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Full length Research Article

A Polyherbal Remedy, PurXcel improves Cadmium-induced Male Reproductive Impairment and Testicular Antioxidant Status in Wistar Rats

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Summary: Cadmium, despite being an environmental pollutant has a wide range of applications and causes oxidative damage to the testes and impairment of male reproductive function. PurXcel, a polyherbal remedy is said to be rich in antioxidants and improves fertility. But there are no scientific records of its effect on Cadmium-induced male reproductive impairment, hence this study. Twenty male wistar rats were randomly assigned into 4 groups of 5 rats each namely control, Cadmium-only, PurXcel-only and Cadnium+PurXcel groups. Daily treatment with PurXcel lasted for 28 days after which blood samples were collected and testes and epididymis harvested for evaluation of relevant parameters. Body weight changes (BWC) as well as weights of testes and epididymis were significantly reduced in Cadmium-only group ($P<0.05$) compared with the control. PurXcel given alone and in combination with Cadmium significantly increased ($P<0.05$) the BWC as well as testicular and epididymal weights in comparison with the Cadmium-only group. Sperm function indices (count, motility, viability and normal morphology) and reproductive hormones (GnRH, FSH, LH and testosterone) activities were significantly decreased ($P<0.05$) in the Cadmium-only group compared with the control but higher in all treated groups ($P<0.05$) compared with Cadmium-only group. Testicular concentrations of MDA and TBARS were significantly increased in the Cadmium-only group compared with control ($P<0.05$) but reduced in treated groups ($P<0.05$) when compared with Cadmium–only group. The activities of testicular SOD, GPx and Catalase as well as total antioxidant capacity were significantly reduced in the Cadmium-only group compared with control ($P<0.05$) but increased in the treated groups compared with Cadmium-only group ($P<0.05$). Testicular morphometric parameters showed decreases in Sertoli cell count, Leydig cell count, Johnson's score, seminiferous tubules diameter and germinal epithelial height ($P<0.05$) in the Cadmium-only group compared with the control but these were significantly higher in the Cadmium+PurXcel than in the Cadmium-only groups ($P<0.05$). A section of the testes also revealed mainly empty luminal cavities and scanty intervening interstitium in the Cadmium-only group compared with the control. There were loosely packed epididymis and which were mainly empty. We conclude that PurXcel improves Cadmium-induced male reproductive toxicity and given alone, it improves testicular antioxidant status in Wistar Rats.

Keywords*: PurXcel, Cadmium, reproductive parameters, male rats, oxidative stress*

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INTRODUCTION

Infertility, defined by the failure of a couple to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse is a disease of the male or female reproductive system (WHO, 2023, Araoye, 2003). Infertility affects millions of people, having economic and social implications. The WHO (2015) estimates that 1 in 6 people of reproductive age, experience infertility in their life time. Fifteen percent (15%) of all couples in the United States of America and about 180 million couples worldwide are said to have infertility issues with 40 – 50% of the cases attributed to male factor (Brugh and Lipshutz, 2004). Male infertility is regarded as the inability of a male to make a fertile female pregnant after 12 months of regular unprotected intercourse (Leslie *et al.*, 2023; Huang *et al*., 2023). About 42.4% of infertility cases is attributed to the male factor in Nigeria (Ikechebulu *et al*., 2003).

Over the past decades, a downward trend in the quality and quantity of male fertility indices has been observed. Sengupta *et al* (2017) and Levine *et al* (2023) reported a decline in sperm count among men. A downward trend in fertility as demonstrated by decreases in morphologically normal sperms, motility and count as well as a decrease in seminal fluid volume among males over the past decades was also reported by Carlsenal *et al* (1992) and Sengupta *et al* (2012).

Causes of male infertility or sub-fertility vary widely but can be related to congenital anatomical factors, testicular dysfunction, endocrinopathies as well as lifestyle and gonadotoxic exposures (Agarwal *et al*., 2020, Eisenberg *et al*., 2023). Research activities in the recent past have drawn the world's attention to the negative effects of environmental toxins or pollutants on male reproductive health. One of such environmental toxins which also has a wide range of application is Cadmium (Chandel and Gyan, 2014; Angelis *et al*, 2017, Predes *et al*., 2010).

Cadmium (Cd) is one of the most toxic industrial and agriculture-associated heavy metasl that has been widely dispersed in the range of applications (Unsal *et al*, 2020; WHO, 2019). It is used as a coating material in PVC and ship building industry. Cadmium products are also used in the production of plastics, battery, petrochemicals, cigarette, glass, ceramics, rubber, paint, fireworks etc. (WHO, 2019). Exposure to Cd is often through industrial and non-industrial links through drinking water and food affected by contamination by these products. Humans are also exposed to Cd through inhalation of dust or air that have been contaminated by industrial activities and vehicle exhaust fumes as well as exposure to cigarette smoke (Pappas, 2011; WHO, 2019; IPCS, 1992)

Exposure to Cadmium has been reported to have detrimental effects on various organs and systems including the cardiovascular, hepatic, respiratory, renal, bone and reproductive systems (Godt, *et al*, 2006, Gore *et al.*, 2015, Gu *et al*., 2022). Exposure to Cadmium has also been associated with derangement in seminal fluid parameters (Abarikwu *et al*.., 2016; Olanijan *et al.*, 2022; Yang *et al.*, 2022) as well as levels of testosterone, follicle stimulating hormone and luteinizing hormone (Almeer *et al*., 2018; Alkhedaide *et al*., 2016; Olaniyam *et al*., 2022) among others. The pathophysiology of Cadmium-induced cytotoxicity is partly blamed on oxidative stress, which is proven by the ameliorating effects of different antioxidants on Cadmium-induced cytotoxicities (El-Neweshy *et al*, 2012; Abarikwu *et al* 2016).

