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Impact of Cannabidiol Oil and Prednisolone on Sex Hormones, Oxidative Stress Markers, Lipid Profile, and Testes Histology on Cadmium Induced Toxicity in Male Wistar Rats

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Abstract
This study investigated the impact of CBD oil and prednisone on reproductive indices and testicular oxidative stress markers in cadmium-induced toxicity in male Wistar rats. Forty rats weighing between 150g to 200g were assigned into 8 (1-8) five rats per group. Group 1 served as control, groups 2-8 received prednisolone(1mg/kg) bw; cadmium (1.5mg/kg); prednisolone (1mg/kg) +CBD-oil (0.2mg/kg); CBD-oil (0.2mg/kg) +cadmium (2mg/kg); prednisolone (3mg/kg) +cadmium (2mg/kg); CBD-oil(0.1mg/kg) and CBD-oil(0.2mg/kg) respectively. The administration was done using gavage for 14 days. Results revealed that testosterone significantly ($p<0.05$) increased in the prednisolone group than in the control group. However, all treated groups significantly decreased than the prednisolone group. Luteinizing hormone significantly increased in treated groups than in control. Follicle-stimulating hormone significantly increased in treated groups except the prednisolone group than in the control group. Total cholesterol significantly decreased across groups than control. Triglyceride significantly increased in the treated groups than in the control. HDL-c significantly increased in CBD-oil (0.1ml) and (0.2ml) groups than control. LDL-c was significantly reduced in the treated groups than in the control. Catalase revealed non-significant differences amongst groups than control. Superoxide dismutase significantly increased in prednisolone, pred+cadmium, CBD-oil (0.1ml), and CBD-oil (0.2ml) groups than control. Malondialdehyde significantly decreased in all treated groups than control. Glutathione peroxidase was significantly reduced in cadmium, pred+CBD-oil, CBD-oil (0.1ml) and CBD-oil (0.2ml) groups than control. Glutathione was significantly reduced in cadmium, pred+CBD-oil, CBD-oil (0.1ml), and CBD-oil (0.2ml) groups than control. Histology of testes revealed interstitial tissue fibrosis, numerous immature spermatozoa, vacuolization, and degeneration of Sertoli cells especially in prednisolone, pred+CBD-oil, CBD-oil (0.1ml), and CBD oil (0.2ml) groups. We conclude that CBD oil, prednisolone, and cadmium administration at different doses induced biochemical alterations, altered male reproductive hormones, and cytoarchitecture of testes. Therefore, if these results are applicable to humans, consumption of CBD oil and prednisolone should be taken with caution as they could alter male reproductive function.
Keywords*:* CBD oil, prednisolone, cadmium, Testosterone, malondialdehyde, catalase
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1. Introduction

In southern Nigeria today, there is increasing use of prednisolone and *cannabis* products like cannabidiol (CBD) oil as a remedy in the management of some illnesses such as obstructive respiratory disorder, depression, and insomnia associated with hypertension, nausea, and vomiting in cancer chemotherapy, anorexia and cachexia in HIV/AIDS patients, neuropathic pain, allergy, inflammation and spasticity in multiple sclerosis (Pollmann & Feneberg 2008). This has raised questions regarding their possible physiologic adverse effects basically on male reproduction and fertility. Cannabidiol (CBD) is the major non-psychoactive component of *Cannabis sativa* (Watson *et al.* 2000). It has been reported that CBD which is an isomer of tetrahydrocannabinol (THC) acts as a balancing force to regulate the strength of the psychoactive agent (THC) and also regulates the body's metabolism of THC by inactivating cytochrome p450 which is an enzyme that metabolizes drugs (Watson *et al.* 2000). Cannabinoid receptors (CB1 and CB2) have been found to be among the G protein-coupled receptor family (Pertwee 1997). The receptor is found to be present on the cell membrane of both intracellular and extracellular

cell membranes (Pertwee 1997). CB1 receptors have been found to be more abundant in the brain while CB2 receptors are structurally different and are mostly found only on cells of the immune system, more prevalent in B-cells, natural killer cells, and monocyte, but may be found on polymorphonuclear neutrophil cells, T8 cells and T4 cells (Pertwee 1997).

Studies have implicated *Cannabis* in male infertility due to its ability to alter the endocrine system (Mobisson *et al.* 2018; Mobisson *et al.* 2022b). The reproductive deformity caused by *Cannabis* was suggested to be via natural cannabinoid receptors interactions in the hypothalamus (Murphy *et al.* 1998). *Cannabis sativa* was reported in animal models to reduce testosterone concentrations (Symons *et al.* 1976), the size and function of testes (Dixit *et al.* 1977). An irregular cycle of ovulation was reported on marijuana and THC-fed female humans and animals which caused Luteinizing hormone secretion fluctuations (Dalterio 1983). The implication of cannabinoid receptors (CB_1) in the reproductive system confirmed that *Cannabis sativa* may negatively affect the system causing morphologic and physiologic changes in reproduction (Ahrens *et al.* 2009). A computational study conducted by Mobisson *et al.* (2022a) reported that CBD caused an inhibition of the androgen receptor, indicating a possible route through which CBD may prevent prostate cancer.

Prednisolone is a synthetic, anti-inflammatory glucocorticoid derived from cortisone. It is used to treat immunosuppression, rheumatism, dermatologic, allergic, ophthalmic, respiratory, hematologic, neoplastic, edematous, gastrointestinal, and acute exacerbations of multiple sclerosis (Bunte *et al*. 2018). Prednisolone reduces inflammation by suppressing the migration of polymorphonuclear leukocytes and reversing increased capillary permeability. It reduces the volume and activity of the immune system causing immunosuppression (Bunte *et al*. 2018). Tissue injury may occur when cells are under prolonged oxidative stress and inflammation (Biswas 2016). Hence, this study aimed to evaluate the effect of CBD oil and prednisone on reproductive indices and testicular oxidative stress markers in cadmium-induced toxicity in male Wistar rats.

2. Materials and Methods

Drugs: prednisolone used for this study was purchased from Unicure pharmaceutical limited, Lagos, Nigeria. The cadmium chloride was purchased from Sigma-Aldrich Limited Germany with EC number 233-296-7. Cannabidiol (CBD) oil was purchased from TEEMU Premium, California, USA.

