



# Mitigating Cadmium-Induced Reproductive Toxicity in Male Wistar Rats: Protective Role of STC30, a Polyherbal Formulation

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## Authors' contributions

This work was carried out in collaboration among all authors. Authors NJN and AEO conceptualized the study. Authors NJN, AEO and OEAI participated in the design of the study. Authors NJN and AEO conducted the experiments and collected the samples. All authors read and approved the final manuscript.

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## ABSTRACT

**Background:** Cadmium, one of the most toxic heavy metals but which finds its presence in many applications and the environment impairs male reproductive function via oxidative stress. There is a growing interest in the development and usage of polyherbal remedies with high antioxidant base. Super Life Total Care (STC30) is one of such remedies. There is however paucity of information on most of its claimed effects and Cadmium-induced male reproductive toxicity.

**Methods:** Twenty male wistar rats were separated into control, Cadmium-only, STC30-only and Cadmium+STC30 groups of 5 rats each. Once daily administration was for 28 days after which blood and testicular samples collected for determination of relevant parameters.

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**Results:** Results showed a significantly reduced ( $p < 0.05$ ) sperm count in the Cadmium-only compared with the control though significantly lower ( $p < 0.05$ ) in the Cadmium+STC30 compared with the STC30-only groups. Sperm motility was significantly reduced in STC30-only but increased in STC30-only compared with control. It was significantly higher ( $p < 0.05$ ) in the STC30-only and Cadmium+STC30 than in the Cadmium-only groups. Sperm viability was significantly reduced in the Cadmium-only compared with control. Teratozotospermia was significantly elevated in the Cadmium-only compared with the control but lower in the Cadmium+STC30 and STC30-only compared with Cadmium-only groups. Serum follicle stimulating hormone, luteinizing hormone and testosterone were significantly reduced ( $p < 0.05$ ) in the Cadmium-only compared with the control groups. Testicular malondialdehyde and thiobarbituric acid were significantly increased ( $p < 0.05$ ) in the Cadmium-only compared with the control groups, but lower in STC30-only and STC30+Cadmium than in the Cadmium-only groups though higher in Cadmium+STC30 than in the STC30-only groups. Testicular superoxide dismutase, glutathione peroxidase and catalase activities as well as total antioxidant capacity were significantly reduced ( $p < 0.05$ ) in the Cadmium-only compared with the control but higher in the Cadmium+STC30 than in the Cadmium-only groups.

**Conclusion:** In conclusion, STC30 ameliorates Cadmium-induced reproductive toxicity and oxidative stress in male wistar rats.

*Keywords:* STC30; cadmium; male; reproductive; impairment; ameliorates.

## 1. INTRODUCTION

Infertility is the inability of a couple to achieve a pregnancy after 12 months of regular unprotected intercourse [1]. It is said to affect over 180 million people worldwide. The 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 affecting 15% of couples of reproductive age globally [3]. Infertility is associated with emotional, economic and social issues especially in societies like ours with strong emphasis on child-bearing [4,5].

Over the past decades, there has been a rapid decline in male reproductive health [6,7]. Though the exact cause for this decline is not clear, some factors have been implicated including stress, lifestyle, changes as well as exposure to environmental and occupational pollutants [8]. Cadmium is one of such environmental pollutants and is an endocrine disruptor [9].

Cadmium (Cd) is said 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 fire works [10]. Environmental exposure to Cadmium is often through drinking water, inhalation in industries, vehicles exhaust fumes, cigarettes smoking as well as consumption of agricultural products from contaminated soils [10,11]. Chronic exposure to Cadmium has been

associated with respiratory, renal, hepatic and reproductive toxicities [12,13,14]. The mechanism of tissue damage by Cadmium is in part attributed to oxidative stress [14,15]. Free radicals generated by lipid peroxidation and other oxidants damage cellular structures and functions [16] which are mitigated by antioxidants. Recent studies show also that tissue damage following exposure to Cadmium could be due to up-regulation of Nitric Oxide Synthase activity which was ameliorated by antioxidant-rich preparations [17,18].

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 [19]. Some have been explored and reported to have therapeutic effects against cytotoxicities either due to their antioxidants contents or some other factors [20,21]. STC30 is one of those plant-based remedies claimed to have positive health benefits. STC30 a polyherbal preparation is a proprietary brand of supplement produced by Superlife world, Kuala Lumpur, Malaysia. Few studies that have been done on STC30 shows it ameliorates carbon tetrachloride ( $CCl_4$ )-induced nephropathy and glomerular function impairment [22] and reduces the serum concentration of c-reactive protein in  $CCl_4$ -induced hepatocellular carcinoma. It is said to boost immunity, rejuvenate and replace damaged cells and improves the redox state of tissues [23]. STC30 contains swiss apple, grapes, glusodin, bilberry, blackcurrant juice-powder and blueberry extracts [24].