PurXcel is a plant-based polyherbal dietary supplement that works using the tri-active technology to improve the body's antioxidant defense system (Lobo *et al*., 2010; Lewis *et al.*, 2013) PurXcel is said to contain 18 bioactive substances which are believed to work synergistically to affect health. These bioactive substances include glutathione, superoxide dismutase, Aloe vera, vitamin C, vitamin E, Selenium, N-acetyl L-cysteine, alpha lipoic acid, *Morringa oleifera*, turmeric, broccoli, *Cordyceps simensis*, milk thistle, blue berry, schisandra, grape seed extract, pomegranate and black pepper extract (https://livepure.com) which have antioxidant and anti-inflammatory activities (Forman *et al.*, 2009; Pickspiteteller, 2007; Dean *et al*., 2011). PurXcel is said to provide nutritional support to the body's antioxidant system (Richie *et al.*, 2015; Carillon *et al*., 2014) and improves reproductive health (Lobo *et al.*, 2011). Though PurXcel is said to contain a wide range of bioactive substances individually known to have pro-health effects, there is paucity of information on the effect of this product on male reproductive parameters either given alone or in Cadmium-induced male reproductive toxicity and hence this study.

MATERIALS AND METHODS

Preparation of Stock Solution of PurXcel: The content of one capsule (435mg) of PurXcel (Live Pure, Frisco, Texas, USA) purchased from Puregen African Nigeria Limited, Lagos, Nigeria 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-Aldriech, Chemical Company, St Louis, MO, USA) in 50ml of distilled water.

Acute toxicity study: The median lethal dose LD_{50} of PurXcel was estimated using Lorke's method (Lorke, 1983) and followed up by the up and down method as described by Erhirhie *et al*, 2018).

Experimental design: Twenty male wistar rats were randomly divided into four groups (n=5) namely control, PurXcel-only, Cadmium-only and Cadmium+PurXcel. Cadmium (as Cadmium Chloride) was administered at 5mg/kg (Da-Costa et al., 2016; Nna et al., 2017). PurXcel was administered daily for twenty eight days at an oral dose of 38.4mg/kg based on the computation of effective dose described by Nair and Jacob (2016). The control group was given 0.5ml of the vehicle daily. All animals were given rat feeds and water ad libitum. Duration of experimentation was twenty eight days.

Collection of samples: At the end of the treatment period, the animals were anaesthetized, blood samples collected from them via cardiac puncture into plain bottles for determination of relevant serum parameters. The animals were then sacrificed and their testes dissected out for assay of necessary parameters.

Determination of body, testes and epididymal weights: The weights of the animals, their testes and epididymis were measuared with an electronic weighing scale (Scout Pro, Ohaus Corporation, USA) while the relative organ weight was computed as Absolute weight of organ/final body weight x 100/1.

Preparation of testicular homogenate: The left testis of each rat was homogenized separately in 50mm Tris-HCl buffer (pH 7.4) containing 1.15% KCl to prepare a 20% (1/5 w/v) tissue homogenate using Potter Elvelyem homogenizer (BEE International, Apion Company, USA). The homogenate was then centrifuged at 10,000g for 10 minutes in a cold centrifuge (4^{o}C) (YSCF0424AR, Multipurpose centrifuge, Guanghzhou, China). The supernatant was then obtained for determination of necessary testicular parameters.

Evaluation of Sperm Count: The method of Raji *et al* (2006) was used to determine sperm count. In brief, the harvested left cauda epididymis was put in 2ml of normal saline and prewarmed to 37^{o}C. Small incisions were then made in the cauda epididymis and released sperms suspended in normal saline. Two hundred microliter (200µL) of the sperm suspension was transferred into both chambers of the improved Neubauer hemocytometer (Hawksley, England) using a Pasteur pipette. The sperms

were counted in five large Thorma squares with the help of a microscope (Leica DM 750, Switzerland).

Sperm viability: The method of Wyrobek *et al.,* (1983) was used to assess sperm viability. In brief 20µl of sperm suspension was stained with 20µL of 0.05% eosin Y-nigrosine and the mixture incubated for 120 seconds at room temperature. The slides were then viewed under microscopy (Leica DM 750, Switzerland) using X400 magnification. Viable sperms were unstained while non-viable ones stained pink.

Sperm motility: This was done using the Makler's counting chamber as demonstrated by Nna *et al*., (2019). A sperm sample obtained from the vas deferens was introduced into 1ml of normal saline and the mixture stirred gently. A drop of the mixture was then dropped on the Makler's chamber (Self Medical Instruments, Israel) and examined microscopically, counting the cells and expressing it as percentage of the total number of spermatozoa.

Determination of sperm morphology: The method described by Narayana et al., (2005) was used to evaluate this parameter. A drop of epididymal sperm suspension used for sperm count was smeared on a glass slide and stained with 1% eosin Y. The slide was air-dried and examined microscopically (Leica DM 750, Switzerland) at X100 magnification.

