDA) and Glutathione (GSH) values were insignificantly decreased in Cd group and increased values of both MDA and GSH were observed in control groups followed by VCO groups and Cd+VCO and VCO+Cd. Morphometric evaluation showed CSA and GED increase in Cd-treated rats compared to other treated groups and the control, while the LD was significantly higher in Cd-treated rats.

The sperm cells reduced significantly ($P<0.05$) in Cd-treated rats, whereas there was insignificant ($P>0.05$) increase number of sperm cells in VCO-treated rats, Control rats, Cd+VCO and VCO+Cd rats.

The percentage of rapid progressive sperm cells in VCO-treated rats was insignificantly ($P>0.05$) higher while that of the Cd was lower in the treated groups. Meanwhile, the rapid motility grading of groups D and E was lower compared with both control and VCO-treated rats but higher than the Cd-treated rats. The percentage of non-progressive sperm cells was significantly ($P<0.05$) higher in Cd rats compared with the rest of the groups. Hence, percentage of dead sperm cells was also higher in Cd-treated rats.

Structural deformities were also observed in the sperm cells where Cd-treated has the highest percentage values of deformities of the head, mid-piece and tail. Hence, the lowest deformities values were recorded in control group and VCO group.

Unique contribution to policy, theory, and practice: Cd induces negative effects on sperm parameters in rat testes. The increased oxidative stress resulted from Cd intoxication in testicular tissue might be responsible for these adverse changes. VCO on the other had protective effect against Cd toxicity, evidenced by increase the SOD, MDA and GSH level in the testicular tissue, and minimal histological changes in the testes of rats treated with VCO+Cd and Cd+VCO.

Keywords: Rat; Testes toxicity; Cadmium; VCO

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Introduction

Cadmium (Cd) is an environmental pollutant ranked eighth in the top 20 hazardous substances and the human activity has markedly increased the distribution of Cd in the global environment. Exposure to Cd produces hepatic, pulmonary, and testicular injury, whereas chronic exposure results in renal and bone injury and cancer, as well as toxicity to other organs. The factors that influence Cd absorption, distribution, and elimination are not well understood, but it is known that Cd is poorly absorbed after oral ingestion [1].

The uptake of Cd through the food chain in aquatic organisms may lead to morphological alterations and pathological disorders. Cadmium is a bivalent cation and is unable to generate free radicals directly; nevertheless, there is increased production of Reactive Oxygen Species (ROS) after cadmium exposure. Cadmium alters antioxidant defense systems and increase production of cellular ROS, such as singlet oxygen, hydrogen peroxide, and hydroxyl radicals [2]. ROS can lead to oxidative stress within cells by reacting with macromolecules causing damages, such as mutation, destruction of protein function and structure, and peroxidation of lipids as well as alterations in gene expression and apoptosis [3]. The effects of cadmium induced oxidative stress in the tissues and cells of animals and plants have been reported [3].

VCO is the oil obtained from fresh and mature kernel of coconut (*Cocos nucifera*) by mechanical or natural means that does not alter the nature properties of the oil. VCO-making should not undergo chemical refining, bleaching, or deodorizing. VCO can be consumed in its natural state without further processing [4]. VCO can also be used for cooking and topical applications such as for hair blackening and wound healing [5]. Examples of processes that can be used to produce VCO are by heating or centrifuging coconut milk, pressing coconut kernel, and letting the air-borne *Acetobacter* spp. to inoculate the coconut milk. Such processes broke the emulsion of the coconut milk, making the oil separated from other components like carbohydrates and proteins [6].

Standard coconut oil is normally produced by firstly drying the kernel (to produce something known as copra) and secondly refining, bleaching and deodorizing the extracted oil. The so-called virgin coconut oil is instead made *via* a 'wet process', either being extracted from coconut milk or from fresh kernel which is not subjected to drying or chemical refining. Coconut oil comprises 99.9% fatty acids; of these, 91.9% are Saturated Fatty Acids (SFA), 6.4% are Monounsaturated Fatty Acid Acids (MUFA) and 1.5% is Polyunsaturated Fatty Acids (PUFA), and coconut oil contains no dietary cholesterol. Advocates of coconut oil claim that some of the specific saturated fatty acids present in coconut oil, those of medium-chain length, actually confer health benefits.