Blackcurrant juice is rich in anthocyanins, polyphenolic compounds, antioxidants, vitamin C, gamma linolenic acid (GLA) and is said to regulate blood flow, improves immunity with antioxidant, antimicrobial and antitumor activities [25,26]. Bilberry has hepatoprotective, antioxidant and anti-inflammatory effects [27]. Glisodin is a known antioxidant, reduces synthesis of lactic acid during exercise [28]. While few researches have been done on STC30, there is paucity of information in literature regarding its effect on Cadmium-induced male reproductive impairment. This is the essence of this study.

## 2. 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. Duration of acclimatization before experimentation was one week. They were allowed free access to animal feed and water.

**Preparation of stock solution of STC30:** To prepare the stock solution of STC30, the content of one capsule (1500mg) was dissolved in 200ml of distilled water.

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

**Experimental Design:** Twenty male wistar rats were randomly divided into 4 groups of 5 rats each and raised in metallic cages which were cleaned regularly. Group 1 served as the control (given portable water), group 2 was the Cadmium-only group, group 3 was STC30-only while group 4 was Cadmium + STC30. Cadmium chloride was administered at a dose of 5mg/kg [29,30], while STC30 was given at a dose of 132.7mg/kg, its effective dose calculated from its therapeutic dose [31]. Cadmium and STC30 were administered daily by gavaging for 28 days. Animals were weighed regularly and the amount of drugs administered adjusted accordingly.

**Acute toxicity study:** The median lethal dose LD50 of PurXcel was estimated using Lorke's method [32] and followed up by the up and down method as described by Erhirhie et al, [33].

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

**Determination of body, testicular and epididymal weights:** This was done weekly with an electronic weighing balance (Scout Pro, Ohaus Corporation, USA).

*Relative organ weight =*

$$\frac{\text{Absolute weight of organ}}{\text{Final body weight}} \times \frac{100g}{1g}$$

### 2.1 Sperm Function Analysis

**Sperm Count:** Sperm count was measured as described by [34]. In brief, the cauda epididymis was immersed in 2ml of normal saline and pre-warmed to 37°C after which small incision were made on it to enable sperm come out from it. The collected sperms were suspended in the normal saline and 200µl of the sperm suspension transferred to both chambers of the improved Neubauer hemocytometer by touching the edge of the cover slip and allowing each chamber to fill up by capillary action. The sperms were then counted in five large Thorma squares using a microscope (Leica DM 750, Switzerland).

**Sperm Motility:** A Makler's chamber was used for this purpose as previously demonstrated by other researcher [35]. Mild pressure was exerted on the vas deferens to obtain sperm suspension which was introduced into 1ml of normal saline and the mixture 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.* [36] was used to assess sperm viability. Twenty microliter (20µl) of sperm suspension was stained with 20µl of 0.05% eosin Y/nigrosin and the mixture 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 total sperms counted.

**Sperm morphology:** Sperm morphology was evaluated as described by Narayana et al [37]. 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 50µl 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 10000g for 10min in a cold centrifuge. The supernatant were obtained and used for determination of necessary testicular parameters

**Determination of serum concentration of FSH:** Serum FSH concentration was evaluated in triplicate using rat FSH ELISA Kits cat No. E.EI-R0391 (Elabscience Biotechnology, Wuhan China) and following manufacture's protocol.

**Determination of serum LH:** Serum LH concentration was determined with rats LH ELISA kit, Cat No. ABIN6574078 (ElabScience Biotechnology, China) and following manufacturer's protocol.

**Determination of testosterone: Rat:** ELISA Kit (ElabScience Biotechnology, China) was used for this assay and following manufacturer's protocol.

**Determination of serum concentration GnRH:** This was done with rat GnRH Kit (ElabScience Biotechnology, China) and following manufacturer's protocol.

## 2.2 Evaluation of Testicular Level of Lipid Peroxidation

**Malondialdehyde (MDA):** The concentration of MDA in testicular homogenate was evaluated using Ohkawa et al method [38] as also described by Chatterjee et al [39] using commercially available reagents. In brief, a 100ml aliquot of testicular homogenate was to a reaction mixture that contains 200ml of 8.1%

(wt/v) Lauryl sulphate, 1.5ml of 20% (wt/v) acetic acid, 1.5ml of 0.8% (wt/v) thiobarbituric acid and 100ml of distilled water. The mixture was then boiled and centrifuged and the absorbance of the supernatant measured spectrophotometrically.

**Thiobarbituric acid reactive substance (TBARS):** The level of TBARS in testicular homogenate was determined by the method of Armstrong and Al- Awadi [40] using commercial reagents. Malondialdehyde as one of the end products of lipid peroxidation reacts with thiobarbituric acid to form a coloured substance whose absorbance is measured spectrophotometrically at 532nm.

## 2.3 Determination of Testicular Activities of Antioxidant Enzymes

**Superoxide dismutase (SOD):** The activity of superoxide dismutase in testicular homogenate was determined according to the method of Sun et al [41] 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 evaluated on the resultant supernatant. 1ml of the reactant (13nM L-methionine, 100nM EDTA, 300µL of 2µM riboflavin and 50nM phosphate buffer, pH 7.8) and the activity read spectrophotometrically at 560nm

**Catalase (CAT):** This was evaluated as described by Chandran et al [42] and is based on an enzyme-catalyzed decomposition of H<sub>2</sub>O<sub>2</sub> which forms a yellowish complex with molybdate whose absorbance is assayed at 405nm.