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 lipid peroxidation

Malondialdehyde (MDA): The concentration of MDA in testicular homogenate was evaluated using Ohkawa *et al* method (Ohkawa et al, 1979) as also described by Chatterjee *et al* (2000) using commercially available reagents. In brief, a 100ml aliquot of testicular homogenate was added to the reaction mixture that contained 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 substances (TBARS): The level of TBARS in telsticular homogenate was determined as described by Armstrong *et al* (1991) 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* (1988) and used by Al-Batran *et al*, (2013) which is based on the ability to inhibit the reduction of nitro tetrazolium-blue and using commercialy available reagents (Sigma-Aldrich, St Louis, USA). 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): Calatase activity was evaluated in testicular homogenate by the method described by Chandran *et al* (2014) using commercially available reagents (Sigma-Aldrich St Louis USA).The method is based on enzyme-catalysed decomposition of H_2O_2 which forms a yellowish complex with molybdate and the absorbance read at 405nm.

Glutathione peroxidase (GPx): This was evaluated in testicular homogenate as described by Luchese *et al* (2009) using H_2O_2 as a substrate.

Total antioxidant capacity (TAC): The TAC was assayed using the method described by Koracevic *et al* (2001). 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 computered. Sertoli cells were counted in 20 seminiferous tubules. Johnsen score was assessed in 10 seminiferous tubules (Johnsen, 1970) as used by Aksu *et al* (2017). 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

Results were presented as mean ± SEM and analysed using statistical package for Social Sciences (SPSS) version 20. One-way analysis of variance (ANOVA) was employed to analyse the data and Tukey Post hoc test used to compare the mean values. Values of $P<0.05$ were considered statistically significant.

RESULTS

Acute toxicity study: There were no mortality or significant behavioural changes up to a dose of 5000mg/kg. The LD_{50} of PurXcel was therefore greater than 5000mg/kg.

Body Weights in different groups: There was significant reduction (P<0.05) in body weight in the Cadmium-only (15.2±5.42) compared with control (28±9.92) groups but higher body weights in the PurXcel-only (25.4±7.23) and Cd+PurXcel (5.6±1.14) than in the Cadmium-only group (P<0.05). This is shown in table 1.

Testes and epididymal weights: The absolute/relative weights of the testes were significantly reduced in the Cadmium-only (1.98±0.19/ 0.92±0.10)) group compared with control (3.56±0.16/ 1.37±0.06) group (P<0.05) but significantly higher in the PurXcel-only (3.52±0.15/ 1.37±0.04) and Cd+PurXcel (2.36±0.17/ 1.00±0.07) than in the Cadmium-only (1.98±0.19/ 0.92±0.10) groups (P<0.05). The absolute/relative weights of the epididymis were significantly reduced (P<0.05) in the Cadmium-only (1.4±0.15/ 0.53±0.06) compared with control (1.5±0.16/ 0.58±0.05) but significantly higher in the PurXcel-only (1.5±0.17/ 0.60±0.06) than in the Cadmium-only (1.4±0.15/ 0.53±0.06) group (P<0.05). These results are shown in Table 1.

Comparison of sperm parameters in different groups

Sperm count: The mean±SEM sperm count ($\times 10^6$/L) of the control, Cadmium–only, PurXcel-only and Cadmium+PurXcel groups were 55.60± 4.57, 29.92±3.47; 62.60± 3.73 and 46.70±4.35 respectively. Sperm count was significantly decreased in the Cadmium-only and Cd+PurXcel compared with control groups (P<0.05) and significantly higher in the PurXcel-only compared with control and Cadmium-only groups (P<0.05). It was also signfiicnalty higher in the Cadmium+PurXcel than in the Cadmium-only groups (P<0.05). These result are shown in Table 2.

Sperm motility: Sperm motility (%) was significantly reduced (P<0.05) in the Cd-only (52.20±3.90) and Cd+PurXcel (67.20 ± 3.12) groups compared with control (76.20±3.11) though significantly higher (P <0.05) in the Cd+PurXcel (67.30±3.12) group compared with control (76.20 ± 3.11) and higher in the PurXcel–only (86.20± 5.12) compared with control (P<0.05). It was also significantly lower in Cd+PurXcel than PurXcel–only groups (P<0.05) as shown in Table 2.

Sperm viability: Sperm viability (%) was significantly reduced (P<0.05) in Cadmium–only (50.80 ± 6.28) compared with control (70.20±4.49) groups. It was however significantly higher (P<0.05) in the PurXcel-only (77.80± 7.36) and Cd+PurXcel (71.40±6.88) than in the Cd-only groups (P<0.05). This is shown in Table 2.

Sperm morphology: The percentage of morphologically abnormal sperms was significantly higher in the Cd–only (28.20±3.27) than in the control (9.60±2.41) and significantly reduced (P<0.05) in the PurXcel–only (7.60±3.05) compared with Cadmium-only (P<0.05) groups. It was significantly higher in the Cd+PurXcel (16.40±4.16) than in the PurXcel–only groups (P<0.05). This is shown in Table 2

Testicular oxidative stress biomarkers

Malomdialdehyde concentration: Testicular concentrations of MDA (mmol/mg protein) were 2.88±0.25, 9.66±0.59, 2.92±0.23 and 4.40±0.44 for control, Cd-only, PurXcel-only and Cd+PurXcel groups respectively. Malondialdehyde was significantly increased in Cd-only and Cd+PurXcel compared with control (P<0.05) but significantly lower in PurXcel-only and Cd+PurXcel than Cd-only groups (P<0.05). It was however higher in the Cd+PurXcel than in the PurXcel–only groups (P<0.05) as shown in Table 3.

