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Cadmium-induced Reproductive Toxicity in Male Wistar Rats: Ameliorative effects of STC30

Justina Nwangwa[1], Ekpe Aribo[1*], Augustine, Udefa[1], Atim, Antai[1]

[1] *Department of Physiology, University of Calabar, Calabar, Nigeria.*

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*** Corresponding author:**
Ekpe Aribo; Department of Physiology, University of Calabar, Calabar, Nigeria.
E- mail: ekpearibo@gmail.com

ABSTRACT

Background: Cadmium-induced male infertility is associated with oxidative stress. However, there is a lack of literature regarding its effects on Cadmium-induced male reproductive toxicity, which is the motivation for conducting this study.
Methods: Twenty male Wistar rats were divided into four groups of five rats each as follows:1) Control; 2) Cadmium-only; 3) STC30-only; and 4) Cadmium+STC30. Daily administration was for 28 days, after which blood and testicular samples were collected for the determination of relevant parameters.
Results: The results demonstrated a significant loss of body weight in the Cadmium-only group compared to the control, Cadmium+STC30, and STC30-only groups. Testicular and epididymal weights were also significantly lower in the Cadmium-only group compared to the control, STC30-only, and Cadmium+STC30 groups. Furthermore, sperm count, viability, and motility were significantly diminished in the Cadmium-only group compared to the control, STC30-only, and Cadmium+STC30 groups. Teratozoospermia was significantly higher in the Cadmium-only group than in the control, Cadmium+STC30, and STC30-only groups. Levels of gonadotropin-releasing hormone, follicle-stimulating hormone, luteinizing hormone, and testosterone were significantly reduced in the Cadmium-only group compared to the control group.
Additionally, testicular malondialdehyde and TBARS levels were significantly elevated in the Cadmium-only group compared to the control, STC30-only, and Cadmium+STC30 groups. Activities of superoxide dismutase, glutathione peroxidase, catalase, and total antioxidant capacity in the testes were significantly lower in the Cadmium-only group compared to the control, STC30-only, and Cadmium+STC30 groups. Histological analysis revealed a significantly reduced Johnsen score in the Cadmium-only and Cadmium+STC30 groups compared to the control, with higher scores in the STC30-only and Cadmium+STC30 groups compared to the Cadmium-only group.
Conclusion: The STC30 compound ameliorated the Cadmium-induced male reproductive toxicity and oxidative stress in Wistar rats.

Keywords: Ameliorative effect, Cadmium; Male rats, Reproductive toxicity, STC30

Introduction

Infertility is the inability of a couple to achieve a pregnancy after 12 months of regular unprotected intercourse [1]. It is believed to affect over 180 million people worldwide. Male factor infertility is responsible for about 20% and is a contributing factor in another 30%-40% of all infertility cases [2].

Infertility is one of the major reproductive issues globally, affecting 15% of couples in reproductive age [3]. Infertility is associated with emotional, economic, and social issues, especially in societies like ours with a strong emphasis on childbearing [4,5].

Over the past decades, there has been a rapid decline in male reproductive health [6,7]. Though the exact cause is not clear, some factors have been implicated, including stress, lifestyle changes, and exposure to environmental and occupational pollutants [8]. Cadmium is one such environmental pollutant and an endocrine disruptor [9]. It is believed to be one of the most toxic heavy metals [10]. It has a range of applications, such as in the coating of polyvinyl chloride pipes, plastics, glass, ceramics, rubber, paint, and fireworks [10]. Environmental exposure to Cadmium is often through drinking water, inhalation in industries, vehicle exhaust fumes, cigarette smoking, and consumption of agricultural products from contaminated soils [10,11]. Chronic exposure to Cadmium has occurred through respiratory, renal, hepatic, and reproductive systems [12,13,14]. The mechanism of tissue damage by

Cadmium is in part attributed to the oxidative stress it causes [14,15].

In recent years, there has been increasing patronage of natural or plant-based remedies with many claims due to their affordability, assumed efficacy, and advertisement [16]. Some have been explored and reported to have therapeutic effects against cytotoxicities either due to their antioxidant contents or some other factors [17,18]. The compound STC30 is one of those plant-based remedies claimed to have positive health benefits. It is a proprietary polyherbal supplement created by Superlife World in Kuala Lumpur, Malaysia. The results of a few studies conducted on STC30 show that it ameliorates carbon tetrachloride (CCl_4)-induced nephropathy and glomerular functions [19] and reduces the serum concentration of c-reactive protein in CCl_4-induced hepatocellular carcinoma. It is believed to boost immunity, rejuvenate and replace damaged cells, and improve the redox state of tissues [20]. The compound STC30 contains Swiss apple, grapes, glisodin, bilberry, blackcurrant juice powder, and blueberry extracts [21].

Blackcurrant juice is rich in anthocyanins, polyphenolic compounds, antioxidants, vitamin C, and gamma-linolenic acid (GLA) and is thought to regulate blood flow and improve immunity with antioxidant, antimicrobial, and antitumor activities [22,23]. Bilberry has hepatoprotective, antioxidant, and anti-inflammatory effects [24]. Glisodin is a known antioxidant that reduces the synthesis of lactic acid during exercise [25]. While few studies have been performed on STC30, there is a lack of information in the literature regarding its effects on Cadmium-induced male reproductive impairment. This is the main research focus of this study and the reason behind conducting this project.