2.1 Laboratory Animals

Forty (40) male Wistar rats, weighing between 150–200g were used for this study. The animals were housed in the Department of Physiology animal house, University of Calabar, Nigeria. Standard animal cages (435 x 290 x150) with wood shavings as bedding were used in housing the animals (n=5). They were allowed *ad libitum* access to rat chow and clean water, and exposed to 12/12-hr light/dark cycle. The animals were acclimatized for 7 days. Animals were kept in line with laid down rules for animal care as approved in Helsinki's 1964 declaration. The animal ethics committee of the University of Calabar permitted our research procedure with authorization number 040PHY3719.

2.2 Experimental Design and Administration of Drugs

The animals were randomly assigned differently into eight separate groups (n = 5). After 7 days of acclimatization, CBD oil, prednisolone, and cadmium administration commenced. The drugs were administered via oral route using an orogastric tube (gavage), once, every day, to animals in treatment groups (2 to 8), using doses outlined in Table 1, while the control group received feed and 0.5ml normal saline as a vehicle. Administration of cadmium chloride solution lasted only 2 days. Afterward, administration of CBD oil and prednisolone commenced for fourteen (14) days before the rats were killed and samples collected for analysis.

Table 1: Study Design and Drugs Administration

Groups	No. of rats	Treatment
Group 1(Control)	5	Feed + 0.5ml of normal saline as a vehicle throughout the experimental period.
Group 2	5	1mg/kg bw of prednisolone
Group 3	5	1.5mg/kg bw of Cadmium
Group 4	5	1mg/kg bw of prednisolone + 0.2mg/kg bw of CBD Oil.
Group 5	5	0.2mg/kg bw of CBD oil + 2mg/kg bw of cadmium
Group 6	5	3mg/kg bw of prednisolone + 2mg/kg of cadmium
Group 7	5	0.1mg/kg bw of CBD Oil low dose
Group 8	5	0.2mg/kg bw of CBD oil high dose

2.3. Evaluation of Serum Reproductive Hormones Levels

Blood collection was via ocular puncture, using chloroform anesthesia, a 5 ml syringe and a 21 G needle were used. The blood samples were introduced into plain capped sample bottles and allowed for 2 hours and centrifuged

thereafter at 1,000 rpm for 5 minutes using a bucket centrifuge (B-Bran Scientific and Instrument Company, England). The settled serum was used for this assay. Serum testosterone, luteinizing hormone, and follicle-stimulating hormone levels were determined using a rat-specific ELISA kit technique as was used by Khaki *et al.* (2009); Mobisson *et al.* (2022b). The rat-specific ELISA kits were purchased from Sunlong Biotech Limited, China with reference numbers; SL0668Ra for testosterone, SL0286Ra for FSH, and SL1093Ra for LH.

Principle: The procedure for ELISA depends on the standard of high specificity of antibodies to bind molecules, for this study diverse reproductive hormone is involved. The antibody is marked with an enzyme since the enzyme-labeled antibody reacts with the hormone. The amount of hormone inside the sample is obtained by placing an enzyme substrate that appears as a colored product. The color intensity is relative to the amount of the hormone bound.

2.5 Serum Lipid Profile Assessment

The serum used for this assay was obtained by the already described procedure above. Serum levels of total cholesterol and other lipid fractions were measured using the Randox test as described below.

2.5.1 Estimation of Serum Total Cholesterol Concentration

Plasma total cholesterol (TC) level was determined by the procedure of Sieldel *et al.* (1985).

Principle: Cholesterol esterase catalyzes the hydrolysis of cholesterol esters into free cholesterol and fatty acid. Cholesterol oxidase then catalyzes the oxidation of free cholesterol to cholestene-3-one and hydrogen peroxide. Phenol and 4-amino-antipyrine then unite with the hydrogen peroxide with peroxidase presence to give a red-colored quinonemine and later read with a colorimeter at 540nm. The color intensity obtained is equal to the amount of total cholesterol.

2.5.2 Evaluation of Serum Triglyceride Concentration (TG)

The amount of serum triglyceride in the samples was determined using the method described by Negele *et al.* (1992).

Principle: A lipoprotein lipase hydrolyses triglycerides to glycerol and fatty acid. The glycerol yielded was phosphorylated to glycerol-3-phosphate by glycerol kinase. Glycerol phosphate oxidase afterward oxidizes glycerol-3-phosphate to produce dihydroxyacetone phosphate and hydrogen peroxide. The chromogen consisting of n-ethyl-n-sulphohydroxypropyl-n-foludine was oxidized thereafter. A quinoneinine dye (purple colored) produced from the reactions was interpreted at 540 nm colorimetrically.

2.5.3 Evaluation of High-Density Lipoprotein–Cholesterol (HDL-C)

High-density lipoprotein-cholesterol was determined using Siedel *et al.* (1985) method. Its principle is the same as previously described total cholesterol evaluation.

2.5.4 Evaluation of Low-Density Lipoprotein –Cholesterol Level (LDL-C)

With the Friedewald *et al.* (1972) association, LDL-cholesterol was measured using the difference between serum total cholesterol, the sum of HDL-cholesterol, and Triglyceride as presented below:

$$LDL\text{-}c = TC - (VLDL\text{-}c + HDL\text{-}c)$$

2.6 Assessment of Testicular Oxidative Stress Markers

The left testis of each rat was homogenized using the Potter-Elvehjem homogenizer. Twenty percent (1/5 w/v) of tissue homogenate was prepared in 50 mm Tris–HCl buffer (pH 7.4) with 1.15% potassium chloride and centrifuged at 10,000 rpm at 4°C for 10 minutes using a bucket centrifuge (B-Bran Scientific and Instrument Company, England). The supernatant was collected for catalase (CAT) assay, using hydrogen peroxide as substrate. At 412 nm, reduced glutathione (GSH) was assayed by the method of Lucchese *et al.* (2009). The activity of glutathione peroxidase (GPx) was examined using hydrogen peroxide as a substrate with regards to the Lucchese *et al.* (2009) method. The concentration of malondialdehyde (MDA) was determined in thiobarbituric acid reacting substances (TBARS) as described by Meenakshi, *et al.* (2007); Okhawa, *et al.* (1979). Thereafter, the mixed reaction produced 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid solution matched to pH 3.5 with sodium hydroxide and 1.5 ml of 0.8% water solution of thiobarbituric acid was introduced to 0.2 ml of 10%(w/v) of homogenate. Using distilled water, the mixture was moved up to 4.0 ml and warmed at 95°C for 60 mins. About 1.0 ml of distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15:1 v/v) was introduced and centrifuged at 4000 rpm after tap water cooling. The crude layer was removed and the absorbance was calculated at 532 nm and measured up to with the results obtained from MDA standards. Value levels were measured from absorption values as normal absorption.