One of the natural sources that contain antioxidants is Virgin Coconut Oil (VCO), oil that comes from fresh old coconut (*Cocos nucifera*), which is processed at low temperatures. Scientifically, VCO has been reported to exert various pharmacological activities such as anti-arthritis and antioxidant, anti-thrombogenicity, antihyperlipidemic, cardioprotective, antimicrobial, antiosteoporosis, hepatoprotective, and antinociceptive and anti-inflammatory. Interestingly, recent clinical studies demonstrated that VCO possesses at least the anti-hypercholesterolemic and anti-Alzheimer.

Moreover, VCO has been taken orally by people throughout Asia, especially in India, due to high medicinal values especially due to its high antioxidant properties and phenolic content. Scientifically, VCO has been reported to exert various pharmacological activities such as anti-arthritis and antioxidant, anti-thrombogenicity [7], antihyperlipidemic, antimicrobial and cardioprotective, anti-osteoporosis [8], hepatoprotective and, antinociceptive and anti-inflammatory. Interestingly, recent clinical studies demonstrated that VCO possesses at least the anti-hypercholesterolemic and anti-Alzheimer activities.

Despite these streams of findings, there is need to carry out scientific investigation on the male sexual parameters of the protective and ameliorative capacities of the VCO to corroborate the ethnomedical use of the VCO as an antioxidant and against other male reproductive dysfunctions. Therefore, this study seeks to evaluate the sperm parameters and testicular effects of virgin coconut oil on cadmium-damaged testes of Wistar rats.

Materials and Methods

Experimental animal

Twenty-five healthy male rats were used during this study. The rats were fed with grower mash under good ventilation and adequate water was provided ad libitum. The rats were acclimatized to the new environment for two weeks before the commencement of the experiment.

Preparation of virgin coconut oil (VCO)

The coconut oil was extracted using a modified wet extraction method described by Nevin and Rajamohan [5,9]. Fresh Coconut was procured; the kernel was separated from the coconut pot and diced into small sizes. The kernel was soaked in water for 48 h and later sieved from the water and blended using an electric blender.

The resultant coconut milk was left for 24 h to facilitate the gravitational separation of the emulsion as described by Onsaard et al. [10] and Nour et al. [11]. Demulsification produced layers of an aqueous phase (water) on the bottom, an emulsion phase (cream) in the middle layer and an oil phase on top as described by Nour et al. [11]. The oil on top was scooped and heated to remove moisture. The obtained oil was then filtered through a fine sieve, stored at room temperature and used for the experiment.

Chemicals

For comparative purposes, dosages of cadmium were equivalent to those used by Chiquoine [12] and Rose et al. [13] and were apportioned according to body weight. Cadmium Chloride (CdCl2. 2H20) was dissolved in distilled water; All other chemicals used were of analytical grade and were from Sigma (USA).

Experimental design

The rats were randomly selected into five groups A, B, C, D and E with group 'A' as the general control, Groups 'B, C, D and E' as the dosed groups. The control group received distilled water every day *via* oral route using oral cannula. Group B received Cadmium (Cd) at dose of 4.56 mg/kg in distilled water for 2 weeks (Cd-treated control) *via* oral route administration using oral cannula, Group C received Virgin Coconut Oil (VCO) at 6.7 ml/kg body weight per day following a modification of the protocols described by Dayrit [14], for 7 weeks (VCO-treated control) *via* oral route using oral cannula, Group D (Cd+VCO) received cadmium for 2 weeks and afterwards virgin coconut oil for 7 weeks, group E (VCO+Cd) received virgin coconut oil for 7 weeks and cadmium for 2 weeks.

Sample collection

The rats were sacrificed using cervical dislocation method, the epididymides were removed to collect semen for sperm analysis (sperm counts, motility and morphology) and testes were also removed, weighed and fixed in Bouin's fluid for routine histologic and histomorphometric studies.