Materials and Methods

Animals: Twenty male Wistar rats were purchased and kept in the Animal House of the Department of Physiology, University of Calabar, in good hygienic condition under a 12-hour day/night cycle. The duration of acclimatization before experimentation was one week. They were allowed free access to animal feed and water.

Preparation of Stock Solution of PurXcel: The content of one capsule (435 mg) of PurXcel (Live Pure, Frisco, Texas, USA) purchased from Puregen African Nigeria Limited, Lagos, Nigeria, was dissolved in 200 ml of distilled water.

Preparation of Stock Solution of Cadmium: This was made by dissolving 50 mg of Cadmium Chloride, $CdCl_2$ (Sigma-Aldrich, Chemical Company, St Louis, MO, USA) in 50 ml of distilled water.

Experimental Design: Twenty male Wistar rats were randomly divided into 4 groups of 5 rats each and raised in metallic cages that were cleaned regularly. Group 1 served as the control (given portable water), group 2 was the Cadmium-only group, group 3 was the STC30-only group, and group 4 was the Cadmium + STC30 group. Cadmium chloride was administered at a dose of 5 mg/kg [26,27], while STC30 was given at a dose of 132.7 mg/kg, derived from its therapeutic effective dose [28]. Cadmium and STC30 were administered daily by gavage for 28 days. Animals were weighed regularly, and the amounts of drugs administered were adjusted accordingly.

Collection of Samples: At the end of the treatment period, the animals were anesthetized with pentobarbital (60 mg/kg), the blood samples were collected from the rats via cardiac puncture, after which animals were sacrificed, and their testes were harvested for the determination of relevant parameters.

Acute Toxicity Study: The LD50 of STC30 was determined using Lorke's method [29], with the up-and-down method employed for follow-up as described in a previous study [30].

Determination of Body, Testicular, and Epididymal Weights: This was done weekly with an electronic weighing balance (Scout Pro, Ohaus Corporation, USA) based on the following formula:

Relative organ weight=Absolute weight of organ×100g

Final body weight 1

Sperm Function Analyses

Sperm Count: Sperm count was measured as described in a previous study [31]. Briefly, the cauda epididymis was immersed in 2 ml of normal saline and pre-warmed to 37°C, after which small incisions were made to enable sperm discharge from it. The collected sperms were suspended in the normal saline, and 200 µl of the sperm suspension was transferred to both chambers of the improved Neubauer hemocytometer. This was achieved by touching the edge of the coverslip, allowing each chamber to fill up by capillary action. The sperms were then counted in five large Thorma squares under a light microscope (Leica DM 750, Switzerland).

Sperm Motility: A Makler's chamber was used to examine sperm motility, as demonstrated by an earlier study [32]. Mild pressure was exerted on the vas deferens to obtain sperm suspension, which was introduced into 1 ml of normal saline, and the mixture was stirred gently. A drop of the suspension was then placed on the Makler's chamber (Self-Medical Instruments, Israel) and examined microscopically (Olympus BX41, Olympus Corporation Tokyo, Japan). The sperm motility was then expressed as a percentage of the total number of spermatozoa.

Sperm Viability: The method of Wyrobek, *et al.* [33] was used to assess sperm viability. Twenty µl of sperm suspension was stained with 20 µl of 0.05% eosin-nigrosin, and the mixture was incubated for 120

seconds at room temperature. The slides were then viewed microscopically using x400 magnification. Viable sperm cells were unstained, while non-viable ones stained pink. The number counted as viable was expressed as a percentage of the total sperms counted.

Sperm Morphology: Sperm morphology was evaluated as described in a study [34]. In brief, a drop of sperm suspension previously prepared for epididymis sperm count was smeared on a glass slide and stained with 1% eosin Y. The slide was air-dried and examined microscopically with x400 magnification. Two hundred sperms were screened for each rat, and the percentage of total, head, middle piece, and tail abnormalities were calculated.

Preparation of Testicular Homogenate: The left testis of each rat was homogenized separately in 50 µl Tris-HCl buffer (pH 7.4) containing 1.15% KCl to prepare a 20% (1/5w/v) tissue homogenate using Potter Elvehjem homogenizer (BEE International, Apion Company, USA). It was then centrifuged at 10000 g for 10 minutes in a cold centrifuge. The supernatants were collected and used to determine the testicular parameters.

Determination of Serum FSH Concentration: Serum follicle-stimulating hormone (FSH) concentration was evacuated in triplicate using rat FSH ELISA Kit catalog#: El-R0391 (Elabscience Biotechnology, Wuhan, China) and following the manufacture's protocol.

Determination of Serum Luteinizing Hormone: Serum luteinizing hormone concentration was determined with Rat LH ELISA kit, Cat No. ABIN6574078 (Elabscience Biotechnology, China) and following the manufacturer's protocol.

Determination of Testosterone: ELISA Kit (Elabscience Biotechnology, China) was used for this assay, and the manufacturer's protocol was followed.

Determination of serum concentration GnRH: This was done with a Rat GnRH Kit (Elabscience Biotechnology, China) following the manufacturer's protocol.

Evaluation of Testicular Lipid Peroxidation

Malondialdehyde: The concentration of malondialdehyde (MDA) in the testicular homogenate was evaluated using Ohkawa, *et al.* method [35] as described in a previous study [36] using commercially available reagents. In summary, a 100 ml aliquot of testicular homogenate was added to a reaction mixture consisting of 200 ml of 8.1% (wt/v) Lauryl sulfate, 1.5 ml of 20% (wt/v) acetic acid, 1.5 ml of 0.8% (wt/v) thiobarbituric acid (TBA), and 100 ml of distilled water. The mixture was then boiled and centrifuged, and the absorbance of the supernatant was measured spectrophotometrically.

