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

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.

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-onlygroups. 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 malomdialdehyde 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], an imbalance in oxidative and antioxidant systems [16]. Free radicals generated by lipid peroxidation and other oxidants damage cellular structures, an effect which is mitigated by antioxidants. This is the basis for using antioxidants to prevent oxidative stress-mediated cytotoxicities [17].

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 [18]. Some have been explored and reported to have therapeutic effects against cytotoxicities either due to their antioxidants contents or some other factors [19,20]. 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, Kualar Lumpur, Malaysia. Few studies that have been done on STC30 shows it ameliorates carbon tetrachloride (CCl_4)-induced nephropathy and glomerular function impairment [21] 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 [22]. STC30 contains swiss apple, grapes, glusodin, bilberry, blackcurrant juice-powder and blueberry extracts [23].

Blackcurrant juice is rich in anthocyanins, polyphenolic compounds, antioxidants, vitamin C, gamma lenolenic acid (GLA) and is said to regulate blood flow, improves immunity with antioxidant, antimicrobial and antitumor activities [24,25]. Bilberry has hepatoprotective, antioxidant and anti-inflammatory effects [26]. Glisodin is a known antioxidant, reduces synthesis of lactic acid during exercise [27]. While few researches have been done on STC30, there is paucity of informationin 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 [28,29], while STC30 was given at a dose of 132.7mg/kg, its effective does calculated from its therapeutic dose [30]. 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 [31] and followed up by the up and down method as described by Erhirhie et al, [32].

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= $\frac{\text{Absolute weight of organ}}{\text{g}} \times \frac{100\text{g}}{}$

Final body weight 1

Sperm function analysis

Sperm Count: Sperm count was measured as described by [33]. In brief, the cauda epididymis was immersed in 2ml of normal saline and pre-warmed to 37^0c 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 [34] Mild pressure was exerted on the vas defenens 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.* [35] was used to asses sperm viability. Twenty microliter (20µl) of sperm suspension was stained with 20µl of 0.05% eosin Ynigrosin 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 [36]. 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 10minules 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 evacuated in triplicate using rat FSH ELISA Kits cat No. E.El-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.

Evaluation of testicular level of lipid of peroxidation

Malondialdehyde (MDA): The concentration of MDA in testicular homogenate was evaluated using Ohkawa et al method [37] as also described by Chatterjee et al [38] 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 telsticular homogenate was determined by the method of Armstrong and Al- Awadi [39] using commercial reagents. Malomdialdehyde 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.

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 [40] which is based on the ability to inhibit the reduction of nitro tetrazolium-blue and using commercialy 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, 300uL of 2uM riboflavin and 50nN phosphate buffer, pH 7.8) and the activity read spectrophotometrically at 560nm

Catalase (CAT): This was evaluated as described by Chandran et al [41] and is based on an enzyme-catalyzed decomposition of H_2O_2 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 Luschese et al [42] using hydrogen peroxide as a substrate.

Total antioxidant capacity: The TAC was assayed using the method described by Koracevic et al [43]. 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 substrstes.

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 [44] as used by Aksu et al [45] 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.

Statistical analysis

The data were presented as mean ± 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±9.92, -15.2±5.45, 12.6±2.30 and 7.8±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±0.19) group was significantly lower ($p<0.05$) than that of the control (3.56±0.21). It was however significantly higher ($p<0.05$) in the STC30-only (3.16±0.23) and Cd+STC30 (2.48±0.13) than the Cd-only group as shown in table 2. The epididymal weight was significantly reduced in Cd-only (1.14±0.15) compared with 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, absolute and relative weights of testes and epididymis

Group	Initial body weight (g)	Final body weight (g)	body weight change (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 ±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

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) compared with the control (55.60±4.57) but significantly higher in the STC30-only (62.60±3.73) and Cd+STC30 than in the Cd-only groups ($p<0.05$) as shown in FTable 2.