Biochemical analysis

The tissues of testes of different groups were homogenized in ice-cold 100 mM phosphate buffer (pH 7.4), using a Potter-Elvehjem homogenizer fitted with a Teflon Plunger. Homogenates were centrifuged at 11,000 g for 20 min and the resulting supernatants were divided into aliquots and stored at –80°C. The Levels of Lipid Peroxides (LPO) were measured in tissue homogenates as Thiobarbituric Acid Reactivity (TBARS). The product of the reaction between malondialdehyde and thiobarbituric acid was measured as described by Thayer [15]. GSH levels in tissue homogenates were measured employing 0.04% to 5,5′-dithiobis-(2-nitrobenzoic acid) in 10% sodium citrate and recording at 410 nm as described by Dutta et al. [16].

Procedure of sperm analysis

The epididymis was removed from the testes of each of the rats, crushed in a container to get the sperm and poured in a container containing the mixture of sodium carbonate, formalin and water. These serve as diluting fluid and for preservation. The semen was put in the Neubauer Counting Chamber and applies coverslip. And put on the microscope to view. The sperm count was checked in millions per liter, motility and morphology in percentage.

Histological slides preparation

Specimens from testicular tissues were fixed in 10% neutral buffer formalin, dehydrated in ascending grades of ethanol alcohols, cleared in xylol, casted, blocked, cut at 2 µm to 5 µm thickness and stained with hematoxylin-eosin for microscopic examination [17].

Statistical analysis

The results are expressed as mean ± Standard Error (SE). Differences between groups were assessed by one-way analysis of variance (Bonferroni test) using the Prism version 5 software package for Windows. Values of P<0.05 were considered significant results.

Results

Effects on mean body weight

The Table 1 shows the mean weight parameters and the standard error of mean of the experimental rats. The weight analysis shows insignificant increase in the rats' body weights from the initial week to the final week of the experiment for all Groups. Hence, there is no significant effect of Cadmium (Cd) and Virgin Coconut Oil (VCO) on the weight of the experimental rats.

Effects on testicular weight

The rats of VCO group show insignificant increase of testicular weight, likewise, the rats of Cd+VCO and VCO+Cd groups when compared with group B that received Cd only. However, the testicles of Cd+VCO and VCO+Cd groups are lesser in weight when compared with the VCO group C (Table 2).

Effects on rats' testicular oxidative status

The activities of Superoxide Dismutase (SOD) were significantly (P<0.05) decreased in Cd group (1.99 ± 0.47 U/L). Meanwhile, highest SOD value was observed in VCO group (7.79 ± 3.37 U/L) followed by the control group (5.77 ± 1.66 U/L) and Cd+VCO) and VCO+Cd groups with values of 3.48 ± 0.79 U/L and 4.52 ± 2.53 U/L respectively. Likewise, Lipid peroxidation (MDA) and Glutathione (GSH) values were insignificantly decreased in Cd groups (1.88 ± 0.61 nmol/ml and 0.42 ± 0.19 µmol/ml) and increased values of both MDA and GSH were observed in control groups (3.33 ± 0.20 nmol/ml and 0.89 ± 0.12 µmol/ml) followed by VCO group (2.93 ± 1.04 nmol/ml and 0.93 ± 1.04 µmol/ml) and Cd+VCO (2.35 ± 1.01 nmol/ml and 0.64 ± 0.04 µmol/ml) and VCO+Cd (2.79 ± 0.46 nmol/ml and 0.71 ± 0.31 µmol/ml) (Table 3).

Analyses of sperm parameters

The sperm cells reduced significantly (P<0.05) in Cd (group B) treated rats (28.01 ± 6.76 × 10^6 Cells/L), whereas there was insignificant (P>0.05) increase number of sperm cells in VCO-treated rats (71.43 ± 13.26 × 10^6 Cells/L), Control rats (67.20 ± 7.09 × 10^6 Cells/L), Cd+VCO (44.53 ± 16.00× 10^6 Cells/L) and VCO+Cd, (60.93 ± 18.90 × 10^6 Cells/L) (Table 4).