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
PurXcel	230.80±6.98	256.20±5.26[a]	25.40±7.23[a]	3.52±0.15[a]	1.37±0.04[a]	1.54±0.17[a]	0.60±0.06
Cadmium + PurXcel	231.40±2.70	237.00±2.74*[ab]	5.60±1.14*[ab]	2.36±0.17*[ab]	1.00±0.07*[a]	1.32±0.13	0.56±0.05

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

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*
PurXcel	62.603.73[a]	86.205.12*[a]	44.605.50*[a]	23.601.67[a]	18.001.58*[a]	77.807.36[a]	7.603.05[a]
Cadmium+ PurXcel	46.704.35*[ab]	67.205.63*[ab]	36.402.41[ab]	15.603.44[b]	15.202.39*	71.406.88[a]	16.4016*[ab]

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

Testicular TBARS: Activities of TBARS (nmol/mg protein) for control, Cd–only, PurXcel–only and Cd+PurXcel groups were 1.76±0.32, 11.02±0.64, 1.98±0.65 and 5.52±0.59 respectively. The activities of TBARS was significantly increased in Cd–only and Cd–PurXcel compared with control (P<0.05) but lower in PurXcel–only and Cd+PurXcel than Cd- only groups (P<0.05). It was however significantly higher in the Cd+PurXcel than in the PurXcel-only groups as shown in Table 3.

Superoxide dismutase (SOD) acivity: The activities (IU/mg protein) of SOD in the control, Cd–only, PurXcel–only and Cd+PurXcel groups were 7.62±0.67, 3.04±0.38, 11.1±1.16 and 8.14±0.84 respectively. Superoxide dismutase was significantly reduced (P<0.05) in Cd–only but higher (P<0.05) in the PurXcel groups compared with control. It was significantly higher in PurXcel–only and Cd+PurXcel than Cd–only group though significantly lower in Cd+PurXcel than PurXcel-only groups as shown in Table 3.

Glutathione peroxidase activity (GPx): The activities of GPx (IU/mg protein) were 4.08±0.39, 1.02±0.16, 6.74±0.34 and 3.24±0.46 for control, Cd–only, PurXcel–only and Cd+PurXcel respectively. The activity of GPx was significantly reduced (P<0.05) in the Cd–only, and Cd+PurXcel (P< 0.05), but increased in PurXcel-only (P < 0.05) groups compared with control. It was significantly higher in PurXcel–only and Cd+PurXcel (P < 0.05) than Cd–only groups though lower in the Cd+PurXcel compared with Cd–only groups as shown in Table 3.

Catalase (CAT) activity: The activities of CAT were 77.7±2.20, 49.82±1.19, 82.03±2.48 and 70.98±2.20 for control, Cd–only, PurXcel–only and Cd+PurXcel groups respectively. The activity was significantly reduced in the Cd–only, and Cd+PurXcel (P< 0.05), but higher in PurXcel-only (P < 0.05) groups than the control. It was higher in PurXcel–only and Cd+PurXcel (P < 0.05) than Cd–only groups (P<0.05) but lower in Cd+PurXcel than in the PurXcel–only (P<0.05) groups as shown in Table 3.

Total antioxidant capacity (TAC): The TAC (nmol uric acid Eq/mg protein) in control, Cd–only, PurXcel-only and Cd-PurXcel were 171.82±5.46,95.82±4.60, 194.46±4.16 and 165.54±4.59 respectively. It was significantly reduced (P<0.05) in the Cd–only but increased (P< 0.05) in PurXcel-only groups compared with the control. TAC was significantly higher in PurXcel–only and Cd+PurXcel groups (P< 0.05) than Cd–only groups though significantly lower (P< 0.05) in the Cd+PurXcel than PurXcel-only groups as shown in Table 3

Histology of testes and epididymis

Johnsen scores: The Johnsen score for control, Cd–only, PurXcel-only and Cd+PurXCel groups were 8.72 ± 0.49, 3.67 ± 0.70, 9.22 ± 0.33 and 6.68 ± 1.16 respectively. It was significantly decreased (P<0.05) in Cd–only and Cd+PurXcel groups compared with control but higher in the PurXcel–only and Cd+PurXcel groups than in the Cd-only group. It was lower in the Cd+PurXcel than in the PurXcel-only group as in Table 4.

Leydig cell count: Leydig cell count (cells/ITR) in the control, Cd–only, PurXcel–only and Cd+PurXcel groups was 4.40 ± 0.41, 2.76 ± 0.30, 4.24 ± 0.24 and 3.66 ± 0.38 respectively. There was significant decrease in Leydig cells in Cd-only group compared with control (P < 0.05) but significantly increased in the PurXcel–only and PurXcel+Cd groups (P< 0.05) compared with the control as shown in Table 4.

Sertoli cell count: Sertoli cell counts (cells/STR) in the control, Cd-only, PurXcel–only and Cd+PurXcel were 9.32 ± 0.38, 2.76 ± 0.30, 9.14 ± 0.78 and 6.28 ± 0.55 respectively. Sertoli cell count was significantly reduced in the Cd–only and Cd+PurXcel groups compared with the control (P< 0.05) but significantly higher (P< 0.05) in the PurXcel–only and Cd+PurXcel than Cd-only groups. It was also significantly lower in Cd+PurXcel than in the PurXcel–only (P< 0.05) groups as shown in Table 4.