**Glutathione peroxidase:** The activity of peroxidase was determined in testicular homogenate using the method described by Lusches et al [43] using hydrogen peroxide as a substrate.

**Total antioxidant capacity:** The TAC was assayed using the method described by Koracevic et al [44]. The TAC assay employs a thermal radical generator which produces a steady flux of radicals in solution. The addition of antioxidants results in competitive inhibition of the substrates.

**Histological studies:** The harvested right testes were cleaned of connective tissues and fixed in Bouin's fluid and then dehydrated with ethanol before being embedded in paraffin blocks. The

blocks were then sectioned and stained with haematoxylin and eosin (H&E) and viewed using light microscope (Leica, DM, 750 Switzerland) at a magnification of x400. The number of Leydig cells per intertubular 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 score was assessed in 10 seminiferous tubules [45] as used by Aksu et al [46] Image Analyser software (Soft Imaging System, VGA, Utilities Version 3.67c) was used to measure seminiferous tubular diameter and germinal epithelial height in 20 seminiferous tubules chosen from serial sections and their averages computed.

## 2.4 Statistical Analysis

The data were presented as mean  $\pm$  SEM. The data were normally distributed. Statistical package for social sciences (SPSS) version 20 was used to analyse the data. One way analysis of variance (ANOVA) was employed to analyse the data and Tukey Post hoc test performed to compare mean values. Values of  $p < 0.05$  were considered statistically significant.

## 3. RESULTS

**Acute toxicity study:** Administration of STC30 produced no mortality or significant behavioral changes upto 5000mg/kg dose in male wistar rats implying that the LD50 of STC30 is above 5000mg/kg.

**Body weight changes:** Body weight changes for control, Cd-only, STC30-only and Cd+STC were 28 $\pm$ 9.92, -15.2 $\pm$ 5.45, 12.6 $\pm$ 2.30 and 7.8 $\pm$ 2.17 respectively. There were significant decreases in weight 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 $\pm$ 0.19) group was significantly lower ( $p < 0.05$ ) than that of the control (3.56 $\pm$ 0.21). It was however significantly higher ( $p < 0.05$ ) in the STC30-only (3.16 $\pm$ 0.23) and Cd+STC30 (2.48 $\pm$ 0.13) than the Cd-only group as shown in Table 2. The epididymal weight was significantly reduced in Cd-only (1.14 $\pm$ 0.15) compared with the control (1.5 $\pm$ 0.16) but significantly higher in the STC30-only (1.56 $\pm$ 0.18) than in the Cd-only group as shown in Table 1.

## 3.1 Comparison of Sperm Parameters

**Sperm count:** The total sperm count ( $\times 10^6/L$ ) was significantly ( $p < 0.05$ ) decreased in the Cd-only (29.92 $\pm$ 3.47) and Cd+STC30 (47.34 $\pm$ 3.88) compared with the control (55.60 $\pm$ 4.57) but significantly higher in the STC30-only (62.60 $\pm$ 3.73) and Cd+STC30 than in the Cd-only groups ( $p < 0.05$ ) as shown in F Table 2.

**Sperm motility:** Sperm motility (%) was significantly decreased in Cd-only (52.20 $\pm$ 3.90) and Cd+STC30 (71.20 $\pm$ 3.11) compared with the control (72.20 $\pm$ 3.11) but significantly higher ( $p < 0.05$ ) in the STC30-only (84.40 $\pm$ 2.88) and Cd+STC30 than the Cd-only groups. It was significantly higher in the STC30-only than the control ( $p < 0.05$ ) as shown in Table 2.

**Sperm viability:** Sperm viability (%) was significantly decreased in the Cd-only (50.80 $\pm$ 6.38) compared with the control (70.20 $\pm$ 4.49) but significantly higher ( $p < 0.05$ ) in the STC30-only (79.60 $\pm$ 4.16) and Cd+STC30 than in the Cd-only groups as shown in Table 2

**Sperm morphology:** The percentage of morphologically abnormal sperms was significantly increased ( $p < 0.05$ ) in the Cd-only (28.20 $\pm$ 3.27) compared with the control (9.60 $\pm$ 2.41) but significantly lower ( $p < 0.05$ ) in the Cd+STC30 (13.60 $\pm$ 2.70) than in the Cd-only groups as shown in Table 2.

## 3.2 Comparison of Male Reproductive Hormones

**Sperm GnRH:** The serum concentration of GnRH (ng/ML) was significantly reduced ( $p < 0.05$ ) in the Cd-only (1.14 $\pm$ 0.15) compared with the control (2.32 $\pm$ 0.32) but significantly higher ( $p < 0.05$ ) in the Cd+STC30 (2.68 $\pm$ 0.41) than in the Cd-only groups. It was significantly increased ( $p < 0.05$ ) in the STC30-only (3.60 $\pm$ 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 (1.98 $\pm$ 0.28) compared with control (3.88 $\pm$ 0.40) but significantly higher ( $p < 0.05$ ) in the Cd+STC30 (4.3 $\pm$ 0.50) than in the Cd-only groups. It was significantly elevated in the STC30-only (6.14 $\pm$ 0.47) compared with control and Cd-only groups as seen in Table 3.