Thiobarbituric Acid Reactive Substance: The level of thiobarbituric acid reactive substance (TBARS) in the testicular homogenate was determined by the method described in a study [37] using commercial reagents. Malondialdehyde, as one of the end products of lipid peroxidation, reacts with thiobarbituric acid to form a colored substance whose absorbance is measured spectrophotometrically at 532nm.

Determination of Testicular Antioxidant Enzymes

Superoxide Dismutase: The superoxide dismutase (SOD) activity in the testicular homogenate was determined according to the method described in a study [38] which is based on the ability to inhibit the reduction of nitro tetrazolium-blue and using commercially available reagents. Briefly, the homogenate supernatant was recentrifuged at 12000 rpm, and the SOD was evaluated on the resultant supernatant. A 1 ml aliquot of the reactant (containing 13 nM L-methionine, 100 nM EDTA, 300 µL of 2 µM riboflavin, and 50 mM phosphate buffer at pH 7.8) was prepared, and the activity was measured spectrophotometrically at 560 nm.

Catalase: This enzyme was evaluated as described earlier [39] and is based on an enzyme-catalyzed decomposition of H_2O_2, which forms a yellowish complex with molybdate whose absorbance is read at 405 nm.

Glutathione Peroxidase: The activity of peroxidase was determined in testicular homogenate using a method described earlier [40], using hydrogen peroxide as a substrate.

Total Antioxidant Capacity: The total antioxidant capacity (TAC) was assayed using a previously described method [41]. The TAC assay employs a thermal radical generator that produces a steady flux of radicals in the solution. The addition of antioxidants results in the competitive inhibition of the substrates.

Histological Studies: The harvested right testes were cleaned of connective tissues and fixed in Bouin's fluid, then dehydrated with ethanol before being embedded in paraffin blocks. The blocks were then sectioned and stained with hematoxylin and eosin and viewed using a light microscope (Leica, DM, 750 Switzerland) at a magnification of x400. The number of Leydig cells per intratubular region and, thereafter, the average Leydig cell count was computed. The average Sertoli cell count was also computed after counting Sertoli cells in 20 seminiferous tubules. Johnsen's score was assessed in 10 seminiferous tubules [42], as described earlier [43]. We use the Image Analyser software (Soft Imaging System, VGA, Utilities Version 3.67c) to measure the seminiferous tubular diameter and germinal epithelial height in 20 seminiferous tubule samples chosen from serial sections, subsequently calculating the averages.

Statistical Analyses: The study data were presented as means ± SEMs, which were normally distributed. The SPSS software (version 20) was used to analyze

the study data. One-way analysis of variance (ANOVA) was also utilized to analyze the data, while Tukey's post hoc test was performed to compare the mean values. The statistical significance among the pair of datasets was set at $P \leq 0.05$.

Results

Acute Toxicity Study: The administration of STC30 produced no mortality or significant behavioral changes up to 5000 mg/kg dose in male Wistar rats, implying that the LD50 of STC30 is above 5000 mg/kg.

Changes in Body Weight: The body weight changes for control, Cd-only, STC30-only, and Cd+STC were 28±9.92, -15.2±5.45, 12.6±2.30, and 7.8±2.17, respectively. There were significant reductions in the animal weights in Cd-only, STC30-only, and Cd+STC30 groups (P<0.05) compared with the control, though significantly higher (P<0.05) in the STC30 and Cd+STC30 than in the Cd-only groups as shown in Table 1.

Testes and Epididymal Weights: The absolute testicular weight in the Cd-only (1.89±0.19) group was significantly lower (P<0.05) than that of the control (3.56±0.21). However, it was significantly higher (P<0.05) in the STC30-only (3.16±0.23) and Cd+STC30 (2.48±0.13) than in the Cd-only group, as shown in Table 1. The absolute epididymal weights (right and left testes) were significantly lower in Cd-only (1.14±0.15) compared to those of the control (1.5±0.16) but significantly higher in the STC30-only (1.56±0.18) than in the Cd-only group, as shown in Table 1.

Table 1. Body weight and absolute and relative weights of testes and epididymis

Group	Initial body weight (g)	Final body weight (g)	body weight changes (g)	Absolute testis weight (g)	Relative testis weight (g)	Absolute epididymis weight (g)	Absolute epididymis weight (g)
Control	231.00 ±8.46	259.00±5.34	28.00±9.92	3.56±0.21	1.37±0.06	1.50±0.16	0.58±0.05
Cadmium	230.00±1.58	214.80±5.45	-15.20±5.45	1.98±0.19	0.92±0.10	1.14±0.15	0.53±0.06
STC30	234.80±5.54	247.40±5.55	12.60±2.30	3.16[a]±0.23	1.28±0.07	1.56[a]±0.18	0.63±0.06
Cadmium +STC30	234.40±3.29	242.20±4.55	7.80±2.17	2.48±0.13	1.02±0.07	1.40±0.12	0.58±0.04

Values are presented as mean ±SEM, n = 5. *= p<0.05 vs control; a = p<0.05 vs Cadmium; b = p<0.05 vs STC30.