Seminiferous tubule diameter: The tubular diameter (µm) in the control, Cd–only, PurXcel–only and Cd+PurXcel were (30.30 ± 3.36, 97.79 ± 3.98, 136.87 ± 3.00 and 120.45 ± 3.34 respectively. It was significantly decreased in Cd–only and Cd+PurXcel (P < 0.05) compared with the control but higher in PurXcel–only and Cd+PurXcel than in the Cd–only groups (P< 0.05). It was also significantly lower in Cd+PurXcel than in the PurXcel–only (P< 0.05) groups as shown in Table 4.

Germinal epithelial thickness: Germinal epithelial thickness (µm) in control, Cd–only, PurXcel–only and Cd+PurXcel groups were 36.63 ± 2.78, 17.46 ± 2.74, 32.62 ± 2.69 and 25.98 ± 3.48 respectively. It was significantly decreased in Cd–only and Cd+PurXcel groups compared with control (P< 0.05) though significantly higher in PurXcel–only and Cd+PurXcel than PurXcel–only groups. It was also significantly lower in Cd+PurXcel than in the PurXcel–only groups as shown in Table 4.

Table 3:
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*
PurXcel	2.92±0.23[a]	1.98±0.47[a]	11.10±1.16*[a]	6.74±0.34*[a]	82.03±2.48*[a]	194.46±4.16*[a]
Cadmium + PurXcel	4.40±0.44*[ab]	5.52±0.59*[ab]	8.14±0.82[ab]	3.24±0.46*[ab]	70.98±1.75[ab]	165.54±4.59*[ab]

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

Table 4:
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*
PurXcel	9.22±0.33[a]	4.24±0.24[a]	9.14±0.78[a]	136.87±3.00[a]	32.62±2.69[a]
Cadmium + PurXcel	6.68±1.16*[ab]	3.66±0.38[a]	6.280. ±55*[ab]	120.45±3.34*[ab]	25.98±3.48*[ab]

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

Male reproductive hormones

Serum gonadotropin-releasing hormone (GnRH): The mean ± SEM serum concentrations of GnRH (Pg/ml) were 2.32±0.32, 1.18±0.15, 3.80±0.84 and 2.34±0.38 for control, Cadmium-only, PurXcel–only and Cd+PurXcel respectively. The result shows a significant decrease (P<0.05) of GnRH in the Cd-only compared with the control groups and increased in the PurXcel–only group compared with control. GnRH was significantly higher (P<0.05) in both PurXcel–only and Cd+PurXcel groups compared with Cd-only group, though lower in the Cd+PurXcel than PurXcel–only groups (P<0.05). This is shown in Fig 1.

Serum testosterone: The concentrations of testosterone (ng/ml) were 3.08 ± 0.40, 6.14 ± 0.14, 1.98 ± 0.28, 6.06± 0.21 and 4.24 ± 0.43 for control, Cd-only, PurXcel-only and Cd+PurXcel respectively. Testosterone was significantly reduced in the Cd-only compaà lolred with the control and higher (P<0.05) in the PurXcel–only compared with the control. It was also higher in the PurXcel-only and Cd+PurXcel (P< 0.05) than in the Cd-only groups but lower in the Cd+PurXcel (P<0.05) than in the PurXcel–only groups as shown in Fig. 2

Serum LH: The mean ± SEM of LH (IU/ml) were 5.20 ± 0.45, 2.76 ± 0.11, 6.74 ± 0.29 and 4.12 ± 0.31 for control, Cd-only, PurXcel-only and Cd+PurXcel respectively. Serum LH was significantly reduced in Cd-only and Cd+PurXcel (P<0.05) compared with control but higher (P<0.05) in PurXcel–only than in the control. It was significantly higher in Cd+PurXcel and PurXcel–only (P<0.05) groups than Cadmium-only groups though it was lower in the Cd+PurXcel than PurXcel-only groups as shown in Fig. 3.

Serum follicle stimulating hormone: Serum levels of FSH (ng/ml) were 6.20±0.53, 2.68±0.30, 7.96±0.18 and 5.13±0.36 for control, Cadmium-only, PurXcel-only and Cadmium+PurXcel groups respectively. Serum FSH levels were significantly reduced in the Cadmium-only and Cadmium+PurXcel (P<0.05) compared with the control but higher in the PurXcel-only than in the control groups. It was significantly higher in the PurXcel-only and Cadmium+PurXcel than in the Cadmium-only groups as shown in Fig. 4.

Photomicrographs of sections of the testes and epididymis in different experiment groups: Plate 1a shows a section of the testis in the control group exhibiting numerous seminiferous tubules of various sizes with intact basement membranes, most of the tubules containing numerous 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 Cd–only group showing uniform seminiferous tubules with an intact basement membrane containing spermatogonia at various stages of maturation. The tubules are mostly 3 to 5 cell layers thick with the luminal cavities mainly empty. The intervening interstitium are scanty with 3 – 5 Leydig cells. The Sertoli cells are up to 9 to 10 per tubule.