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

<b>Group</b>	<b>Initial body weight (g)</b>	<b>Final body weight (g)</b>	<b>body weight change (g)</b>	<b>Absolute Testis weight (g)</b>	<b>Relative testis weight (g)</b>	<b>Absolute epididymis weight (g)</b>	<b>Absolute epididymis weight (g)</b>
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 ±0.23	1.28 ±0.07	1.56 ±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	76.20	32.40	21.20	22.60	70.20	9.60
	±4.57	±3.11	±1.67	±3.77	±2.30	±4.49	±2.41
Cadmium	29.92	52.20	22.40	17.00	12.80	50.80	28.20
	±3.47	±3.90*	±2.88*	±2.12	±1.92*	±6.38*	±3.27*
STC30	60.94	84.40	42.40	23.20	18.80	79.60	8.40
	±4.07	±2.88*,a	±3.51*,a	±2.77 <sup>a</sup>	±1.79 <sup>a</sup>	±4.16 <sup>a</sup>	±2.30 <sup>a</sup>
Cd+STC30	47.34	71.20	39.60	18.40	13.20	70.80	13.60
	±3.88	±3.11 <sup>a,b</sup>	±4.39 <sup>a,b</sup>	±3.91	±2.77* <sup>b</sup>	±4.27 <sup>a,b</sup>	±2.70 <sup>a,b</sup>

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 3. Sex hormones concentration in the different experiment groups**

	GnRH	TEST	LH	FSH
Control	2.32	3.88	5.20	6.20
	±0.32	±0.40	±0.45	±0.53
Cadmium	1.14	1.98	2.76	2.68
	±0.32*	±0.28*	±0.11*	±0.30*
STC30	3.68	6.14	6.26	7.40
	±0.44*,a	±0.47*,a	±0.42*,a	±0.62*,a
Cd+STC30	2.68	4.34	4.32	4.96
	±0.418*,a,b	±0.50 <sup>a,b</sup>	±0.38*,a,b	±0.59*,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 4. Antioxidant activity of the different experimental groups**

	MDA	TBARS	SOD	GPx	CAT	TAC
Control	2.88	1.76	7.62	4.08	77.79	171.82
	±0.25	±0.32	±0.67	±0.39	±2.20	±5.46
Cadmium	9.66	11.02	3.04	1.02	49.82	95.82
	±0.59*	±0.64*	±0.38*	±0.16*	±1.19*	±4.60*
STC30	2.76	1.84	12.34	6.12	81.56	191.22
	±0.30 <sup>a</sup>	±0.32 <sup>a</sup>	1.22*,a	±0.26*,a	±2.13*,a	±3.09*,a
Cd+STC30	4.20	5.72	9.10	4.02	71.99	165.90
	±0.26*,a,b	±0.68*,ab	±0.37*,a,b	±0.26 <sup>a,b</sup>	±1.85 <sup>a,b</sup>	±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

**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 (4.32±0.38) compared with control (5.20±0.45) but increased (p<0.05) in the Cd+STC30 (4.37±0.38) compared with Cd-only (2.76±0.11). It was significantly higher in the STC30-only (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 (4.96±0.59) compared with the control (6.20±0.53), but

significantly higher ( $p<0.05$ ) in the STC30-only ( $7.40\pm 0.62$ ) and Cd+STC30 ( $4.96\pm 0.53$ ) than in the Cd-only groups. It was significantly increased ( $p<0.05$ ) in the STC30-only group compared with the control as shown in Table 3.

### 3.3 Comparison of Lipid Peroxidation

**Testicular malondialdehyde (MDA) concentration:** The concentration of MDA (nmol/mg protein) was significantly increased ( $p<0.05$ ) in the Cd-only ( $9.66\pm 0.59$ ) compared with the control ( $2.88\pm 0.25$ ), but significantly lower ( $p<0.05$ ) in the Cd+STC30 ( $4.2\pm 0.65$ ) than in the Cd-only groups as shown in Table 4.

**Testicular concentration of TBARS:** The level of TBARS (nmol/mg protein) was significantly increased ( $p<0.05$ ) in the Cd-only ( $11.02\pm 0.63$ ) compared with control ( $1.76\pm 0.32$ ), but significantly lower ( $p<0.05$ ) in the STC30-only ( $5.52\pm 0.59$ ) than in the Cd-only group as in Table 4.