Table 2. Sperm count, motility, viability, and morphology

Group	Sperm count	Motility	RPFM	SPFM	RM	Sperm viability	Abnormal Morphology
Control	55.60±4.57	76.20±3.11	32.40±1.67	21.20±3.77	22.60±2.30	70.20±4.49	9.60±2.41
Cadmium	29.92±3.47	52.20±3.90*	22.40±2.88*	17.00±2.12	12.80±1.92*	50.80±6.38*	28.20±3.27*
STC30	60.94±4.07	84.40±2.88*[,a]	42.40±3.51*[,a]	23.20±2.77[a]	18.80±1.79[a]	79.60±4.16[a]	8.40±2.30[a]
Cd+STC30	47.34±3.88	71.20±3.11[a,b]	39.60±4.39[a,b]	18.40±3.91	13.20±2.77*[,b]	70.80±4.27[a,b]	13.60±2.70[a,b]

Values are presented as mean ± SEM, n = 5. * = p<0.05 vs control; a = p<0.05 vs Cadmium; b = p<0.05 vs STC30

Comparison of Sperm Parameters

Sperm Count: The total sperm count (x10^6/L) was significantly (P<0.05) decreased in the Cd-only (29.92±3.47) and Cd+STC30 (47.34±3.88) groups compared with the control (55.60±4.57); however, it was significantly higher in the STC30-only (62.60±3.73) and Cd+STC30 groups than in the Cd-only group (P<0.05), as shown in Table 2.

Sperm Motility: Sperm motility (%) was significantly reduced in the Cd-only (52.20±3.90) and Cd+STC30 (71.20±3.11) groups compared with the control (72.20±3.11); nevertheless, it was significantly higher (P<0.05) in the STC30-only (84.40±2.88) and Cd+STC30 groups than in the Cd-only group. It was significantly higher in the STC30-only than the control (P<0.05), as shown in Table 2.

Percent Sperm Viability: The sperm viability percentage was significantly lower in the Cd-only group (50.80±6.38) compared with the control (70.20±4.49); however, it was significantly higher (P<0.05) in the STC 30-only (79.60±4.16) and Cd+STC30 groups than in the Cd-only group, as shown in Table 2.

Sperm Morphology Examinations: The percentage of morphologically abnormal sperms was significantly increased (P<0.05) in the Cd-only group (28.20±3.27) compared with the control (9.60±2.41); nonetheless, it was significantly lower (P<0.05) in the Cd+STC30 group (13.60±2.70) than in the Cd-only group, as shown in Table 2.

Comparison of Male Reproductive Hormones

Sperm GnRH: The serum level of GnRH (ng/ML) was significantly reduced (P<0.05) in the Cd-only group (1.14±0.15) compared with the control (2.32±0.32); however, it was significantly higher (P<0.05) in the Cd+STC30 group (2.68±0.41) than in the Cd-only group. It was significantly increased (P<0.05) in the STC30-only group (3.60±0.44) compared with control and Cd-only groups, as shown in Table 3.

Serum Testosterone: The concentration of serum testosterone (ng/ml) was significantly reduced in the Cd-only group (1.98±0.28) compared with control (3.88±0.40); nonetheless, it was significantly higher (P<0.05) in the Cd+STC30 group (4.3±0.50) than in the Cd-only group. It was significantly elevated in the STC30-only group (6.14±0.47) compared with the control and Cd-only groups, as seen in Table 3.

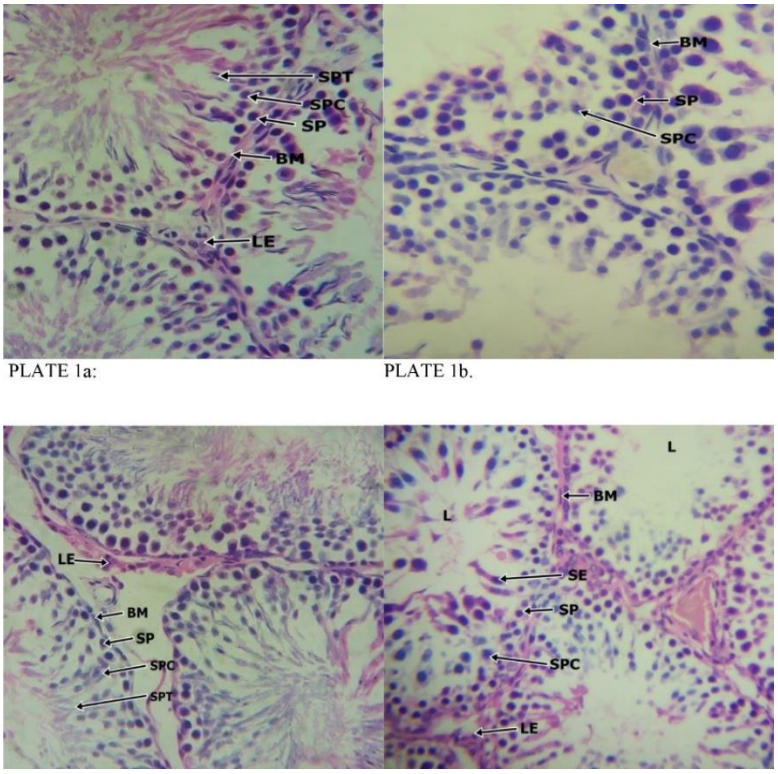

PLATE 1a: PLATE 1b.