2.7 Histological Examination of the Testis

The testis of the control and treated rats were carefully detached, all connective tissues removed, and put into Bouin's solution [0.2% picric acid/2% (v/v) formaldehyde in PBS]. Different segments were obtained and stained with hematoxylin and eosin (H & E) stains. The tiny slides were labeled correctly. With x500 magnifications, the photomicrographs were taken under a light microscope (Leica DM 750, Switzerland).

2.8 Statistical Analysis

All results are presented as mean ± SEM, n=5. One-way analysis of variance (ANOVA) was utilized in comparing the difference within groups, followed by post hoc multiple comparisons. Computer software SPSS version 17.0 and Excel analyzer were used for the analysis. The level of significance was set at $p<0.05$.

3 Results

3.1 Comparison of Mean Serum Reproductive Hormones in Control and Different Experimental Groups.

Figure 1 below showed a significant increase ($p<0.05$) in serum testosterone concentration in rats treated with prednisolone alone compared to control. However, there was a significant ($p<0.05$) decrease in all treated groups compared to the group treated with prednisolone alone. Though, there was a significant reduction in the group treated with pred+CBD oil compared to the group treated with cadmium. Furthermore, rats treated with CBD oil (0.2ml) had a significant increase compared to control. Figure 2 below showed mean serum luteinizing hormone concentration significantly increased in all treated groups compared to the control. Furthermore, groups treated with prednisolone alone, pred+Cd, and CBD oil (0.2ml) were markedly increased compared to control and all other groups. Figure 3 below showed mean serum follicle stimulating hormone concentration was significantly ($p<0.05$) increased in treated groups except group treated with prednisolone alone compared to control. Although, the group treated with CBD oil significantly increased compared to all other groups.

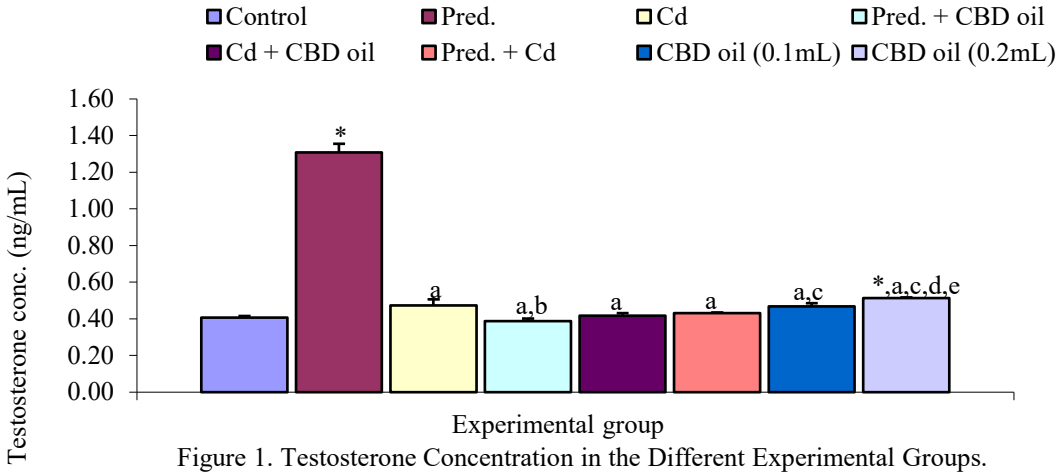

Figure 1. Testosterone Concentration in the Different Experimental Groups.

Values are expressed as mean ±SEM, n = 5.
* = $p<0.05$ vs control;
a = $p<0.05$ vs Pred.
b = $p<0.05$ vs Cd

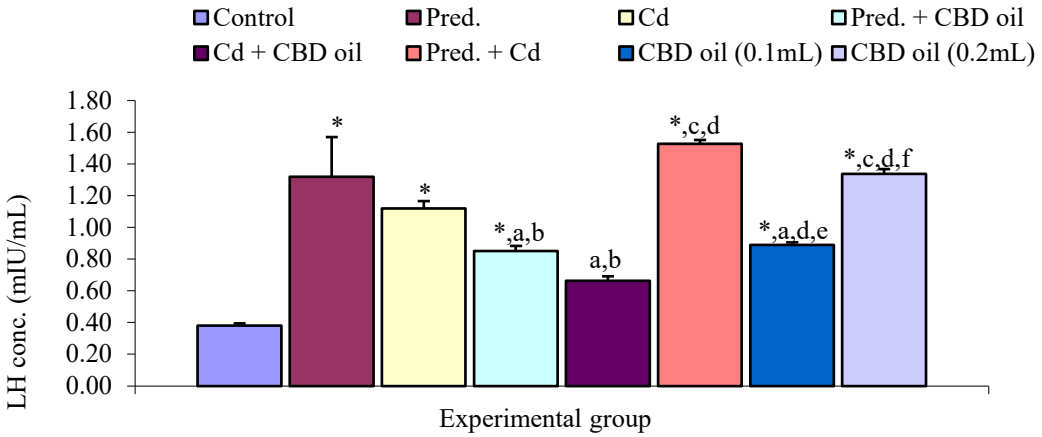

Figure 2. Luteinizing hormone (LH) concentration in the different experimental groups.
Values are expressed as mean ±SEM, n = 5.
* = p<0.05 vs control;
a = p<0.05 vs Pred.
b = p<0.05 vs Cd
c = p<0.05 vs Pred. + CBD oil