The percentage of rapid progressive sperm cells in VCO treated rats was insignificantly (P>0.05) higher (50.00% ± 20.82%) while that of the Cd was lower (20.00% ± 10.00%) in the treated groups. Meanwhile, the rapid motility grading of Cd+VCO and VCO+Cd groups was lower (36.67% ± 13.33% and 45.00% ± 20.21%) compared with the control (53.33% ± 12.02%) and VCO-treated rats but higher than the Cd-treated rats. The percentage of non-progressive sperm cells was significantly (P<0.05) higher in Cd rats compared with the rest of the groups. Hence, percentage of dead sperm cells was also higher in Cd-treated rats (Table 5).

The percentage of normal morphology of sperm cells were found to be high in Control, VCO and VCO+Cd treated rats whereas the values were low in the Cd and Cd+VCO groups with the lowest value

Table 1: Comparison of weekly body weight (g).

GROUP	CONTROL A	CADMIUM B	VCO C	CADMIUM+VCO D	VCO+CADMIUM E
WEEK 1	166.7 ± 22.05	158.3 ± 8353	141.7 ± 16.68	150.0 ± 25.01	158.3 ± 22.05
WEEK 2	192.0 ± 8.51 *0.42	166.7 ± 22.05 *0.34	150.0 ± .5774 *0.06	158.3 ± 22.05 *0.28	183.3 ± 30.05 *0.83
WEEK 3	200.0 ± 28.87 *0.74	175.0 ± 28.87 *0.41	150.0±0.5774 *0.16	166.3 ±8.172 *0.33	183.3±30.05 *0.69
WEEK 4	200.0 ± 14.43 *0.64	183.3 ± 22.05 *0.64	158.7 ± 8.172 *0.19	166.3 ± 16.84 *0.21	191.7±22.05 *0.830
WEEK 5	200.0 ± 14.43 *0.80	183.3 ± 22.05 *0.67	175.0 ± 14.43 *0.29	183.0 ± 8.505 *0.49	200.0 ± 28.87 *1.00
WEEK 6	200.3 ± 24.84 *0.59	183.7 ± 16.84 *0.50	192.0 ± 8.505 *0.66	191.7 ± 22.05 *0.77	200.0 ± 28.87 *1.00
WEEK 7	208.0 ± 22.05 *0.75	200.0 ± 0.5774 *1.00	192.0 ± 8.505 *1.00	191.7 ± 22.05 *0.77	208.3 ± 28.87 *0.53

**$P \leq 0.05$ = significant; otherwise; *$P > 0.05$ = insignificant when compared with the control, values are expressed as mean ± SEM

Table 2: Shows Testicular Weight (g).

GROUP	CONTROL A	CADMIUM B	VCO C	CADMIUM+VCO D	VCO+CADMIUM E
Right Testes	1.24 ± 0.22	0.81 ± 0.31 *0.33	1.48 ± 0.04 *0.37	1.10 ± 0.18 *0.64	0.92 ± 0.19 *0.33
Left Testes	1.09 ± 0.08	0.78 ± 0.12 *0.08	1.23 ± 0.29 *0.69	1.04 ± 0.15 *0.78	0.82 ± 0.35 *0.46
Average Testicular Weight (g)	1.17 ± 0.15	0.79 ± 0.22 *0.21	1.36 ± 0.17 *0.53	1.03 ± 0.17 *0.71	0.87± 0.27 *0.39

**$P \leq 0.05$ = significant; otherwise, *$P > 0.05$ = insignificant when compared with the control, values are expressed as mean ± SEM

Table 3: Showing oxidative stress analysis of rats' testes.