Figure 1. Sections of testis in a) control group, b) cadmium group, c) STC30 group, and d) Cd + STC30 group, x400 magnification.
BM-basement membrane, SP-spermatogonia,
SPC-spermatocytes, SPT-spermatid,
SZ-spermatozoa.
L-Lumen
LE-Luminal epithelium

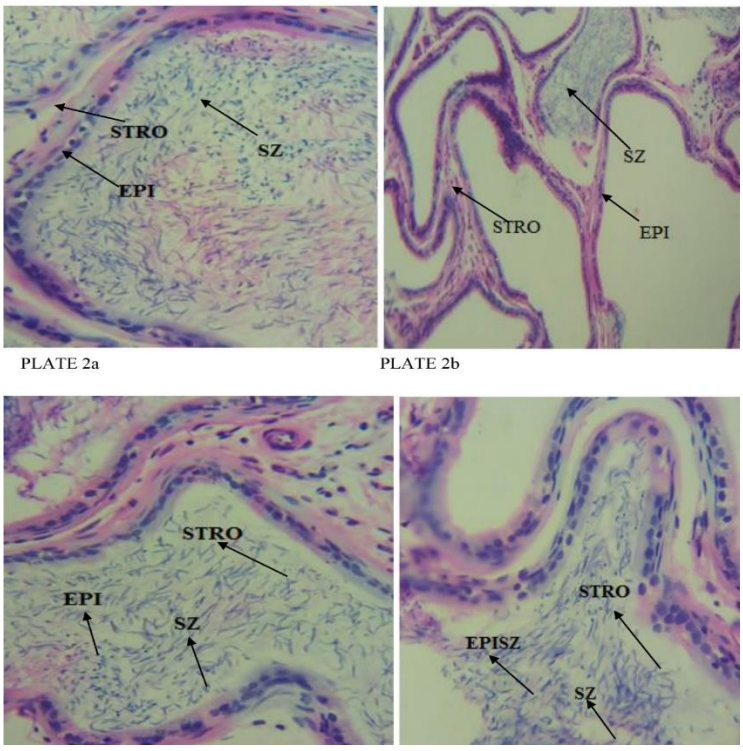

PLATE 2a PLATE 2b

Figure 2. Sections of the epididymis in a) control group, b) cadmium group, c) STC30 group, and d) Cd + STC30 group, x400 magnification. STRO = loose stroma, EPI = lining epithelium, SZ = spermatozoa

Serum LH: Serum concentration of LH (µ/ml) was significantly reduced ($p<0.05$) in the Cd-only (2.76±0.11) and Cd+STC30 groups (4.32±0.38) compared with control (5.20±0.45); however, it increased ($P<0.05$) in

the Cd+STC30 group (4.37±0.38) compared with Cd-only group (2.76±0.11). It was significantly higher in the STC30-only group (P<0.05) than in the control and the Cd-only groups, as shown in Table 3.

Serum FSH: The serum concentration of FSH was significantly reduced (P<0.05) in the Cd-only (2.68±0.30) and Cd+STC30 groups (4.96±0.59) compared with the control (6.20±0.53); nevertheless, it was significantly higher (P<0.05) in the STC30-only (7.40±0.62) and Cd+STC30 groups (4.96± 0.53) than in the STC30-only group (7.40±0.62). It was also significantly higher in the Cd+STC30 group than in the Cd-only group. It was significantly increased (P<0.05) in the STC30-only group compared with the control, as shown in Table 3.

Comparison of Lipid Peroxidation

Testicular Malondialdehyde Concentration: The concentration of MDA (nmol/mg protein) was significantly increased (P<0.05) in the Cd-only group (9.66±0.59) compared with the control (2.88±0.25); however, it was significantly lower (P<0.05) in the Cd+STC30 group (4.2±0.65) than in the Cd-only group, as shown in Table 4.

Testicular TBARS Concentration: The level of TBARS (nmol/mg protein) was significantly increased (P<0.05) in the Cd-only group (11.02±0.63) compared with control (1.76±0.32); nevertheless, it was significantly lower (P<0.05) in the STC30-only group (5.52±0.59) than in the Cd-only group, as presented in Table 4.

Comparison of Testicular Antioxidant Status

Superoxide Dismutase Activity: The activity of SOD was significantly reduced (P<0.05) in the Cd-only group (3.05±0.38) compared with control (7.62±0.06); nonetheless, it was higher (P<0.05) in the STC30-only (12.34±1.2) and Cd+STC30 groups (9.1±0.37) than in the Cd-only group. It was significantly increased in the STC30-only group compared with control (P<0.05), as shown in Table 4.

Glutathione Peroxidase: Testicular activity (µ/mg protein) of glutathione peroxidase (GPx) was significantly reduced (P<0.05) in the Cd-only (1.02±0.16) and Cd+STC30 groups (P<0.05) compared with the control (4.08±0.39). However, it increased in the Cd+STC30 group (P<0.05) compared with the Cd-only group. The GPx was significantly increased in the STC30-only group (6.12±0.26) compared with control and Cd-only groups (P<0.05), as shown in Table 4.

Catalase Activity: Testicular catalase (CAT) activity (IU/mg protein) was significantly reduced in the Cd-only (49.82±1.19) and Cd+STC30 groups (71.99±1.85) compared with the control (77.79±2.20); nevertheless, it was higher in the Cd+STC30 group than in the Cd-only group. The CAT level was significantly increased in the STC30-only group compared with the control (P<0.05), as shown in Table 4.