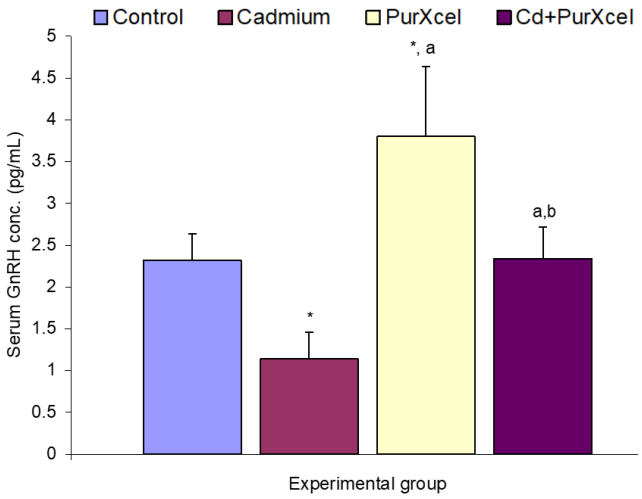

Figure 1
Serum gonadotropin-releasing hormone concentration in the different experimental group.
Values are presented as mean ±SEM, n = 5.
** = p<0.05 vs control; a = p<0.05 vs Cadmium ; b = p<0.05 vs PurXcel*

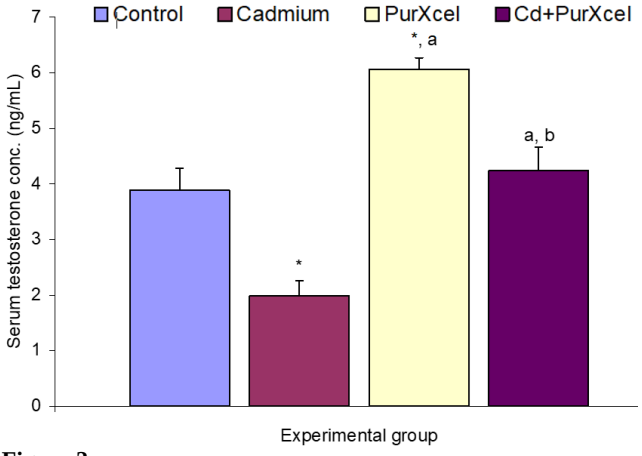

Figure 2
Serum testosterone concentration in the different experimental group.
Values are presented as mean ±SEM, n = 5.
** = p<0.05 vs control; a = p<0.05 vs Cadmium;*
b = p<0.05 vs PurXcel

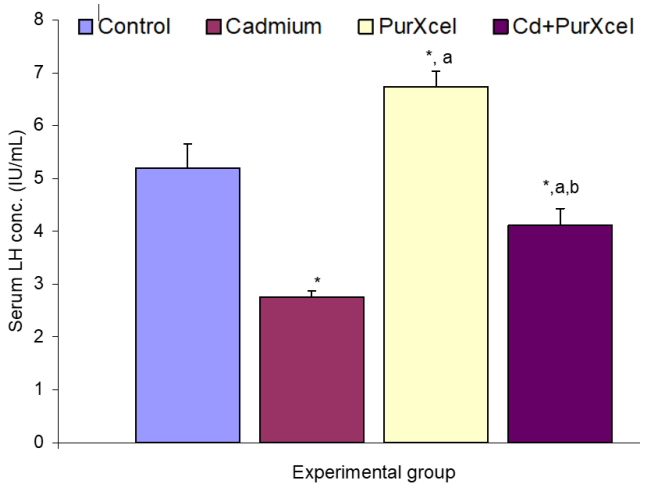

Figure 3
Serum LH concentration in the different experimental group.
Values are presented as mean ±SEM, n = 5.
** = p<0.05 vs control; a = p<0.05 vs Cadmium;*
b = p<0.05 vs PurXcel

Plate 1c is a section of testis in PurXcel–only group showing numerous widely spaced seminiferous tubules with intact basement membrane. The spermatogonic cells are in various stages of maturation. The luminal cavities are filled with spermatozoa.

Plate 1d shows a section of testis in Cd+PurXcel group which shows closely packed seminiferous tubules with intact basement membrane. The tubules contain proliferating spermatogomia at various stages of development. The cells are 3 - 5 cell layers thick consisting of spermatogonia, spermatocytes, and moderate amounts of spermatids and spermatozoa.

Plate 2a is a section of the epididymis in the control group showing prominent tubules separated by a loose stroma with an intact basement membrane. The lumens are filled with spermatozoa.

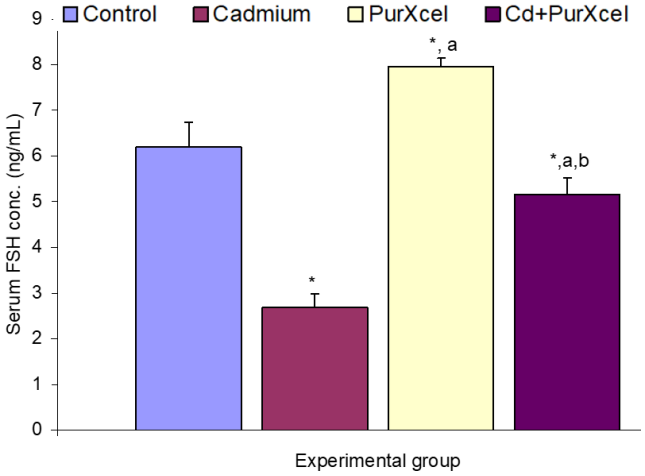

Figure 4
Serum FSH concentration in the different experimental group.
Values are presented as mean ±SEM, n = 5.
** = p<0.05 vs control; a = p<0.05 vs Cadmium;*
b = p<0.05 vs PurXcel