Sperm motility: Sperm motility (%) was significantly decreased in Cd-only (52.20±3.90) and Cd+STC30 (71.20±3.11) compared with the control (72.20±3.11) but significantly higher($p<0.05$) in the STC30-only (84.40±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±6.38) compared with the control (70.20±4.49) but significantly higher ($p<0.05$) in the STC 30-only (79.60±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±3.27) compared with the control (9.60±2.41) but significantly lower ($p<0.05$) in the Cd+STC30 (13.60±2.70) than in the Cd-only groups as shown in Table 2.

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 ±2.77a	18.80 ±1.79a	79.60 ±4.16a	8.40 ±2.30a
Cd+STC30	47.34 ±3.88	71.20 ±3.11a,b	39.60 ±4.39a,b	18.40 ±3.91	13.20 ±2.77*, b	70.80 ±4.27a,b	13.60 ±2.70a,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 male reproductive hormones

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

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±0.62) and Cd+STC30 (4.96± 0.53) than in the STC30-only (7.40±0.62), and in the Cd+STC30 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.

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 control
a = $p<0.05$ vs Cadmium
b = $p<0.05$ vs STC30

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±0.59) compared with the control (2.88±0.25), but significantly lower ($p<0.05$) in the Cd+STC30 (4.2¥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±0.63) compared with control (1.76±0.32), but significantly lower ($p<0.05$) in the STC30-only (5.52±0.59) than in the Cd-only group as in Table 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±0.38) compared with control (7.62±0.06) but higher ($p<0.05$) in the STC30-only (12.34±1.2) and Cd+STC30 (9.1±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 (μ/mg protein) of GPx was significantly decreased ($p<0.05$) in the Cd-only (1.02±0.16) and Cd+STC30 group ($p<0.05$) compared with the control (4.08¥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±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±1.19) and Cd+STC30 (71.99±1.85) groups compared with the control (77.79±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±4.60) compared with control (171.82±5.50) to be higher ($p<0.05$) in the Cd+STC30 (165.9±4.06) and STC30-only (191.22±3.09) than Cd-only groups as shown in Table 4

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 ±0.30[a]	1.84 ±0.32[a]	12.34 1.22*[,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 ±0.26[a,b]	71.99 ±1.85[a,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

Testicular morphometric parameters

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

Leydig cell count: The Laydig cell count (cells/ITR) was significantly reduced ($p<0.05$) in the Cd-only (1.88±0.25) compared with the control (4.46±0.41) but increased ($p<0.05$) in the STC30-only (4.12±0.20) and Cd+STC30 (3.30±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±0.30) and Cd+STC30 (6.10±0.58) compared with control (9.32±0.38) though higher ($p<0.05$) in STC30-only (9.22±0.41) and Cd+STC30 than Cd-only groups as shown in Table 5.

Seminiferous tubules diameter: The seminiferous tubules diameter (μm) was significantly reduced ($p<0.05$) in the Cd-only (97.79±3.98) and Cd+STC30 (118.94±3.83) compared with the control (130.30±3.16) but significantly higher in the STC30-only (138.49±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 (μm) was significantly decreased ($p<0.05$) in the Cd-only (17.46±2.74) and Cd+STC30 (22.99±2.90) compared with the control (36.63±2.78) but higher ($p<0.05$) in the STC30-only (33.98±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

	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 ±0.29[a]	4.12 ±0.20[a]	9.22 ±0.41[a]	138.49 ±2.28[a]	33.98 ±2.30[a]
Cd+STC30	6.54 ±0.63*,[a,b]	3.30 ±0.51[a,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/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 shaps. 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 intestitium 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.