Total Antioxidant Capacity: The TAC (nmol uric acid Eq/mg protein) was significantly lower (P<0.05) in the Cd-only group (95.8±4.60) compared to the control group (171.82±5.50). In contrast, the TAC levels were significantly higher (P<0.05) in both the Cd+STC30 (165.9±4.06) and STC30-only (191.22±3.09) groups than in the Cd-only group, as indicated in Table 4.

Testicular Morphometric Parameters

Johnsen Score: The Johnsen score was significantly lower (P<0.05) in the Cd-only (3.67±0.70) and Cd+STC30 (6.54±0.63) groups compared to the control group (8.72±0.49). However, the Johnsen score was significantly higher (P<0.05) in both the Cd+STC30 and STC30-only (9.30±0.29) groups than in the Cd-only group, as shown in Table 5.

Leydig Cell Count: The Leydig cell count (cells/ITR) was significantly lower (P<0.05) in the Cd-only group (1.88±0.25) compared to the control group (4.46±0.41). However, it increased (P<0.05) in both the STC30-only (4.12±0.20) and Cd+STC30 (3.30±0.51) groups when compared to the Cd-only group, as shown in Table 5.

Sertoli Cell Count: The Sertoli cell count (cells/SFT) was significantly reduced (P<0.05) in the Cd-only (2.76±0.30) and Cd+STC30 (6.10±0.58) groups compared to the control group (9.32±0.38). Nevertheless, it was significantly higher (P<0.05) in the STC30-only (9.22±0.41) and Cd+STC30 groups than in the Cd-only group, as indicated in Table 5.

Seminiferous Tubules Diameter: The diameter of the seminiferous tubules (µm) was significantly decreased (P<0.05) in the Cd-only (97.79±3.98) and Cd+STC30 (118.94±3.83) groups compared to the control group (130.30±3.16). Conversely, the diameter was significantly greater in the STC30-only (138.49±2.28) and Cd+STC30 groups compared to the Cd-only group, and it was also significantly larger (P<0.05) in the STC30-only group compared to the control group, as shown in Table 5.

Germinal Epithelial Height: The germinal epithelial height (µm) was significantly reduced (P<0.05) in the Cd-only (17.46±2.74) and Cd+STC30 (22.99±2.90) groups compared to the control group (36.63±2.78). In contrast, it was significantly higher (P<0.05) in both the STC30-only (33.98±2.30) and Cd+STC30 groups relative to the Cd-only group, as indicated in Table 5.

Table 3. Sex hormones concentration in the different experiment groups

	GnRH	TEST	LH	FSH
Control	2.32±0.32	3.88±0.40	5.20±0.45	6.20±0.53
Cadmium	1.14±0.32*	1.98±0.28*	2.76±0.11*	2.68±0.30*
STC30	3.68±0.44*,a	6.14±0.47*,a	6.26±0.42*,a	7.40±0.62*,a
Cd+STC30	2.68±0.418*,a,b	4.34±0.50a,b	4.32±0.38*,a,b	4.96±0.59*,a,b

Values are presented as mean ± SEM, n = 5. * = p<0.05 vs the controls;
a = p<0.05 vs Cadmium; b = p<0.05 vs STC30

Table 4. Antioxidant activity of the different experimental groups

	MDA	TBARS	SOD	GPx	CAT	TAC
Control	2.88±0.25	1.76±0.32	7.62±0.67	4.08±0.39	77.79±2.20	171.82±5.46
Cadmium	9.66±0.59*	11.02±0.64*	3.04±0.38*	1.02±0.16*	49.82±1.19*	95.82±4.60*
STC30	2.76±0.30a	1.84±0.32a	12.341.22*,a	6.12±0.26*,a	81.56±2.13*,a	191.22±3.09*,a
Cd+STC30	4.20±0.26*,a,b	5.72±0.68*,ab	9.10±0.37*,a,b	4.02±0.26a,b	71.99±1.85a,b	165.90±4.06*,a,b

Values are presented as mean ± SEM, n = 5. * = p<0.05 vs control; a = p<0.05 vs Cadmium
b = p<0.05 vs STC30.

Table 5. Testicular morphometric indices of the different experimental groups

	Johnsen's Score	Leydig cell count	Sertoli cell count	Tubular diameter (Microns)	Germinal epithelial height
Control	8.72±0.49	4.40±0.41	9.32±0.38	130.30±3.16	36.63±2.78
Cadmium	3.67±0.70*	1.88±0.35*	2.76±0.30*	97.79±3.98*	17.46±2.74*
STC30	9.30±0.29a	4.12±0.20a	9.22±0.41a	138.49±2.28a	33.98±2.30a
Cd+STC30	6.54±0.63*,a,b	3.30±0.51a,b	6.10±0.58*,a,b	118.94±3.83*,a,b	22.99±2.90*,a.b

Values are presented as mean ± SEM, n = 5. * = p<0.05 vs control
a = p<0.05 vs Cadmium; b = p<0.05 vs STC30

Histology of Testis and Epididymis

Plate 1a is a section of the testes in the control group, showing numerous seminiferous tubules of different sizes and shapes with intact basement membranes. Most of the tubules' lumens were filled with spermatozoa. There were 10-12 Sertoli cells per tubule and 3-5 Leydig cells per interstitium.