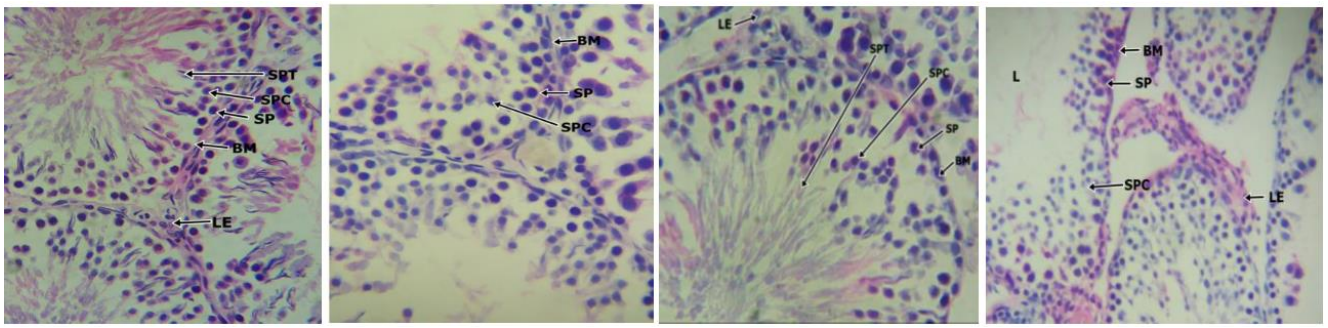

Plate 1:
Section of testis showing seminiferous tubules in a) control group, b) cadmium group, c). PurXcel group and d). Cd + PurXcel group, x400 magnification.
BM-basement membrane, SP-spermatogonia, SPC-spermatocytes, SPT- spermatid, L – Lumen; LE – Luminal endothelium

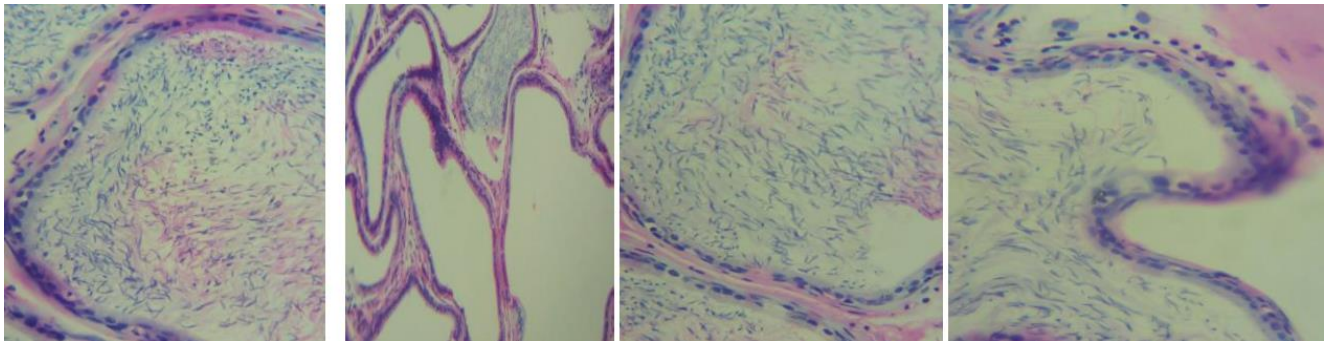

Plate 2:
Section of epididymis in a) control group; b) cadmium group; c) PurXcel group and d) Cd + PurXcel group, x400 magnification
STRO = loose stroma, EPI = lining epithelium, SPZ = spermatozoa

Plate 2b shows a section of epididymis in the Cd–only group and shows loosely packed, fibrotic epididymal tubules with an intact basement membrane. The tubules contain scanty spermatozoa with majority of the tubules being empty.

Plate 2c is a photomicrograph of the section of epididymis in the PurXcel–only group and shows prominent tubules separated by stroma. The tubules are dilated with intact basement membrane. The lumens contain dense spermatozoa.

Plate 2d is a section of epididymis of the Cd+PurXcel groups and shows widely spaced tubules with intact basement membrane and abundant intervening stroma. The tubules contain scanty spermatozoa with majority of the tubules being empty while few contain degenerated spermatozoa.

DISCUSSION

The study investigated the effect of PurXcel which is said to contain antioxidants and improves reproductive health on Cd–induced male reproductive toxicity associated with oxidative stress.

The non-significant differences in the initial body weights of the rats means they were weight-matched. The observed progressive weight loss in the Cadmium-only group is similar with the finding of Nna *et al* (2017) and Olaniyan *et al,* (2021).The decrease in body weight might have been due to the toxic nature of the metal (Godt *et al*., 2006, Aitken and Curry, 2010). The rather increase in body weight that was noted in the PurXcel–only group and Cd+PurXcel compared with Cd–only groups suggests the ability of PurXcel to counter the mechanisms responsible for the weight loss.

Our results show decreased testicular and epididymial weights in the Cd–only group, a finding consistent with that made by El-neweshy *et al* (2012) and Nna *et al.,* (2017) which they attributed to necrosis and degeneration of the testis. PurXcel given alone or in combination with Cd improved these weights likely because of their possible antioxidant effects occasioned by the numerous antioxidant factors it contains. Necrosis and degeneration of tissues are associated with oxidative stress (Choi *et al.*, 2009).