### 3.4 Comparison of Testicular Antioxidant Status

**Superoxide dismutase (SOD) activity:** The activity of SOD was significantly reduced ( $p<0.05$ ) in the Cd-only ( $3.05\pm 0.38$ ) compared with control ( $7.62\pm 0.06$ ) but higher ( $p<0.05$ ) in the STC30-only ( $12.34\pm 1.2$ ) and Cd+STC30 ( $9.1\pm 0.37$ ) than in the Cd-only groups. It was significantly increased in the STC30-only group compared with control ( $p<0.05$ ) as shown in Table 4.

**Glutathione peroxidase (GPx):** Testicular activity ( $\mu$ /mg protein) of GPx was significantly decreased ( $p<0.05$ ) in the Cd-only ( $1.02\pm 0.16$ ) and Cd+STC30 group ( $p<0.05$ ) compared with the control ( $4.08\pm 0.39$ ) but increased in the Cd+STC30 group ( $P<0.05$ ) compared with the Cadmium-only groups. It was significantly increased in the STC30-only ( $6.12\pm 0.26$ ) compared with control and Cd-only groups ( $p<0.05$ ) as shown in Table 4.

**Catalase activity:** Testicular Catalase activity (IU/mg protein) was significantly reduced in the Cd-only ( $49.82\pm 1.19$ ) and Cd+STC30 ( $71.99\pm 1.85$ ) groups compared with the control ( $77.79\pm 2.20$ ) group though higher in the Cd+STC30 than in the Cd-only group. It was also significantly increased in the STC30-only

compared with the control ( $p<0.05$ ) as shown in Table 4.

**Total antioxidant capacity:** The total antioxidant capacity (nmol uric acid Eq/mg protein) was significantly reduced ( $p<0.05$ ) in the Cd-only group ( $95.8\pm 4.60$ ) compared with control ( $171.82\pm 5.50$ ) to be higher ( $p<0.05$ ) in the Cd+STC30 ( $165.9\pm 4.06$ ) and STC30-only ( $191.22\pm 3.09$ ) than Cd-only groups as shown in Table 4.

### 3.5 Testicular Morphometric Parameters

**Johnsen score:** The Johnsen score was significantly reduced ( $p<0.05$ ) in the Cd-only ( $3.67\pm 0.70$ ) and the Cd+STC30 ( $6.54\pm 0.63$ ) groups compared with the control ( $8.72\pm 0.49$ ) but significantly higher ( $p<0.05$ ) in the Cd+STC30 and STC30-only ( $9.30\pm 0.29$ ) than in the Cd-only groups as shown in Table 5.

**Leydig cell count:** The Leydig cell count (cells/ITR) was significantly reduced ( $p<0.05$ ) in the Cd-only ( $1.88\pm 0.25$ ) compared with the control ( $4.46\pm 0.41$ ) but increased ( $p<0.05$ ) in the STC30-only ( $4.12\pm 0.20$ ) and Cd+STC30 ( $3.30\pm 0.51$ ) compared with Cd-only groups as shown in Table 5.

**Sertoli cell count:** The Sertoli cell count (cells/SFT) was significantly reduced ( $p<0.05$ ) in the Cd-only ( $2.76\pm 0.30$ ) and Cd+STC30 ( $6.10\pm 0.58$ ) compared with control ( $9.32\pm 0.38$ ) though higher ( $p<0.05$ ) in STC30-only ( $9.22\pm 0.41$ ) and Cd+STC30 than Cd-only groups as shown in Table 5.

**Seminiferous tubules diameter:** The seminiferous tubules diameter ( $\mu$ m) was significantly reduced ( $p<0.05$ ) in the Cd-only ( $97.79\pm 3.98$ ) and Cd+STC30 ( $118.94\pm 3.83$ ) compared with the control ( $130.30\pm 3.16$ ) but significantly higher in the STC30-only ( $138.49\pm 2.28$ ) and Cd+STC30 groups than in the Cd-only group. It was also significantly increased ( $p<0.05$ ) in the STC30-only compared with the control group as shown in Table 5.

**Germinal epithelial height:** The germinal epithelial height ( $\mu$ m) was significantly decreased ( $p<0.05$ ) in the Cd-only ( $17.46\pm 2.74$ ) and Cd+STC30 ( $22.99\pm 2.90$ ) compared with the control ( $36.63\pm 2.78$ ) but higher ( $p<0.05$ ) in the STC30-only ( $33.98\pm 2.30$ ) and Cd+STC30 groups compared with Cd-only groups as shown in Table 5.



**Table 5. Testicular morphometric indices of the different experimental groups**

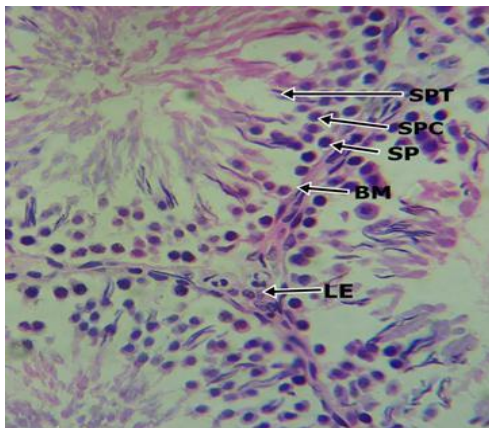
	<b>Johnsen's Score</b>	<b>Leydig cell count</b>	<b>Sertoli cell count</b>	<b>Tubular diameter (Microns)</b>	<b>Germinal Epithelial Height</b>
Control	8.72	4.40	9.32	130.30	36.63
Cadmium	±0.49	±0.41	±0.38	±3.16	±2.78
STC30	3.67	1.88	2.76	97.79	17.46
	±0.70*	±0.35*	±0.30*	±3.98*	±2.74*
Cd+STC30	9.30	4.12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

Values are presented as mean ±SEM, n = 5.