Plate 1b is a section of the testes in the Cd-only group, showing seminiferous tubules, which were mostly 3-5 cell layers thick and only empty tubular lumens. The intervening interstitium was scanty, with few Leydig and Sertoli cells.

Plate 1c is a section of the testes in the STC30-only group, showing prominent seminiferous tubules of different sizes and shapes. The tubules had intact basement membranes containing proliferating spermatogonia were moderately distended. The luminal cavities were filled with spermatids and spermatozoa and contained 10-12 Sertoli cells. The intervening intestitium had 3-5 Leydig cells.

Plate 1d is a section of testis in the Cd+STC30 group. It shows closely packed seminiferous tubules with an intact basement membrane. The tubules containing proliferating spermatogonia were 3-4 layers thick, and the cells were moderately packed.

Plate 2a shows a section of the epididymis in the control group exhibiting prominent tubules separated by loose stroma. The tubules were lined by flattened epithelial cells with an intact basement membrane, and the lumens were filled with spermatozoa.

Plate 2b is a section of the epididymis in the Cd-only group showing loosely packed tubules with an intact basement membrane. The tubules contained scanty spermatozoa, most of them being empty.

Plate 2c is a section of epididymis showing prominent tubules that were dilated and lined by flattened epithelial cells with an intact basement line. The lumens were mostly filled with spermatozoa.

Plate 2d is a section of the epididymis in the Cd+STC30 group. It shows closely packed tubules with an intact basement membrane separated by a scanty stroma. Most of the lumens of the tubules contained few spermatozoa.

Discussion

This study aimed to assess the effect of STC30 on male reproductive dysfunction induced by Cadmium in weight-matched rats. The significant weight loss in the Cd-only rats, when compared to the control group, aligns with findings from earlier research [32,44]. This weight reduction may be attributed to the cytotoxic effects of Cd, leading to cellular and tissue breakdown [10,45]. The observed positive weight changes after coadministration of Cd and STC30 indicate that STC30 antagonized the mechanism by which the weight loss was caused by Cadmium. It could also mean that STC30 had the potential to cause weight gain through mechanisms that have yet to be identified, as it could be observed that STC30, when given alone to normal rats, causes a weight increase.

In our study, both testicular and epididymal weights were lower in the Cd-only group, similar to the findings in [41], as well as in the Cd+STC30 group compared to the control. This reduction may be attributed to the atrophy of the structures damaged by Cd, which is supported by the observed structural changes (reduced Johnsen score, sparse interstitium, and fewer sperm cells) in the histological analyses. The coadministration of Cd and STC30 improved these changes, resulting in

a significant increase in testicular and epididymal weights in the Cd+STC30 group compared to the Cd-only group.

The sperm count was significantly lower in the Cd-only group compared to the control, which aligns with earlier findings [15]. When STC30 was administered alongside Cadmium, the sperm count significantly improved; however, when STC30 was given alone, it did not significantly affect the count compared to the control.

Cadmium administration significantly reduced sperm motility compared to the control, a finding consistent with previous reports [42]. However, this motility improved with the coadministration of STC30. Furthermore, STC30 significantly increased sperm motility when administered alone compared to the control, indicating that STC30 enhances sperm motility in both normal and Cd-induced rats. The significant decrease in the percentage of viable sperm in the Cd-only group compared to the control was improved with concurrent administration of Cadmium and STC30, suggesting that STC30 can counteract the mechanisms by which Cd reduces sperm viability. Although the percentage of viable sperm in the STC30-only group was significantly higher than in the Cd-only group, it did not differ significantly from the control group, indicating that STC30 does not affect sperm viability in normal rats.

The results indicated a significantly higher percentage of abnormal sperm cells in the Cd-only group, consistent with findings from a previous study [15]. After the concurrent administration of Cd and STC30, the percentage of teratozoospermia was significantly reduced compared to the Cd-only group, although it remained higher than in the control group. This suggests that STC30, at the administered dose, improved sperm morphology induced by Cd but not to a normal level. The presence of teratozoospermia may have contributed to the decreased sperm motility observed in both the Cd-only and Cd+STC30 groups. No significant differences were found between the STC30-only and control groups, indicating that STC30 does not affect sperm morphology in normal rats.

Cadmium chloride administration significantly lowered plasma GnRH levels, suggesting that the cytotoxic effects of this metal [12,13], also impact GnRH-secreting neurons in the hypothalamus. This effect was mitigated by the coadministration of Cd and STC30. The concentration of GnRH was higher in the STC30-only groups, indicating that STC30 improves serum GnRH even in normal rats for reasons yet to be determined.

Follicle-stimulating hormone and LH are produced by gonadotropes in the anterior pituitary gland and are regulated by GnRH [46]. The observed decrease in serum levels may result from Cd-induced pituitary toxicity [41] or inadequate GnRH stimulation of the gonadotropes. These hormones are crucial for normal testicular function, including spermatogenesis, and may account for the low sperm count observed in this study [43]. The administration of STC30 alongside Cd improved FSH and LH concentrations, although they remained lower than those in the control group, suggesting that the effects of STC30 might not have completely restored tissue function following Cd toxicity. The STC30 also improved serum FSH and LH in normal rats.

Testosterone is produced by Leydig cells in the testes under the influence of LH [45]. The decrease in serum testosterone levels observed in the Cd-only group aligns with findings from [48]; however, this was improved when Cd was administered alongside STC30. The reduction in testosterone levels may have resulted from direct Cd toxicity to the testes, or the lower levels of FSH and LH noted in this study. Additionally, STC30 given alone to normal rats also elevated testosterone levels, suggesting that STC30 enhances testicular steroidogenesis.