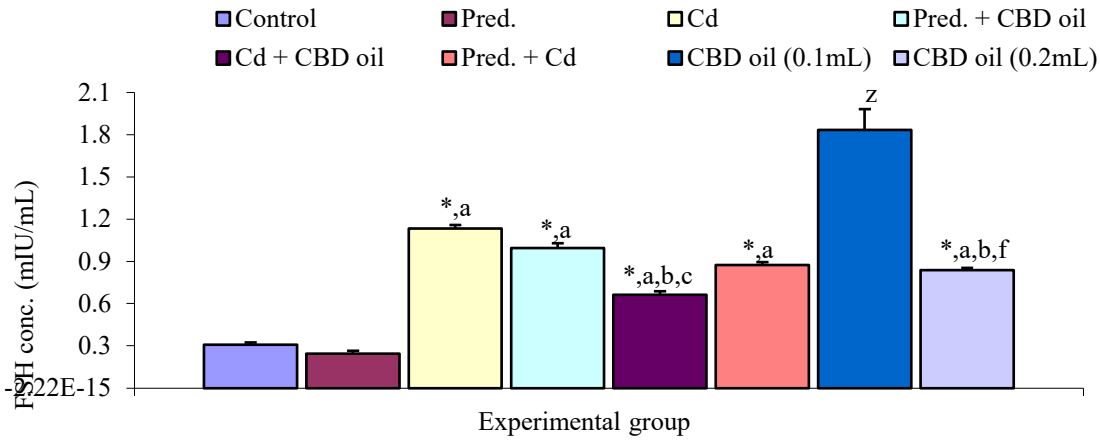

Figure 3. Follicle stimulatiing hormone (FSH) concentration in the different experimental groups.
Values are expressed as mean ±SEM, n = 5.
* = p<0.05 vs control;
a = p<0.05 vs Pred.
b = p<0.05 vs Cd
c = p<0.05 vs Pred. + CBD oil

3.2 Comparison of Lipid Profile Markers in Control and Different Experimental Groups

In Table 2 below, the mean serum total cholesterol (TC) was significantly (p<0.05) reduced in all treated groups compared to control. However, the group treated with cadmium was significantly reduced compared to the prednisolone-treated group. Furthermore, the group treated with cadmium+CBD oil significantly increased compared to prednisolone, cadmium, and pred+CBD oil-treated groups. The mean serum triglyceride (TG) was significantly (p<0.05) increased in pred+CBD oil, cadmium+CBD oil, pred+cadmium, and CBD oil (0.2ml) treated groups compared to control. Though, the groups treated with cadmium+CBD oil and CBD oil (0.2ml) significantly (p<0.05) increased compared to cadmium treated group. The mean serum high-density lipoprotein cholesterol (HDL-c) concentration was significantly (p<0.05) increased in the CBD oil (0.1ml) treated group compared to the control and other treated groups except the CBD oil (0.2ml) treated group. Although, CBD oil

treated group significantly increased compared to all treated groups except CBD oil (0.1ml). Mean serum low-density lipoprotein cholesterol (LDL-c) concentration was significantly ($p<0.05$) decreased in the cadmium and pred+CBD oil-treated group compared to control. The group treated with pred+CBD oil significantly increased compared to the cadmium group. The group treated with cadmium+CBD oil significantly increased compared to prednisolone, cadmium, and pred+CBD oil-treated groups.

Table 2. Comparison of Lipid Profile Markers in Control and Different Experimental Groups

Parameters	Group 1 (Control)	Group 2 prednisolone	Group 3 cadmium	Group 4 pred + CBD oil	Group 5 cadmium + CBD oil	Group 6 pred+ cadmium	Group 7 CBD oil (0.1ml)	Group 8 CBD oil (0.2ml)
TC (mmol/l)	**4.27±0.23**	**3.33±0.28***	**2.33±0.19*, a**	**3.27±0.09*, b**	**4.10±0.26a, b,c**	**3.70±0.12b**	**3.70±0.06b**	**3.87±0.33b**
TG (mmol/l)	**1.53±0.09**	**2.05±0.29**	**1.77±0.06**	**2.15±0.04***	**2.41±0.31*, b**	**2.22±0.18***	**2.06±0.20**	**2.45±0.19*, b**
HDL-c (mmol/l)	**1.22±0.06**	**1.26±0.13**	**1.33±0.18**	**1.29±0.19**	**1.32±0.12**	**1.52±0.06**	**1.72±0.08*, a,b,c,d**	**1.89±0.04y**
LDL-c (mmol/l)	**3.50±0.12**	**3.02±0.29**	**2.10±0.13***	**2.86±0.11*, b**	**3.71±0.21a, b,c**	**3.31±0.10b**	**2.93±0.02b, d**	**3.15±0.33b**

*Values are expressed in mean ± SEM, n = 5. *=represents values with significant differences.*
** = p<0.05 vs control; a = p<0.05 vs Pred.; b = p<0.05 vs Cd; c = p<0.05 vs Pred. + CBD oil;*
d = p<0.05 vs Cd + CBD oil; y= p<0.05 vs all groups except with CBD oil (0.1mL).

3.3 Comparison of Testicular Oxidative Stress Markers in Control and Different Experimental Groups.

Figure 4 below showed the concentration of testicular catalase did not reveal any significant difference statistically among groups compared to control. Although, the group treated with CBD oil (0.2ml) was significantly increased compared to the group treated with Cd+CBD oil. Figure 5 below showed the concentration of testicular superoxide dismutase was significantly ($P<0.05$) increased in groups treated with prednisolone, pred+Cd, CBD oil (0.1ml), and CBD oil (0.2ml) respectively compared to control and other treated groups. However, groups treated with pred+CBD oil and Cd+CBD oil were significantly decreased compared to prednisolone and cadmium groups. Figure 6 below showed the concentration of testicular malondialdehyde was significantly ($P<0.05$) decreased in all treated groups compared to control. Although, groups treated with pred+CBD oil and CBD oil (0.2ml) were significantly increased compared to other treated groups. Figure 7 below showed testicular glutathione peroxidase was significantly reduced in groups treated with cadmium, pred+CBD oil, CBD oil (0.1ml), and CBD oil (0.2ml) compared to control and other treated groups. Although, groups treated with Cd+CBD oil and pred+Cd were significantly increased compared to cadmium and pred+CBD oil-treated groups. Figure 8 below showed testicular reduced glutathione was significantly reduced in groups treated with cadmium, pred+CBD oil, CBD oil (0.1ml), and CBD oil (0.2ml) compared to control and other treated groups. Although, groups treated with Cd+CBD oil and pred+Cd were significantly increased compared to cadmium and pred+CBD oil-treated groups.