\* = p<0.05 vs control

a = p<0.05 vs Cadmium

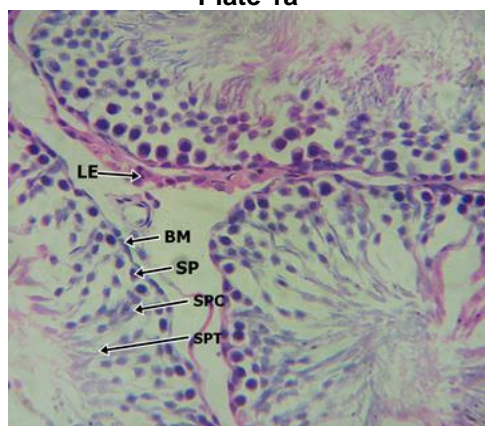
b = p<0.05 vs STC30



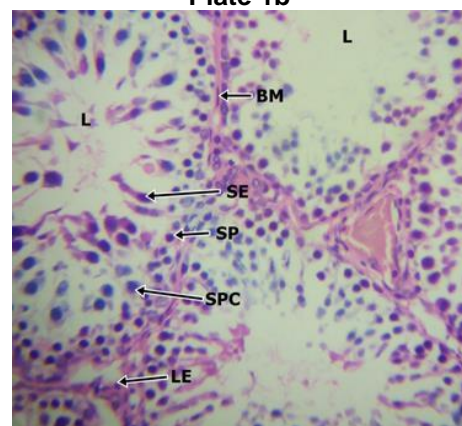
**Plate 1a**



**Plate 1b**



**Plate 1c**



**Plate 1d**

**Plate 1. Section 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

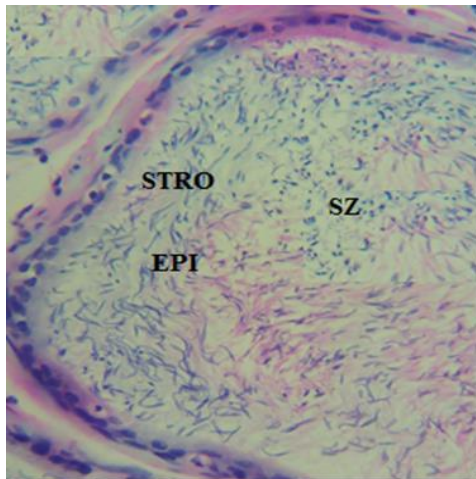


Plate 2a

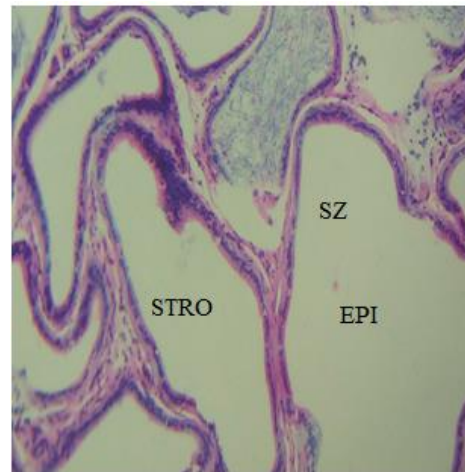


Plate 2b

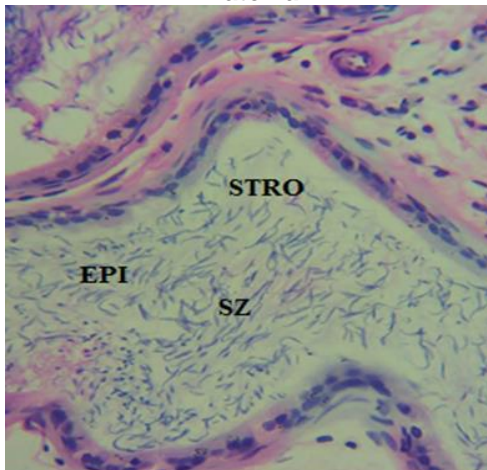


Plate 2c

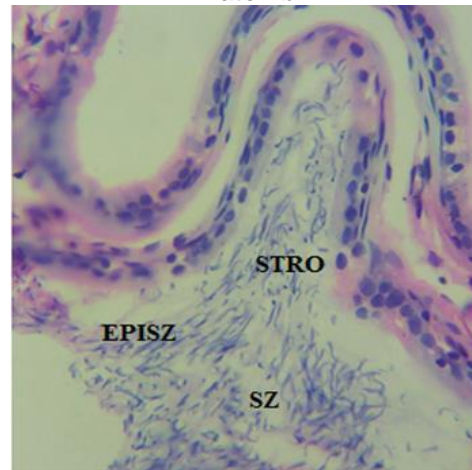


Plate 2d

**Plate 2. Section of 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.*

### 3.6 Histology of Testis/Epididymis

Plate 1a is a section of the testis in the control group showing numerous seminiferous tubules of different sizes and shapes with intact basement membrane. Most of the tubules lumen are filled with spermatozoa. There are 10-12 Sertoli cells per tubule and 3-5 Leydig cells per interstitium.