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

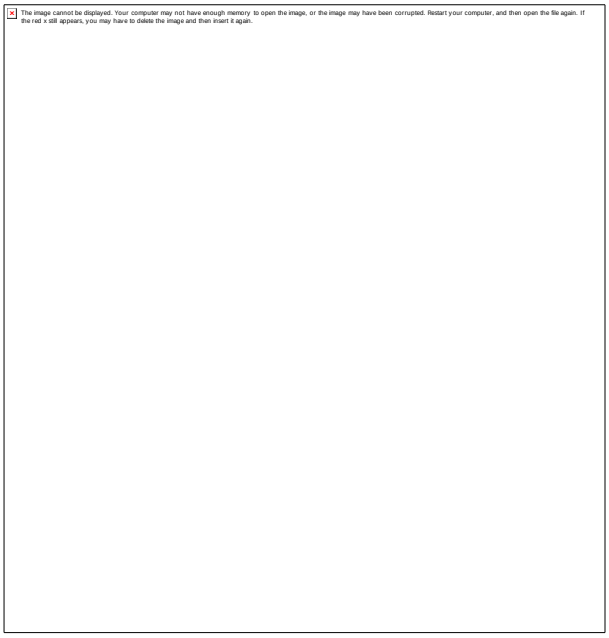
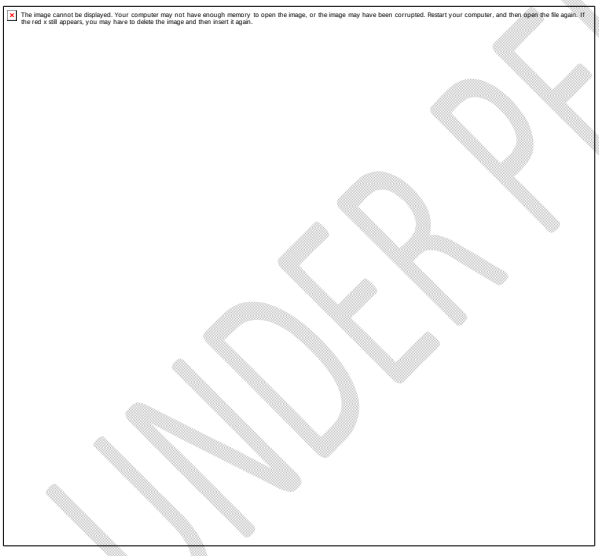


PLATE 2a

PLATE 2



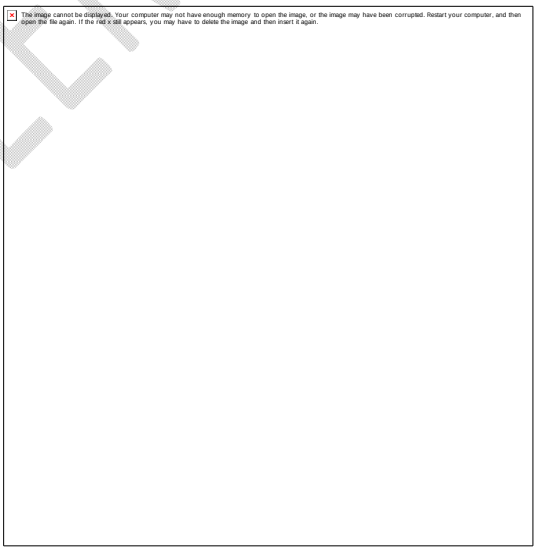


PLATE 2c

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.

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 [34,46]. This effect could be attributed to the cytotoxicity of Cd which results in the breakdown of many cells and tissues [10,47]. The positive weight changes that were noticed following the coadministration of Cd and STC30 suggests that STC30 antagonizeos 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 [43] 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-adminstration 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.

Adminstration of Cd significantly reduced spem motility compared with the control, an observation similar to that of previous studies [42] 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 teratozootospermia 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 dismorphology induced by Cd but not to a normal level. The teratozotospermia 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 teratozotospermia 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 [48]. The decrease in the serum levels that were observed in this study might have been due to Cd-induced pituitary toxicity [43] 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 spern count seen in this study [45]. The administration of STC30 together with Cd improved the FSH and LH concentrations though still less tha functin in the control suggesting that the effects of STC30 may not completely restore tissueon 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 [49]. Its reduction in serum in the Cd-only group is similar to the findings by [50] 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. Malondialdyhyde and TBARS are final products of lipid peroxidation in tissues [50]. 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 [47]. 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 [51]

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 it's stores of antioxidants [52]. 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 [53]. 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 quantity the characteristics of sperm cell and the spermatogenic apparatis[44]. 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 [54] 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-omly 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 interstutium with few Leydig cells. However, following co-administration of Cd with STC30, the section showed improvements like closely pack 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.

Ethical Approval

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

Availability of datasets

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

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.