GROUP	CONTROL A	CADMIUM B	VCO C	CADMIUM+VCO D	VCO+CADMIUM E
SOD (U/L)	5.77 ± 1.66	1.99 ± 0.47 **0.04	7.79 ± 3.37 *0.39	3.48 ± 0.79 *0.37	4.52 ± 2.53 *0.60
MDA (nmol/ml)	3.33 ± 0.20	1.88 ± 0.61 *0.19	2.93 ± 1.04 *0.07	2.35 ± 1.01 *0.08	2.79 ± 0.46 *0.32
GSH (µmol/ml)	0.89 ± 0.12	0.42 ± 0.19 *0.33	0.93 ± 1.04 *0.97	0.64 ± 0.04 **0.03	0.71 ± 0.31 *0.16

**$P \leq 0.05$ = significant; otherwise, *$P > 0.05$ = insignificant, values are expressed as Mean ± SEM

Table 4: Microscopic sperm count ($X10^6$ Cells/L).

GROUP	CONTROL A	CADMIUM B	VCO C	CADMIUM+VCO D	VCO+CADMIUM E
Microscopic Sperm Count (X)	67.20 ± 7.09	28.01 ± 6.76 **0.04	71.43 ± 13.26 *0.92	44.53 ± 16.00 *0.61	60.93 ± 18.90 *0.89

**$P \leq 0.05$ = significant; otherwise, *$P > 0.05$ = insignificant, values are expressed as Mean ± SEM, ($x10^6$ Cells/L)

Table 5: Motility grading (%).

GROUP	CONTROL A	CADMIUM B	VCO C	CADMIUM+VCO D	VCO+CADMIUM E
Rapid progressive	53.33 ± 12.02	20.00 ± 10.00 *0.90	50.00 ±20.82 *0.89	36.67 ± 13.33 *0.41	45.00 ± 20.21 *0.74
Slow progressive	20.00 ± 5.77	17.33 ± 3.53 *0.30	13.34 ± 3.38 *0.38	16.33 ± 2.85 *0.63	10.00 ± 0.5774 *0.16
Non progressive	13.35 ± 3.18	29.07 ± 6.84 *0.05	18.33 ± 10.93 *0.70	26.67 ± 6.667 *0.15	22.50 ± 10.10 *0.45
Dead Sperm Cells	13.32 ± 3.18	33.60 ± 6.84 *0.06	18.33 ± 10.93 *0.70	20.33 ± 9.83 *0.55	22.50 ± 10.10 *0.45

**$P \leq 0.05$ = significant; otherwise, *$P > 0.05$ = insignificant, values are expressed as Mean ± SEM

Table 6: Morphology grading (%).

GROUP	CONTROL A	CADMIUM B	VCO C	CADMIUM+VCO D	VCO+CADMIUM E
Normal spermatozoa	53.00 ± 3.51	30.00 ± 0.57 **0.03	50.00 ± 0.58 *0.45	36.33 ± 3.18 **0.03	50.50 ± 0.29 *0.52
Head defect	37.00 ± 3.51	57.33 ± 3.18 **0.01	40.00 ± 0.57 *0.45	53.67 ± 3.18 **0.03	40.17 ± 0.44 *0.42
Mid piece defect	5.00 ± 0.58	7.00 ± 17.24 *0.12	5.00 ± 0.57 *1.00	5.00 ± 57 *1.00	5.50 ± 0.65 *0.48
Tail defect	5.00 ± 0.57	5.67 ± 0.57 *1.00	5.00 ± 0.57 *1.00	5.00 ± 0.57 *1.00	5.50 ± 0.69 *0.48

**$P \leq 0.05$ = significant; otherwise, *$P > 0.05$ = insignificant, values are expressed as Mean ± SEM

Table 7: Histomorphometric Parameters in Experimental Rats' Testes.

GROUP	CONTROL A	CADMIUM B	VCO C	CADMIUM+VCO D	VCO+CADMIUM E
CSA (10^7 µm)	8.39 ± 1.57	4.30 ± 4.21 **0.01	8.62 ± 2.40 *0.21	5.80 ± 1.65 *0.08	5.96 ± 5.4 *0.10
LD (10^2 µm)	1.26 ± 0.05	1.64 ± 0.08 **0.02	1.18 ± 0.11 *0.51	1.44 ± 0.11 *0.22	1.37 ± 0.23 *0.67
GED (10^3 µm)	0.55 ± 0.05	0.42 ± 0.12 *0.09	0.60 ± 0.05 *0.67	0.47 ± 0.03 *0.24	0.54 ± 0.00 *0.13

CSA: Cross Sectional Area; LD: Lumen Diameter; GED: Germinal Epithelium Diameter

**$P \leq 0.05$ = significant; otherwise, *$P > 0.05$ = insignificant, values are expressed as Mean ± SEM

recorded in Cd-treated group (Table 6).