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

Plate 1c is a section of the testis in the STC30-only group showing prominent seminiferous tubules of different sizes and shapes. The tubules have intact basement membrane and contain

proliferating spermatogonia and are moderately distended. The luminal cavities are filled with spermatids and spermatozoa and contain 10-12 Sertoli cells. The intervening interstitium has 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 are 3-4 layers thick. The cells are moderately packed.

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

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

Plate 2c is a section of epididymis showing prominent tubules which are dilated and lined by flattened epithelial cells with an intact basement line. The lumen are 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 scanty stroma. Most of the lumen of the tubules contain few spermatozoa.

#### 4. DISCUSSION

This study evaluated the effect of STC30 on male reproductive dysfunction induced by Cadmium in rats which were weight-matched.

The significant decrease in weight in the Cd-only rats compared with the control is similar to findings in previous studies [35,47]. This effect could be attributed to the cytotoxicity of Cd which results in the breakdown of many cells and tissues [10,48]. The positive weight changes that were noticed following the coadministration of Cd and STC30 suggests that STC30 antagonizes the mechanism by which the weight loss was orchestrated by Cadmium. It could also mean that STC30 has a potential to cause weight gain by yet to be identified mechanism as could be observed that STC30 given alone to normal rats causes a weight increase.

From our study, testicular and epididymal weights were decreased in the Cd-only, similar to the findings by Koracevic et al [44] and Cd+STC30 groups compared with the control. This decrease could have been due to atrophy of the structures damaged by Cd which is supported by the structural changes (reduced Johnsen score, scanty interstitium and sperm cells) seen in the histologies. Co-administration of Cd and STC30 improved these changes resulting in the significant increase in the testicular and epididymal weights in the Cd+STC30 compared with the Cd-only groups.

The significantly decreased sperm count in the Cd-only group compared with the control is similar to the findings by Abarikwu et al [15]. STC30 administered together with Cd

significantly improved the count but when administered alone, STC30 did not significantly affect the count compared with the control.

Administration of Cd significantly reduced sperm motility compared with the control, an observation similar to that of previous studies [43] but this was improved following co-administration with STC30. STC30 also significantly increased sperm motility when given alone compared with the control suggesting that STC30 improves sperm motility in both normal and Cd-induced rats

The significant reduction in the percentage of viable sperms noted in the Cd-only group compared with the control group was ameliorated following co-administration of the Cd with STC30 implying that STC30 possess the ability to antagonize the mechanism(s) by which the viability was reduced by Cadmium. The percentage of viable sperms in the group only administered STC30, though significantly higher than that of the Cd-only group, was not significantly different from the control showing that STC30 does not have effect on sperm viability in normal rats.

The results show a significantly increased percentage of abnormal sperm cells in the Cd-only group which is in line with previous report [15]. Following co-administration of Cd with STC30, the percentage of teratozoospermia was significantly reduced compared with the Cd-only rats though still higher than in the control group. This means that STC30 at the dose given, improves sperm dimorphology induced by Cd but not to a normal level. The teratozoospermia might have been at least in part responsible for the decrease in sperm motility observed in the Cd-only and Cd+STC30 groups. The observed lack of significant differences in teratozoospermia between the STC30-only and the control groups suggests that STC30 might have no effect on sperm morphology in normal rats.

Cadmium Chloride administration markedly reduced the plasma concentration of GnRH suggesting that in the cytotoxicity of this metal [12,13], the GnRH-secreting neurons in the hypothalamus are not spared. This effect was ameliorated by the co-administration of the Cd with STC30. The concentration of GnRH was higher in the STC30-only groups indicating that, STC30 improves serum GnRH even in normal rats for yet to be determined reason.

Follicle stimulating hormone and LH are produced by gonadotropes in the anterior pituitary gland under the influence of GnRH [49]. The decrease in the serum levels that were observed in this study might have been due to Cd-induced pituitary toxicity [44] or from insufficient GnRH stimulation of the gonadotropes. These hormones are essential for normal testicular function including spermatogenesis and might have been responsible for the low sperm count seen in this study [46]. The administration of STC30 together with Cd improved the FSH and LH concentrations though still less than in the control suggesting that the effects of STC30 may not completely restore tissue on following Cd toxicity. The STC30 also improves serum FSH and LH in normal rats.