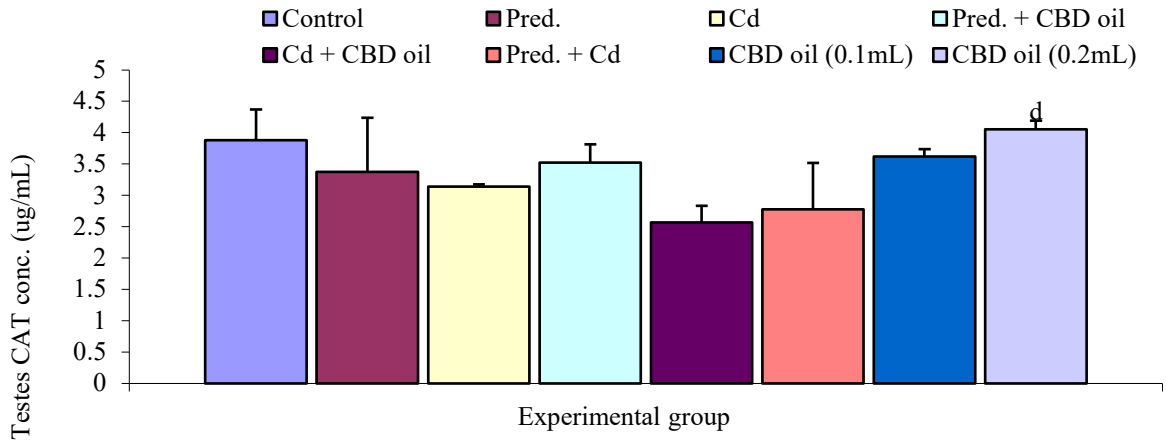

Figure 4. Testicular Catalase (CAT) Concentration in the Different Experimental Groups.

Values are expressed as mean SEM, n = 5.
d = p<0.05 vs Cd + CBD oil

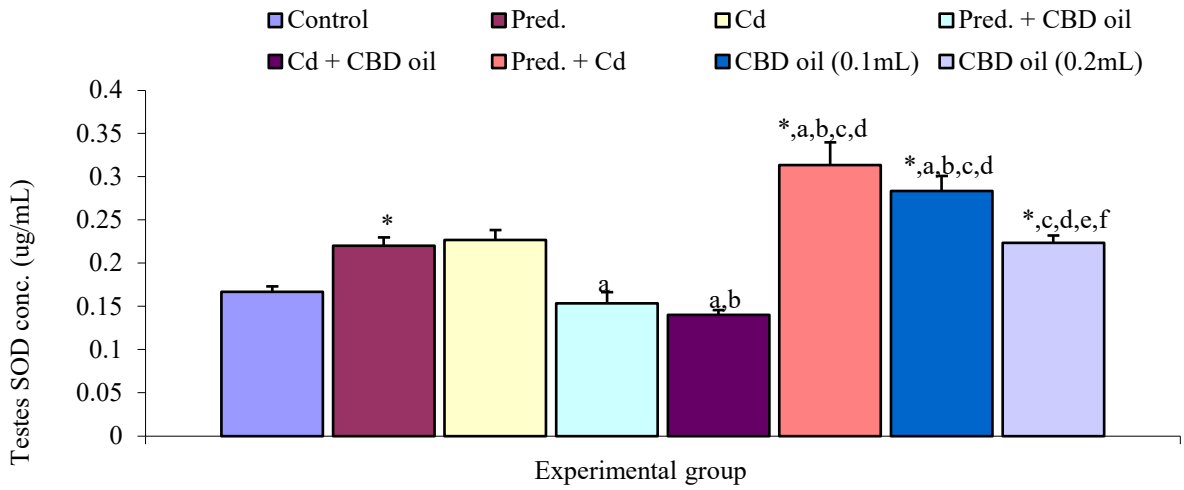

Figure 5. Testicular Superoxide Dismutase (SOD) Concentration in Control and Different Experimental Groups.
Values are expressed as mean ±SEM, n = 5.
* = p<0.05 vs control;
a = p<0.05 vs Pred.
b = p<0.05 vs Cd
c = p<0.05 vs Pred. + CBD oil

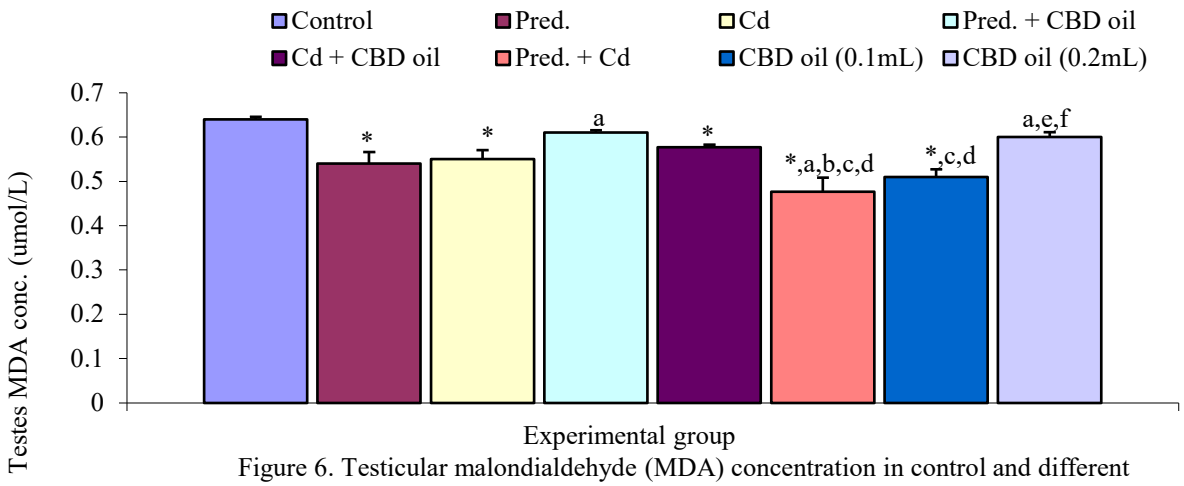

Figure 6. Testicular malondialdehyde (MDA) concentration in control and different experimental groups.
Values are expressed as mean ±SEM, n = 5.
* = p<0.05 vs control;
a = p<0.05 vs Pred.
b = p<0.05 vs Cd
c = p<0.05 vs Pred. + CBD oil