REFERENCES

1. Practice Committee of the American Society for Reproduction medicine. Definition of infertility and recurrent pregnancy loss. Fertility and Sterility 2008;90(5suppl):560
2. Leslie SW, Soon-Sutton TL, Khan MAB. Male infertility. 2023. StalPearls Treasure Island (FL)
3. vander Borght M and Wyns C. Fertility and Infertility: Definition and epidemiology. Clinical Biochemistry. 2018;62:2-10.
4. Kumar N and Singh AK. Trends of male factor infertility, an important cause of infertility: A review of literature. Journal of Human Reproductive Sciences. 2015;8(4)191
5. Uadia PO and Emokpe AM. Male Infertility in Nigeria: A neglected reproductive health issue requiring attention. Journal of basic and Clinical Reproductive Sciences 2015;4(2):45-52
6. Ashuri T and Princhevski A. Witnessing as a field. In Media Witnessing Testimony in the age of mass communication. 2009; Palgrave Macmillan, UK.
7. Auger J, Kunstmann JM, Zyglik F, Jouannet P. Decline in sperm quality among fertile men in Paris during the past 20 years. New England Journal of Medicine 1995;332:281-5
8. Sengupta P. Environmental and occupational exposure of metals and their roles in male reproductive functions. Drug and Chemical Toxicology 2013;36:353-68
9. Takiguchi M and Yoshihara S. New aspects of Cadmium as endocrine disruptor. Environmental Physiology and Toxicology 2006;13(2):107-116.
10. Unsal V, Dalkiran T, Gock, Kolukc U. The role of natural antioxidants against reactive oxygen species produced by cadmium toxicity: a review. Advanced Pharmaceutical Bulletin. 2020;10(2))184-202.
11. Pappas RS. Toxic elements in Tobacco and in cigarette smoke: Inflammation and sensitization. Metallomics 2011;3(11)1181-1198
12. Jarup I, Berglund M, ELinder CG, Nordberg G and Vanter G. Health effects of cadmium exposure – a review of the literature and a risk estimate. Scandinavian Journal of Work, Environmental and Health. 1998;24(1):1-51
13. Godt J, Scheidig F, Grosse-siestrup C, Esche V, Brandenburg P, Reich A, Groneberg DA. The toxicity of Cadmium and resulting hazards for human health. Journal of Occupational Medicine and Toxicology 2006;1:22. doi:10.1186/1745-6673-1-22

14. El-Neweshy MS, EL-Maddawy ZK, EL-Sayed YS. Therapeutic effect of date palm (phoenix dactyllfera L) on Cadmium-induced testicular toxicity. Androcologia. 2012;1-10. Doi:10:1111/and.12025
15. Abarikwu SO, Olufemi PD, Lawrence CJ Wekere FC, Ochulor AC and Barckuma AM. Rutin, an antioxidant flavonoid induces glutathione and glutathione peroxidase activities to protect against ethanol effects in Cadmium-induced oxidative stress in the testis of adult rats. Andrologia 2017;49(7) e 12696.
16. Aribo EO, Uquetan SU, Phillip AE, Uduak AO. Ameliorative effect of Quercetin and Omega-3 Fatty Acids on Haematological and Liver function Impairment induced by Mosquito Coil smoke in male Wistar Rats. *Nigerian Journal of Parasitology*. 2024, 25(1)126-134
17. Aribo EO, Sunday VE, Udokang NE and Urom SE. Selenium and Omega-3 Fatty Acids Ameliorate Highly Active Anti-Retroviral Therapy (HAART)-induced Reproductive Impairment in Male Wistar Rats. *Nigerian journal of Physiological Sciences.* 2023, 38(June 2023)29-35
18. Neerghen-Bhujum, V.S., Munogee, N. and Coolen, V. Antioxidant and antiinflammatory efficacies of Polyherbal formulations and elixirs traditionally used in Mauritius against rheumatoid arthritis. Journal of Herbal Medicine. 2013;4(1). DOI:10.1016/j.hermed.2013.11.002
19. Zhai Q, Narbad A. and Chen W. Dietary strategies for the treatment of Cadnium and Lead toxicity. Nutrients 2015;7 (1): 552-571.
20. Richie JP, Nichenametla S, Neidig W, Calcagnoito A, Haley JS, Schell AD and Muscat JE. Randomized controlled trial of oral glutathione supplementation on body stores of glutathione. *Europian Journal of Nutrition* 2015;54(2) 251-263
21. Ekpo JI and Johnson JT. Effect of Gonoderma lucidum, astaxanthin, Liv-52HB and STC30 on C-reactive protein concentrations of animal model with CCl_4-induced hepatocellular carcinoma. Chemical and Pharmaceutics Research 2021;3(1) 1-5
22. Erhirhie EO, Okafor NJ, Nwafor CM, Agaegbo OC, and James OO. Toxicological evaluation of popular Polyherbal Remedy – STC30 on wistar rats. *Iranian Journal of Toxicology* 2023;17(3) 1-9.
23. www.superlifeworldph.com.
24. Barnes MJ, Perry BG, Hurst RD, and Lomiwes D. Authrocyanin-rich New-Zealand Black currant extract supports the maintenance of forearm blood flow during prolonged sedentary setting. *Frontiers in Nutrition* 2020;7:74.