The increase in testicular MDA levels, consistent with earlier research [15], and TBARS in the Cd-only group indicates that Cadmium promotes lipid peroxidation in the testes. Malondialdehyde and TBARS are the final products of lipid peroxidation [49] in these tissues. These levels were reduced when Cd was combined with STC30, indicating that STC30 mitigates this effect, though not to pre-exposure levels. There were no significant differences in these parameters between the control and STC30-only groups, indicating that STC30 had no effect on testicular lipid peroxidation in normal rats' testes. The increased lipid peroxidation with the release of reactive oxygen species might have been partly responsible for tissue toxicity and impairment.

Superoxide dismutase, CAT, and GPx are natural enzymatic antioxidants used to indirectly assess the oxidative status of tissues [44]. The significant reduction in these enzymes in the Cd-only group compared to the Cd+STC30 group, as observed in this study, supports findings from a previous study [15], indicating heightened oxidative processes. These effects were significantly ameliorated when Cd was administered concurrently with STC30. The testicular activities of these enzymes were significantly higher when STC30 was given to normal rats than the control rats, indicating that STC30 administered alone to normal rats improves the redox or antioxidant status of the testis. This observation could be attributed to the rich content of antioxidants in STC30 [21].

The significant reduction in testicular TAC in the Cd-only compared to the control indicated that Cd increased lipid peroxidation and generation of reactive oxygen species while depleting the testis of its antioxidant stores [50]. This event was, however, ameliorated following the administration of Cd together with STC30. The TAC measures the synergistic interactions of endogenous enzymatic and non-enzymatic antioxidant systems [51]. The differences in TAC could be the result of augmented

antioxidants and lower lipid peroxidation from administered STC30. Our result also demonstrated a higher TAC in normal rats treated with STC30 compared with the control, which could be due to the augmented antioxidants in STC30.

The reduced Johnsen score in the Cd-only group significantly improved when Cd was co-administered with STC30, although it remained lower than that in the control group. This indicates that STC30 alleviates the effects of Cd on the Johnsen score, but not completely. Johnsen score is used to assess the characteristics of the sperm cell and the spermatogenic apparatus [42]. When STC30 alone was given to normal rats, it did not have a significant effect on Johnsen score.

The significantly reduced Leydig cell count in the Cd-only rats compared with the control aligns with findings from [52], which can be attributed to factors such as low serum FSH levels and direct testicular toxicity. As noted in our results, though combination treatment with Cd and STC30 significantly improved the count, it was still lower compared to the Cd-unexposed group of rats. This shows the limited ability of STC30 to correct Leydig cell reduction from Cd toxicity. Given alone, STC30 showed no significant effect on Leydig cell count in exposed rats.

The diameter of the seminiferous tubules, which was reduced in the rats receiving only Cd, improved with the concurrent administration of Cd and STC30, though not to the levels seen in the unexposed or control groups. STC30 given alone significantly increased the seminiferous tubule diameter compared to the control or unexposed groups. This indicates that STC30 can significantly enhance seminiferous tubule diameter in both normal and Cd-exposed rats, countering the pathological processes that lead to tubule narrowing.

Germinal epithelial heights were significantly reduced in both the Cd-only and Cd+STC30 groups compared to the control; however, they were higher in the Cd+STC30 group than in the Cd-only group. This suggests that STC30 improves Cd-induced germinal epithelium damage, but not completely. The administration of STC30 to normal rats did not affect their germinal epithelial heights. The histopathological changes that occurred in the testes following the administration of Cd were ameliorated by the combined administration of STC30 and Cd. The testicular section of the rats was administered only Cd narrowed luminal cavities that were mostly empty, with a sparse interstitium and few Leydig cells. However, following the co-administration of Cd with STC30, the section showed improvements, with seminiferous tubules closely packed, three to four layers thick, and lumens filled with spermatogonia at various developmental stages. The section of the testis of the rats administered with only STC30 did not show any significant differences from those of the controls.

In the epididymis, the Cd-only group exhibited scanty lumens; however, this was improved with the joint administration of Cd and STC30, which showed significant filling of the lumen with spermatozoa. The epididymal sections from rats given only STC30 did not display significant changes in histological architecture compared to the control group, suggesting that STC30 alone may not have a significant impact on the histological structure of the epididymis tissue.

Conclusions

The results of this study indicated that the combined administration of STC30 mitigated Cd-induced male reproductive impairment, enhanced redox status, and improved testicular histological structure in Wistar rats. In normal rats, STC30 enhanced sperm motility, increased levels of male reproductive hormones (GnRH, FSH, LH, and testosterone), and boosted testicular antioxidant enzymes, including SOD, CAT, and GPx, as well as total antioxidant capacity.

Conflict of Interests

The authors declare that there is no competing interest with any entity in conducting this study.

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Compliance with Ethical Guidelines

Ethical consent was granted by the Animal Research Ethics Committee of the Faculty of Basic Medical Sciences, University of Calabar, Calabar (Approval No. 256/PHS/2013).

Authors' Contributions

JN and EA conceptualized the study. JN, EA, AU, and AA participated in the study design. JN and AU conducted the experiments and collected the samples. All authors participated in the content of the manuscript and its approval.

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