Structural deformities were also observed in the sperm cells where Cd group has the highest percentage values of deformities of the head (57.33% ± 3.18%), mid-piece (7.00% ± 17.24%) and tail (5.67% ± 0.57%). Hence, the lowest deformities values were recorded in Control group and VCO group (Table 6).

Histomorphometric analysis

An insignificantly increase (P>0.05) in the Cross-Sectional Area (CSA) of seminiferous tubule was observed in VCO group, while CSA values were insignificantly (P>0.05) decreased in Cd+VCO group, VCO+Cd group and significantly (P<0.05) decrease in Cadmium group. Similarly, the Luminal Diameter (LD) and Germinal Epithelium Diameter (GED) of the seminiferous tubules show insignificant increase value in VCO group and decrease values in Cd+VCO group and VCO+Cd group with more decrease value in Cd group (Table 7). These results showed a poor morphometric grading in cadmium treated groups and a good morphometric result in VCO treated groups.

Histological analysis of the testes

Histological findings in the control group depicted no alteration in the histomorphological presentation as seen across the testicular

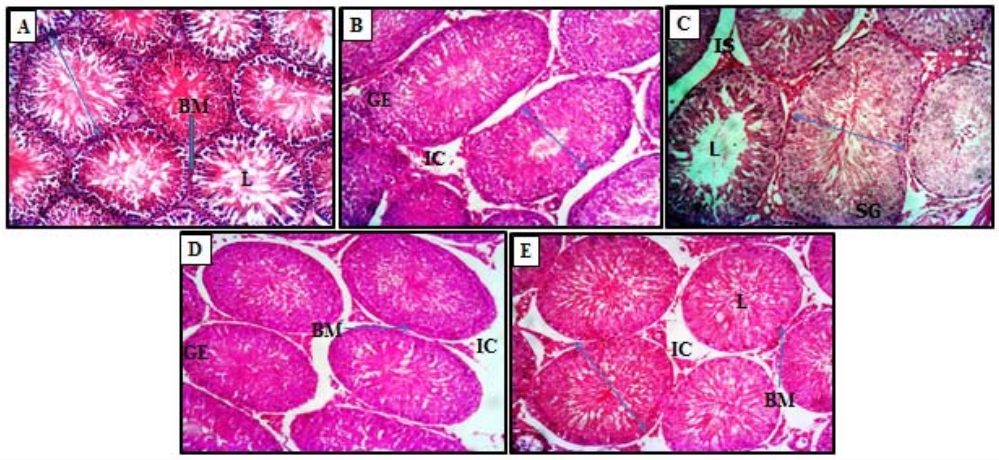

Figure 1: Photomicrographs showing rat testicular cyto-architecture of the seminiferous epithelium with Basement Membrane (BM), the Lumen (L) and Interstitial Space (IS) containing Interstitial Cells (IC), Germinal Epithelium (GE) are well demonstrated across the testicular sections. H&E stain, X100.

profiles. A normal cytoarchitecture of the testes in rats showing a normal shape and arrangement of seminiferous tubule with intact basement membrane and Leydig cells with progressive proliferation of spermatogenic cells to produce matured spermatozoa (Figure 1A).

Cd-treated rats showed irregular shape of seminiferous tubules and wide interstitial spaces with detached basal membrane (Figure 1B). VCO-treated rats showed no visible lesions or the lesion was very mild (Figure 1C). Cd+VCO group and VCO+Cd group showed seminiferous tubules with detached basal membrane and wide interstitial spaces (Figure 1D, 1E).