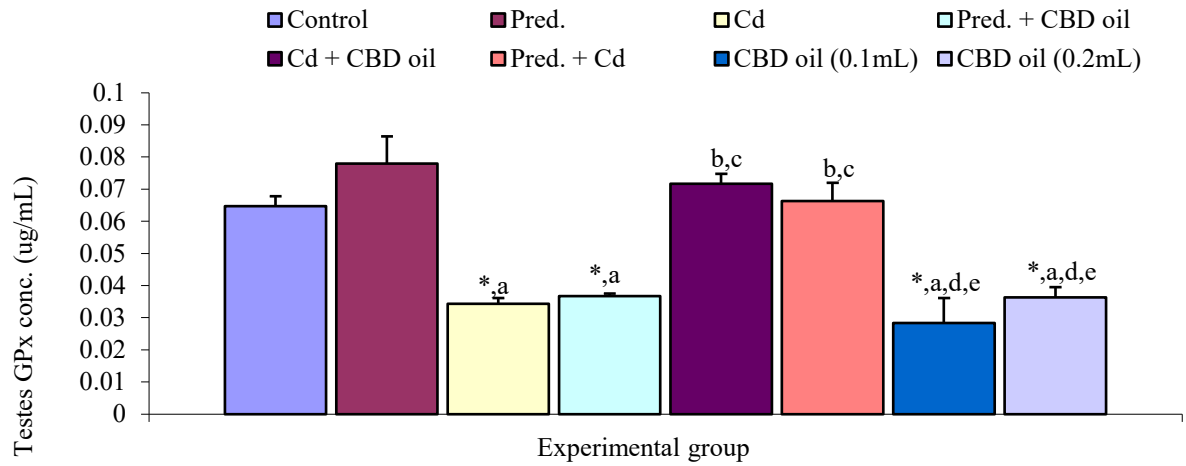

Figure 7. Testicular Glutathion Peroxidase (Gpx) Concentration in Control and Different Experimental Groups.
Values are expressed as mean ±SEM, n = 5.
* = p<0.05 vs control;
a = p<0.05 vs Pred.
b = p<0.05 vs Cd
c = p<0.05 vs Pred. + CBD oil

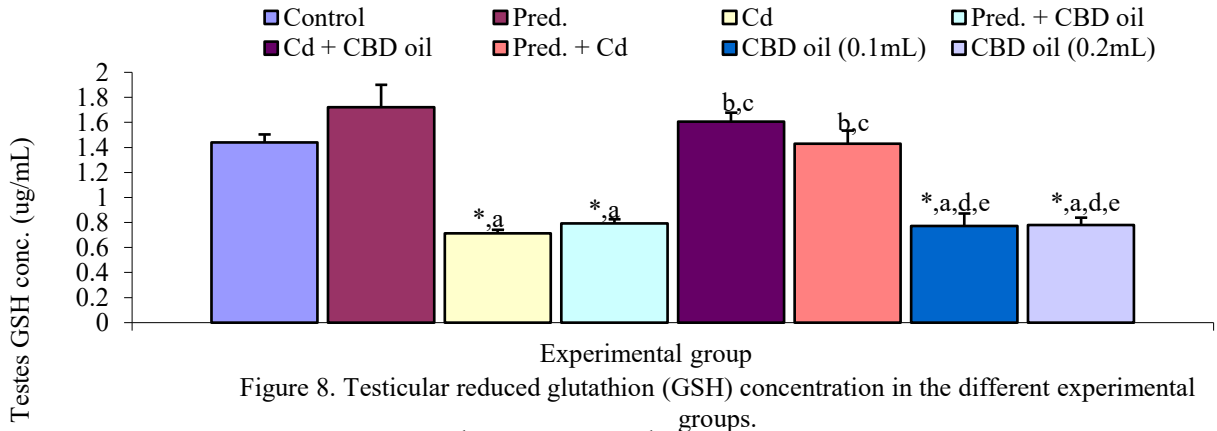

Figure 8. Testicular reduced glutathion (GSH) concentration in the different experimental groups.
Values are expressed as mean ±SEM, n = 5.
* = p<0.05 vs control;
a = p<0.05 vs Pred.

3.4 Histological Examination of Testes in Control and Treated Groups After Drug Administration

Plate 1a below showed a photomicrograph of the control (group 1) with spermatogonia lined by stratified germinal epithelium (SGE) indicating a developmental stage of spermatozoa. There is the presence of mature spermatozoa (MS) in the lumen of seminiferous tubules with interstitial cells of Leydig (ICL) lying in between the seminiferous tubules. Plate 1b (group 2) showed a photomicrograph of the prednisolone group with intact stratified germinal epithelium (SGE) of seminiferous tubules and non-prominent interstitial cells of Leydig (ICL). There are numerous immature spermatozoa (IMS). Fibrosis of interstitial tissue (FIT) is seen. Plate 1c (group 3) showed cadmium treated group with no visible histopathologic change. Plate 1d (group 4) showed a pred+CBD-oil treated group with vacuolization and degeneration of Sertoli cells; clearing of the lumen of seminiferous tubules indicates an impairment of the spermatogenic process. Plate 1e (group 5) showed a cadmium+ CBD oil group with no visible histopathologic change. Plate 1f (group 6) showed pred+cadmium with no visible histopathologic change noticed. Plate 1g (group 7) showed CBD oil (0.1ml) group with disorganized structure of spermatogenic cells and degeneration of mature spermatocytes. No spermatozoid tails in the lumen indicate disruption of spermatogenesis. Plate 1h (group 8) showed CBD oil (0.2ml) group with immature seminiferous tubules.