Testosterone is produced by the Leydig cells of the testis under the influence of LH [50]. Its reduction in serum in the Cd-only group is similar to the findings by [51] but this was improved when the Cd was administered together with STC30. The decreased level of testosterone could have been due to direct Cd toxicity on the testis or the low FSH and LH also noted in this study. STC30 given alone to normal rats also increases the testosterone level implying that STC30 upgrades testicular steroidogenesis.

The increase in testicular levels of MDA and TBARS in the Cd-only groups is similar to the finding from previous studies [15] indicating that Cd increases lipid peroxidation in the testis. Malondialdehyde and TBARS are final products of lipid peroxidation in tissues [51]. These levels were reduced following Cd combination with STC30 inferring that STC30 ameliorates this effect though not to pre-exposure level. There were no significant differences in these parameters between the control and STC30-only groups indicating that STC30 has no effect on testicular lipid peroxidation in normal rats testes. The increased lipid peroxidation with release of reactive oxygen species might have been partly responsible for tissue toxicity and impairment.

Superoxide dismutase, CAT and GPx are natural enzymatic antioxidants and so are used indirectly to measure oxidation status of a tissue [48]. The significant reduction in these enzymes in the Cd-only compared with the Cd+STC30 seen in our study agrees with previous observations [15] which is a reflection of increased oxidative processes. This effects were significantly ameliorated when Cd was co-administered with

STC30. The testicular activities of these enzymes were significantly higher when STC30 was given to normal 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 [52].

The significant reduction in testicular TAC in the Cd-only compared with the control indicates that Cd increases lipid peroxidation and generation of reactive oxygen species (ROS) while depleting the testis of its stores of antioxidants [53]. This was however ameliorated following administration of Cd together with STC30. Total antioxidant capacity measures the synergistic interactions of endogenous enzymatic and non-enzymatic antioxidant system [54]. The differences in TAC could be the result of augmented antioxidants and lower lipid peroxidation from administered STC30. Our result also demonstrates a higher TAC in normal rats treated with STC30 compared with the control which could be due to the augmented antioxidants in STC30.

The decreased Johnsen score in Cd-only group was significantly increased when Cd was co-administered with STC30 though it was still lower than control (which means that STC30 relieves Cd effect on Johnsen score but not totally. Johnsen score is used to quantify the characteristics of sperm cell and the spermatogenic apparatus [45]. STC30 given alone to normal rats does not have a significant effect on Johnsen score.

The significantly reduced Leydig cell count in the Cd-only rats compared with the control is similar to previous findings [55] which can be attributed to among other things, the low serum, FSH 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 Cadmium-unexposed group of rats. This shows the limited ability of STC30 to correct Leydig cell reduction from Cd toxicity. Given alone, STC30 does not show any significant effect on Leydig cell count in exposed rats.

Seminiferous tubules diameter which was reduced in the rats administered only Cd was improved following co-administration of Cd with STC30, though not as much as in the unexposed group or control. STC30 given alone significantly

increased the seminiferous tubules diameter compared with the control or unexposed group. This indicates that STC30 given alone to normal and Cd-administered rats, significantly improves seminiferous tubules diameter and antagonizing the pathological process that lead to the narrowing of the tubules.

Germinal epithelial heights were significantly decreased in the Cd-only as well as Cd+STC30 compared with the control but higher in the Cd+STC30 than in the Cd-only groups. This suggests that STC30 improves Cd-induced germinal epithelium damage but not perfectly. Administration of STC30 to normal rats does not affect on their germinal epithelial heights.

The histopathological changes that occurred in the testis following administration of Cd were ameliorated by co-administration of STC30 with Cd. The testicular section of the rats administered only Cd has narrowed luminal cavities which were mainly empty and scanty interstitium with few Leydig cells. However, following co-administration of Cd with STC30, the section showed improvements like closely packed seminiferous tubules. 3-4 layers thick and lumen fully packed with spermatogonia at various stages of development. The section of the testis of the rats administered with only STC30, does not show any remarkable difference from that of the control.

A section of the epididymis which in the Cd-only group showed scanty lumens was improved in by the joint administration of Cd with STC30 and shows significant filling of the lumen with spermatozoa. The epididymal section of the rats administered STC30 only did not show any significant changes in the histoarchitecture of the epididymis in relation to the control group suggesting that, STC30 administered alone may not have any significant effect on the histoarchitecture of the epididymis.

## 5. CONCLUSION

We conclude that, co-administration of STC30 ameliorates Cd-induced male reproductive impairment, redox status and testicular histoarchitecture in wistar rats. Given to normal rats, STC30 improves sperm motility, male reproductive hormones (GnRH, FSH, LH and testosterone) as well as testicular antioxidants (SOD, CAT and GPx) levels and total antioxidant capacity.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

## AVAILABILITY OF DATASETS

Data used in this study are available from corresponding author on reasonable request.

## TYPE OF STUDY

Original Research study. The data used for this manuscript are from a PhD thesis of the first author at the University of Calabar, Calabar.

## ETHICAL CONSIDERATIONS

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).

## CONSENT

It is not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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