Semen analysis is the cornerstone for male fertility evaluation (WHO, 2003). Therefore, alterations in any of the constituents will impair sperm function leading to infertility. Our study shows derangement in sperm function indices (count motility, viability and morphology) in the Cadmium-only group compared with the control which is in line with the findings made by Asadi *et al* (2014), Oliveira *et al.,* (2009) and Abarikwu *et al.,* (2016). The improvement in these indices following administration of PurXcel alone or in combination with Cadnium shows the ability of PurXcel to improve sperm function indices and to combat the negative effects of Cadnium on sperms. This might have been made possible due to supplemented antioxidants in PurXcel (Richie *et al.,* 2015) snce a major mechanism by which these indices are disrupted is oxidative stress (Abarikwu *et al.*, 2016; El-Neweshy *et al.*, 2012).

Administration of Cadmium resulted in significant decreases in serum GnRH, luteinizing hormone follicle stimulating hormone and testosterone compared with the control producing a hypogonadotropic hypogonadism. This effect on FSH, LH and testosterone secretion is similar to the report by Olaniyan *et al* (2021) and Almeer et al (2018). Cadmium is a known endocrine disruptor (Takiguchi and Yoshihara, 2006). The decrease in serum GnRH could have been due to a primary toxic effect on the GnRH–secreting cells in the hypothalamus.

The reduced levels of FSH and LH might have been due to insufficient stimulation of the gonadotropes in the anterior pituitary by the low GnRH levels (Guyton and Hall, 2011). The low levels of testosterone might have been due to testicular toxicity or the low LH and FSH which are necessary for proper functioning of the testis and secretion of testosterone (Guyton and Hall, 2011). The concentrations of these hormones significantly increased when Cd was co-administered with PurXcel demonstrating the ability of PurXcel to ameliorate the effect of Cd. PurXcel administered alone significantly improved the serum concentration of these hormones compared with the control and the Cadmium+PurXcel groups indicating that PurXcel improves male reproductive hormones function. These effects could be attributed to its rich phytonutrients (Livepure.com).

Oxidative stress is a major mechanism implicatied in Cadmium-induced cytotoxicity (Abarikwu *et al.,* 2016, Takeshima *et al.,* 2021). Our observed increases in MDA and TBARS and decreased levels of GPx, SOD, CAT and TAC in the Cd-only group compared with control supports other studies that Cd induces oxidative stress in tissues (Turner and Lysiak, 2008; Abarikwu *et al.,* 2016). Oxidative stress is evaluated indirectly by measuring the final products of lipid peroxidation (example, MDA and TBARS) and concentration of antioxidants (GPx, SOD and CAT) as well as TAC (Aprioku, 2013, Zim and Schlegel, 1996). Lipid peroxidation (MDA and TBARS) though lower, in the PurXcel-only and the Cd+PurXcel than in the Cd–only groups, it was significantly reduced in the Cd+PurXcel compared with the Cadmium-only group indicating that the ability of PurXcel to relief lipid peroxidation could be limited. Increases in the levels of SOD, GPx CAT and TAC in same groups were observed and supports the fact that PurXcel alone or even if administered with Cd could relief or improve oxidative processes in testicular tissue.

Johnsen score (graded 1 – 10) provides a connection between the results of seminal analysis and those of testicular biopsy/histology and considers the thickness of the germinal epithelium, number and type of sperm cells seen/spermatogenesis as well as presence or absence of Sertoli cells etc (Johnsen, 1970; Gune *et al.,* 2019). The reduced Johnsen score noted in the Cd-only rats compared with the control is similar to the observation by Mohammad *et al.*, (2019) which positively relates with impaired spermatogenesis by Cd as earlier reported. The tubular diameter, Sertoli cells, germinal epithelial height and Leydig cells were also decreased in Cd-only group compared with the control. However, there were improvements in Johnsen score following co-administration of Cd with Purxcel though significantly lower in this group than in the control. This indicates that Purxcel improves Johnsen score indices in Cd-induced testicular toxicity but does not have significant effect on these indices in normal/healthy testes.

A section of the epididymis in the control group (Plate 5) shows prominent tubules separated by loose stroma and an intact basement line. The lining epithelium is columnar with thin lamina propria and muscular layer. The lumens are filled with spermatozoa. In the epididymal section of the Cadmium-only group (Plate 6), the tubules have an intact basement membrane and are loosely packed. The tubules are largely empty containing scanty spermatozoa. Plate 7 (PurXcel-only) is a section of epididymis showing prominent dilated tubules with an intact basement membrane and lumens filled with spermatozoa. The epididymal section of the Cd+Purxcell group (plate 8) shows widely spaced tubules with an intact basement membrane and abundant intervening stroma. The tubules contain scanty spermatozoa and most of them are empty.

Findings from this study indicate that given alone to normal rats, Purxcel does not significantly affect lipid peroxidation (MDA, TBARS) but improves the antioxidative status (SOD, CAT, GPx, TAC) of the testis. In Cadmium-administered rats, PurXcel ameliorates oxidative stress in the rats testes. Purxcel given to normal rats does not affect sperm parameters except motility which it increases. Administered alone, Purxcel increases the serum levels of male reproduction hormones and when given to Cd-administered rats, it improves the levels of these hormones and Johnsen score.

In conclusion, PurXcel ameliorates Cadmium-induced male reproductive toxicity and and oxidative stress and improves sperm motility and testicular redox status when administered alone

Ethical Approval: *The ethical approval for this study was obtained from the Animal Research Ethics Committee of the Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria (Approval number 256PHY2103).*

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