Discussion

Many herbal extracts are known to exert protective action against noxious effects of toxicants/drugs on organs by their ability to decrease oxidative damage-mediated pathologies [18]. Coconut oil is known as a natural health product [19,20]. Histopathology is considered the most reliable parameter for the detection of toxic effects on male reproduction [21,22]. Therefore, macroscopic, histological, morphometric and sperm examination was carried out to determine the degree of testicular tissue damage produced by cadmium and degree of amelioration by virgin coconut oil. However, in the previous study, it was found that the ingestion of different concentrations of virgin coconut oil did not affect the body weight, body measurements and total number and area of adipocytes of the animals. In addition, the consumption of virgin coconut oil did not affect food intake [23]. In the present study, there was no significant difference in the percentage weight difference of the rats in the control and treatment groups. Meanwhile, findings concerning the effect of coconut oil on weight loss and food consumption have been contradictory [24,25].

In this present study, there was insignificant increase in the testicular weight of rats treated with Virgin Coconut Oil (VCO) compared to the control rats and other treated groups with Cadmium (Cd). Research has shown that blockage of the efferent ducts by cells sloughed from the germinal epithelium or the efferent ducts themselves can lead to an increase in testicular weight due to fluid accumulation [26], an effect that could offset the effect of depletion of the germinal epithelium on testicular weight. Although, the group of rats with Cd+VCO showed slight increase in testicular weight compared to the rats fed with Cd-only which probably due to the ameliorative effect of VCO. This result is in agreement with the previous study of Maina et al. [27] that revealed no significant difference in mean testicular weight between the control and coconut oil-treated rats. Meanwhile, a decrease in the mean testicular weight observed in the rats treated with Cd-only however, showed that an increase or decrease in relative or absolute weight of an organ after administering a chemical or drug is an indication of the toxic effect of that chemical. Also, the weight of male reproductive organs usually provides a useful reproductive risk assessment in experimental studies [28] and testicular size is the best primary assessment for spermatogenesis, since the tubules and germinal elements account for approximately 98% of the testicular mass.

Cadmium (Cd) accumulates in male reproductive organs, in both humans and animals [29]. Numerous studies have confirmed that the testis is more sensitive to Cd than other important organs [30,31]. Cd-induced testicular toxicity is caused by the interactions between complex networks [32], involving the inhibition of oxidative stress [33] which leads to an increase in germ cell apoptosis [34] and/or distortion of the blood-testis barrier with subsequent germ cell loss, testicular edema, and hemorrhage [30,35].

VCO has been reported to have active and large amounts of polyphenols and tocopherols which are powerful counter measures against lipid peroxidation [36] in tissues. Therefore, as compelling evidence suggests that Cd amplifies oxidative stress status in rat testes, the current study therefore demonstrates the possible protective role of VCO in the mitigation of cadmium ravages on testicular parameters.

Brucefife [37] reported that coconut oil has an antioxidant effect. Antioxidants stabilize testicular membranes by decreasing lipid peroxidation and presumably abnormal sperm which will enhance gonadal function [38]. Eskenaziet al. [39] also reported higher antioxidant intake is associated with higher sperm numbers and motility which corroborates the results of this research.

It has also been reported that oxidative stress by free radical toxicity caused by Cd affected infertility. Moreover, Gupta and Kara found a significant elevation of LPO in testis tissue of rat treated with Cd. Stajn et al. [40] and Patra et al. [41] reported that different doses of Cd increase organ Lipid Peroxidation (LPO) in many organs including male sex organs and brought about changes

in the antioxidant defense system. Disruption of cell junctions and the blood-epididymis barrier has been determined as the main target of cadmium toxicity in epididymis, leading to deficient sperm maturation and motility [42,43]. In a study there was dose dependent increase in percentage of motile sperm, increased sperm count, viability and percentage of normal morphology which is an indication that coconut oil extract could enhance spermatogenesis [44]. Increase in morphologically normal sperms results and increased motility as normal intact sperm are prerequisite for high speed with straight forward motility in rats treated with VCO as observed in the present study is in agreement with Gandini et al. [45] who postulated that sperm function is strictly correlated with sperm morphology which is a predictor of fertility potential in man. In addition, the present study showed that the number of sperms with normal morphology was increased in the treated group with the highest number being in the VCO group followed by the groups treated with VCO+Cd and the number was significantly less in Cd-treated group when compared to the control.