25. Song G, Shen X, Wang F, Li Y and Zheng X. Black currant anthocyanins improve lipid metabolism and modulate gut microbiota in hgh-fat diet-induced obese mice. *Molecular Nutrition and Food Research* 2021;65(6) 2001090
26. Gaspar DP, Lechtenberg M and Hansel A. Quantity assurance of bilberry fruits (vaccinnium myrtillus) and billbery-containing dietary supplements. *Journal of Agricultural and Food Chemist* 2021;69:2213-2225
27. Manchali SKN, Chidanelsera MV, and Patil BS. Nutritional composition and health benefits of botanical types of melon - (*Cacumis melo L*). Plants 2021;10(9): 1755
28. Da-Costa R, Botana D, Pinero S, Proverbio F, and Martin R. Cadmium inhibits motility activities of plasma membrane Ca^{2+}- ATPase and axonemal dymen-ATPase of human spermatozoa-Andrologia. 2016;48(4) 646-9.
29. Nna VU, Ujah GA, Mohamed M, Etim KB, Igba BO, Augustine ER and Osim EE. Cadmium chloride-induced testicular toxicity in male wistar rats: prophylactic effect of quercetin and assessment of testicular recovery following cadmium chloride withdrawal. Biomedicine and Pharmacotherapy 2017;94:109-123
30. Nair, A.B and Jacob, S.A. Simple Practice guide for dose conversion between animals and human. Journal of Basic and Clinical Pharmacy. 2016;7(2):27-31
31. Lorke DA. A new approach to practical acute toxicity testing. Archieves of Toxicology 1983;54: 275-287.
32. Erhirhie EO, Ihekewreme CP and Ilodigwe EE. Advances in acute toxicity testing: strength weakness and regulatory acceptance. Interdisciplinary Toxicology 2018;11(1): 5-12.
33. Raji, Y., Oloyo, A.K. and Morakinyo, A.O. Studies on the Reproductive Activities of Ricimus communis seeds in male albino Wistar Rats. Asian Journal of Andrology. 2006;8:115-121
34. Nna VU, Bakar A, Ahmad A and Mohamed M. Down-regulation of steroidogenesis-related genes and its accompanying fertility decline in streptozotocin-induced diabetic male rats: effect of metformin Andrology, 2019, 7(1) 110=123.
35. Wyrobek, A.J., Gordon, L.A., Buckhart, J.G., Francis, M.W., Kapp, R.W., Letz, G and Wharton, M.D. HB and STC30 on renal function parameters of animal models ‘with CCl4- induced hepatocellular carcinoma. Journal of Advances in Medicine and Medical Research.1983; 33(21):175-182
36. Narayana, K., Prashanthi, N., Nayanatava, A., Kumar, H.H., Abhilash, K and Bairy, K. L. Effects of methyl parathion (0.0-dimethyl 0.4- nitro phenyl phosphorothioate) on rat sperm morphology and sperm count but not fertility are

associated with decreased ascorbic acid levels in testis. Mutation Research. 2005;588:28-34