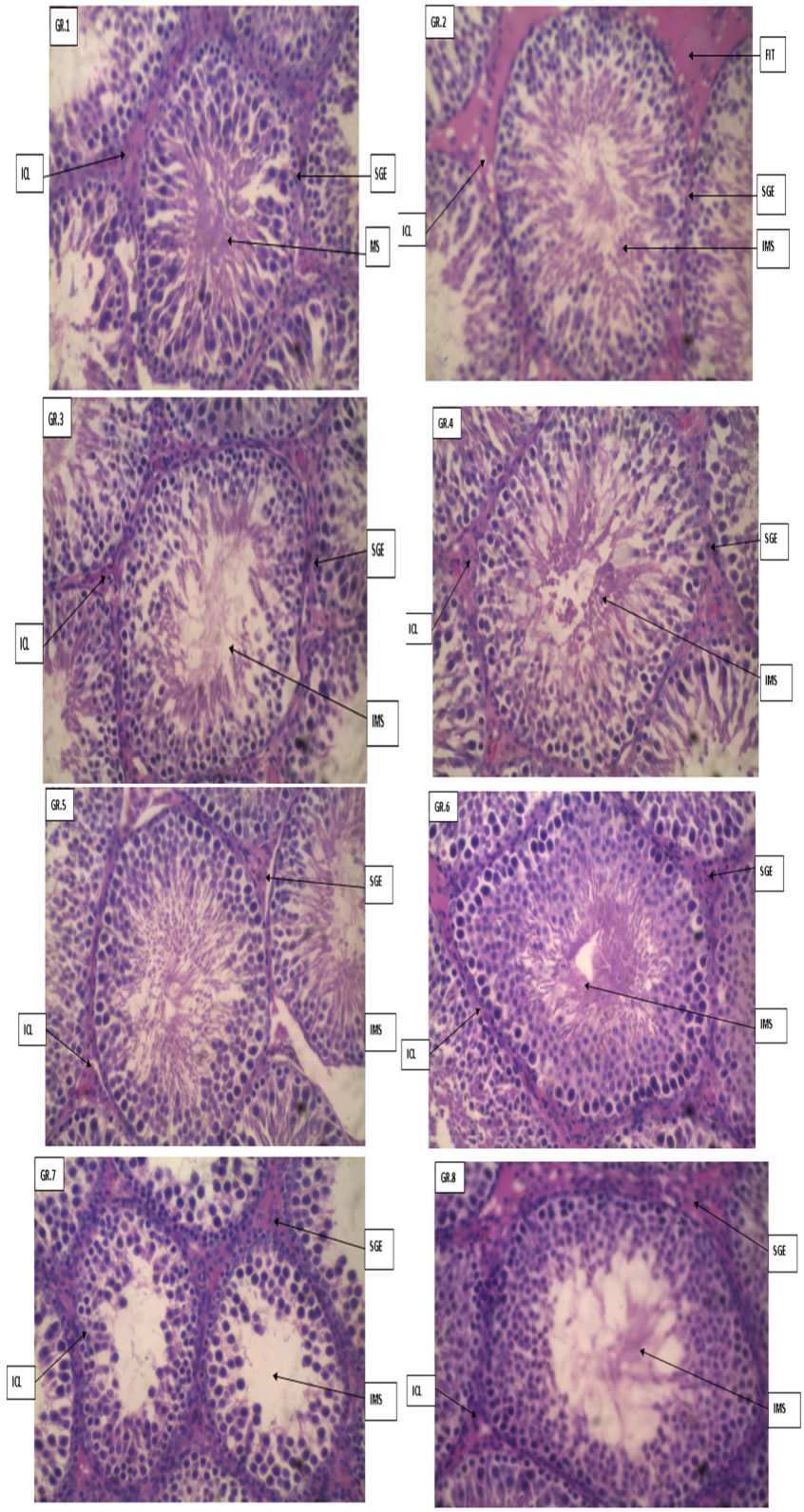

Plate 1 (1 to 8): Photomicrographs of the Testis of Different Experimental Groups After CBD Oil, Prednisone, and Cadmium Administration.
Magnification: X500
Key: ICL= Interstitial Cells of Leydig, SGE= Stratified Germinal Epithelium, MS= Mature Spermatozoa**,** IMS= Immature Spermatozoa, FIT= Fibrosis of Interstitial Tissue

4 Discussions

Presently, there is increasing use of combined cannabidiol oil and prednisolone in Southern Nigeria in the management of Asthma and other respiratory obstructive diseases. Nevertheless, prednisolone is a known anti-inflammatory and auto-immune drug used in the management of inflammatory disorders. Conversely, its combined use with cannabidiol oil has generated questions concerning its likely adverse effects, especially on reproductive function. This study investigated the impact of CBD oil and prednisolone use on cadmium-induced toxicity in male rats. The parameters assessed include male reproductive hormones, lipid profile markers, testicular oxidative stress markers, and histological examination of the testes.

4.1 Evaluation of Serum Male Reproductive Hormone Concentration After Drug Administration

Anterior pituitary hormones such as FSH and LH control testicular functions. FSH controls spermatogenesis whereas LH is important for testosterone production necessary for male reproductive functions (Guyton & Hall 2012). At the end of drug administration, notable changes in serum levels of male sex hormones were observed. The significant increase in serum testosterone in the prednisolone-fed group may possibly be due to the prednisolone administered as it is a steroid that may increase testosterone synthesis in testicular Leydig cells and may also be due to an elevated level of LH in this group. LH is necessary for testosterone production from the interstitial cells of Leydig (Guyton & Hall 2012). However, the decreased FSH level in this group may likely be an indication of excessive spermatogenesis which may have inhibited anterior pituitary FSH synthesis via short loop negative feedback effect. The significant increase in testosterone concentration in the prednisolone-fed group may possibly be linked to a significant increase in serum triglyceride (substrate for testosterone synthesis) as was reported by Yujeong *et al.* (2021). However, the significant decrease in testosterone recorded in other treated groups compared with the prednisolone group could be linked to the dose-dependent effect of drugs administered which could have negatively affected testosterone production and spermatogenesis. Jakubovic *et al.* (1979), reported that excessive consumption of cannabinoids may affect testicular Leydig cells to reduce testosterone production, hence affecting spermatogenesis. The alteration in serum levels of male sex hormones in test rats may possibly be an indication of infertility (Dissanayaka *et al.* 2019).