Reproductive system was mainly controlled by Hypothalamic-Pituitary-Gonad (HPG) axis and Cadmium was endocrine-disrupting metal able to affect homeostasis, reproduction, and also impair the function of HPG axis. Moreover, it was also able to disrupt hormone synthesis and damage plasma protein binding [46]. Cadmium was found to compete with acetylcholine to bind acetylcholinesterase enzyme. Cadmium was also found to inhibit acetylcholine and cause vasoconstriction. Long term vasoconstriction would give rise to poor blood circulation and keep oxygen and nutrition from supplying to cells, thus it would impede metabolism. Less supply of oxygen and nutrition in reproductive organs would eventually hamper spermatogenesis process [47]. Thus, the results from this study showed significant reduction of normal sperm morphology and significant increase in sperm defects and consequently loss of progressivity of spermatozoa in Cd-treated rats except where VCO was used to mitigate the action(s) of Cd.

Histopathologically, the testicular tissue showed varying degrees of distortion in Cd-treated rats and also Cd+VCO treated rats. Many tubules did not corroborate a significant change of germinal epithelium and interstitial tissue. The others appeared markedly affected with degeneration of interstitial cells and basement membrane when compared with the control. Meanwhile, these findings are consistent with earlier reports of Cd mediated histological changes in the testes, epididymis, and accessory sex organs [48,49]. On the other hand, several experimental studies have presented the significant atrophy of the epididymis, decrease in the diameter of the lumen, and alkalization of the epididymis and vas deferens after Cd administration [43,50].

Reversal of the changes by VCO in VCO+Cd and Cd+VCO - treated rats is consistent with the results of some investigators [51] who recorded that the adjuvant treatment of VCO had a protective effect on the seminiferous epithelium as demonstrated by the lowering of degeneration and restitution of normal epithelial lining. The histological analysis revealed improved spermatogenesis in treated group with VCO compared to the control. There was no adverse histological change; rather there was marked proliferation of spermatogenic cell lines. The seminiferous tubules were moderately enlarged as well as the central lumen. This result is in agreement with the report of Victor et al. [44], who added that no changes histologically with VCO-treated Wistar rats, transformation from primordial cells to spermatids and further to spermatozoa.

Any structural alteration may adversely affect function of the epididymal epithelium, which could impact sperm maturation [42]. Heavy metal and pesticide exposure are potential risk factors for adverse reproductive health outcomes, including poor semen quality [52-54].

Statistical differences were found in the seminiferous ductal diameters among the experimental animals in all the groups. It shows that rats given VCO had the highest value of CSA and GED followed by the rats given VCO+Cd and Cd+VCO, while the LD of the Cd-treated rats had the highest values. Statistical differences were markedly significant in seminiferous diameters of the Cd experimental rat testes. Russell et al. [55] reported that increase in the height of the seminiferous tubule epithelium could increase the sperm production process. Decreased ET and ST diameter are a result of reduced metabolic activity of the germinal cells as well as cell numbers with the consequence that interstitial spaces widen with edematous appearance [56]. Several studies that have examined Cadmium effects on semen parameters and other morphometric features of the testis [57,58], have supported our overall result indicating cadmium-induced distortions do occur by changes in morphology and morphometric indices of the testis. These ravages were ameliorated by VCO, possibly due to its rich antioxidative properties as previously stated by Arunima [59].

Conclusion

The exposure to Cd induces toxicity, morphometric changes and negative effects on sperm parameters in rat testes. The increased oxidative stress resulted from Cd intoxication in testicular tissue might be responsible for these adverse changes. VCO had protective and ameliorative effects against Cd toxicity, evidenced by increase in the SOD, MDA and GSH level as oxidative makers, and minimal histological, morphometric and sperm parameters changes in the testes of rats treated with VCO+Cd and Cd+VCO respectively.

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