37. Ohkawa, H., Ohishi, N. and Yagi, K. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. Annals of Biochemistry. 1979;95:351-358
38. Chatterjee PK, Cuzzocrea S, Brown PA, Zacharowski K, Stewart KN, Mota-Filipe H, Thiememann C. Tempol, a membrane-permeable radical scavenger, reduces oxidant stress-mediated renal dysfunction and injury in the rat. Kidney International Journal 2000;58:658-673.
39. Armstrong D and Al-Awadi F. Lipid peroxidation and retinopathy in streptozotocin induced diabetes. Free Radical Biology and Medicine. 1991;11:433-
40. Sun, Y., Overly,L.W. and Li, Y. A simple method for Clinical Assay of Superoxide dismutase. Clinical Chemistry. 1988;34:497-500
41. Chandran G, Sirajudeen K, Yusoff N, Syamimi N, Swamy M, Samarendra MS. Effect of the antihypertensive drug enalapril on oxidative stress markers and antioxidant enzymes in kidney of spontaneously hypertensive rats. Oxidative Medicine and Cellular Longevity 2014;1-10. http://doi.10://55/2014/608512
42. Luschese C, Printon S, Nogueira CW. Brains and Lungs of rats are differently affected by cigarette smoke exposure: antioxidant effect of an organoselenum compound. Pharmacological Research 2009;59:194-201.
43. Koracevic D, Koracevic G, Djondjevic V, Andrejevic and Cosic V. Method for the measurement of antioxidant activity in Human Fluids. Journal of Clinical Pathology. 2001;54:356-361.
44. Johnsen SG. Testicular biopsy serone count – a method for registration of spermatogenesis in human testis: Normal values and results in 335 hypogonadal males. Hormones 1970;1:2-25.
45. Aksu EH, Kandemir FM, Omur AD, Ozkaraka M, Kukukler S and Comakli S. Rutin ameliorates Gsplatin-induced reproductive damage via suppression of oxidative stress and apoptosis in adult male rats. Andrologia 2017;49(1) e12593
46. Olaniyan OT, Ojewale AO, Eweoya OO, Adedoyin AA, Adesanya OO, Adeoye AO, Okeniran OS. Modulatory role of vitamin E proton pump (ATPase) activity of Cadmium chloride-induced testicular damage I in wistar rats. BioMedical Research International. 2021 http://doi.org//.10.1155/2021/4615384.
47. Aitken RJ and Curry BJ. Redox regulation of human sperm functions: from the physiological control of capacitation to the etiology of infertility and DNA damage in the germ line. Antioxidant Redox Signal. 2011;14(3): 367-381.

48. Guyton AC and Hall JE. Guyton and Hall Textbook of Medical Physiology, 2011;W.B. Saunders Philadelphia PA.
49. Chen P, Zirrkin BR, and Chen H. Stain Leydig cells in the adult testis: characterization, regulations and potential Applications. Endocrine Reviews 2020;4(1): 22-32.
50. Almeer RS, Soliman D, Kassab RB, Al-Basher G, Alarifi S, Alkahtanic S, Ali D, Metwally D and Abdel Moneim AE. Royal gelly abrogates Cadnium-induced oxidative challenge in mouse testis: Involvement of the Nrf_2 pathway. *International Journal of Molecular Sciences* 2018;19(12) 3979.
51. Draper HH, and Hadley M. A review on recent studies on the metabolism of exogenous and endogenous malondialdehyde. Xenobiotica. 1990;20(9) 901-907.
52. Aprioku JS. Pharmacology of free radicals and the impact of reactive oxygen species on the testis. Journal of Reproduction in Fertility. 2013;14:158-172.
53. Zini A and Schlegel PN Catalase mRNA expression in the male rat reproductive tract. Journal of Andrology. 1996;17(5):473-480.
54. Yang S, Chen S, Liang C, Shi Y, Chen Q. Effects of Cadmium exposure on Leydig cells and blood vessles in mouse testis. International Journal of Environmental Research and Public Health 2022;19(4): 2416.