4.2 Assessment of Serum Lipid Profile After Drug Administration

A significant increase in serum concentration of HDL-c was observed in groups treated with CBD-oil (0.1ml) and (0.2ml) compared to control and other groups may be an indication that CBD-oil posses' cardiovascular beneficial components as reported by Mobisson *et al.* (2019); Mobisson *et al.* (2022b). This is also in line with Hossein *et al.* (2007) who reported that HDL-c concentration was not affected by *Cannabis* seed consumption in guinea pigs. The significant decrease in serum LDL-c and total cholesterol levels across groups compared to control may be linked to decrease testosterone concentration. LDL-c and total cholesterol serve as a substrate for steroid hormone synthesis (Guyton and Hall 2012).

4.3 Evaluation of Testicular Antioxidant Activity After Drug Administration

The alteration in the concentration of testicular antioxidant enzymes (CAT, SOD, GSH, and GPx) observed in treated groups may likely be an indication of oxidative damage. Antioxidant enzymes such as GPx, SOD, and CAT are vital in improving human health by neutralizing cell damage due to free radicals and reactive oxygen species from metabolic reactions and chronic diseases (Skrzydlewska *et al.* 2005). Antioxidants provide protection against oxidative damage to tissues and proteins (Sun 1990). The significant increase in CAT in rats fed with CBD oil (0.2ml) may possibly be linked to the toxic effects of hydrogen peroxide generated by various reactions and environmental agents (Michiels *et al.* 1994). The effectiveness of GSH depends upon its cofactor availability and extent of lipid peroxidation (Pryor *et al.* 1975). Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde (Moore & Robert 1998). An increase in free radicals causes overproduction of MDA (Pryor *et al.* 1975). Therefore, the significant decrease in testicular MDA in treated rats may be attributed to enhanced antioxidant defenses as evident in increased testicular SOD levels. SOD catalyzes the reduction of superoxide anion into hydrogen peroxide, which is subsequently detoxified by CAT and GPx at both intra and extracellular levels. This result is in agreement with Omar *et al.* (2013), which reported decreased MDA in *Cannabis* fed mice, and in contrast with Mobisson *et al.* (2022b), which reported increased testicular MDA in *C. sativa*-fed rats. The significant decrease in the concentration of testicular GSH in groups treated with cadmium, pred+CBD-oil, CBD-oil (0.1ml), and CBD-oil (0.2ml), may likely be due to insufficient availability of selenium for GSH synthesis in the rats and toxic effect of dose dependant drugs administered. Conversely, the significant increase in testicular GSH in groups treated with Cd+CBD-oil and Cd +Pred may be linked to an increasing antioxidant defense mechanism.

The alterations in the concentration of these antioxidant enzymes and decreased testicular MDA in treated rats compared to control may be attributed to the altered cellular architecture of the testis. This is because oxidative stress affects sperm function by altering membrane fluidity, permeability and impairing sperm functional

competence (Collodel *et al.* 2015). Hence, it is of the view that the reproductive toxicities seen in this study may possibly be due to oxidative stress from reduced antioxidant enzymes that could alter the process of spermatogenesis.

4.4 Histological Examination of Testes After Drug Administration.

The pathologic changes observed in testicular photomicrographs of rats treated with prednisolone, pred+CBD-oil, CBD-oil (0.1ml), and CBD oil (0.2ml) (plate 1b, 1d, 1g, and 1h) may likely be due to reduction in the production of sperm cells and damage to seminiferous tubules. Furthermore, the toxic effect of CBD oil and prednisolone could have caused the destruction of testicular Sertoli cells, causing DNA damage and a resultant decline in sperm production as reported by Peiris *et al.* (2019). The numerous immature spermatozoa, vacuolization, and degeneration of Sertoli cells; clearing of the lumen of seminiferous tubules are indications of an impaired spermatogenic process. The damage to testicular tissues particularly the seminiferous tubule in these groups was suggested to be a direct effect of cannabinoids in testes (Galieque *et al.* 1995), via CB1 receptors (Gerard *et al.* 1991). This is in line with a report by Arnab *et al*. (2011) that exposure to *Cannabis* caused an absence of spermatozoa in tubules. This could also be attributed to the decreased spermatogenic function of the testes due to the spermatogenesis-reducing property of *Cannabis* (Mobisson *et al.* 2018). The hormonal changes, histological alterations, and altered antioxidant levels are pointers that prednisolone and CBD oil could possibly induce infertility in male rats.

5 Conclusion

Administration of CBD oil, prednisolone, and cadmium at different doses caused significant changes in male sex hormone (FSH, LH, and Testosterone), lipid profile, testicular antioxidant levels in male rats, and altered cytoarchitecture of testes. These alterations may negatively influence spermatogenesis, testosterone synthesis, and cytoarchitecture of the testes. Thus, if these findings are applicable to humans, caution should be taken in the administration of medical *Cannabis* (CBD) and prednisolone for the treatment of certain illnesses.

Author Contributions

Mobisson Samuel Kelechi and Agona Odeh Obembe designed the study and wrote the study protocol. Mobisson Samuel Kelechi, Imoh Emmanuel Ukoh, James Boohondah Woha, Harris Opusunju Boma, Ibe Fidelis Udochukwu, Monye Justin Bonaparte, and Abaka OtoAbasi Sunday performed laboratory experiments and literature searches. Mobisson Samuel Kelechi drafted the manuscript; Iheanyichukwu Wopara and Onyebuagu Peter Chukwuma worked on data analysis. Iheanyichukwu Wopara performed the statistical analysis. All authors read and approved the final manuscript.

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