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Protective Effects of *Urtica dioca* Against Cyclophoshamide-Induced Testicular Damage and Endocrine Loss in Rat Testis

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Abstract: **In this study, the toxic effect of cyclophosphamide (CYP) on sperm morphology, testicular histology and oxidant–antioxidant balance, and protective roles of *Urtica dioca* (UD) was investigated. For this purpose, 25 healthy, adult, male albino rats were divided into five groups; five animals in each group. The control group was treated with placebo, the disease control was treated with CYP 10mg/kg body weight, the remaining groups were treated with UD 1000mg/kg only, CYP 10mg/kg + UD 500mg/kg and CYP 10mg/kg + UD 1000mg/kg for 14days. At the end of the experimental period, body weight of the animals were checked, all the groups were sacrificed blood samples collected and organs harvested for histopathological analysis after which the enzymatic and non-enzymatic stress biomarkers, hormone profile, inflammatory markers, heamatological indices, hepatic-renal functionality biomarkers, lipid profile levels, morphological abnormalities of sperm and testicular histopathological changes were examined. CYP induced decrease in testicular activities of catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) and glutathione peroxidase (GPx) level, whereas levels of malondialdehyde (MDA) increased markedly and was confirmed by histopathological result. Serum levels of testosterone, FSH and LH were considerably reduced. Co-treatment with UD, ameliorated the biochemical changes in the testis, improved the hormone levels and alleviated the histological injury. CYP-induced lipid peroxidation lead to the structural damages in spermatozoa and testicular tissue of rats, and UD have shown to have a protective effect on these types of damage.**

***Keywords: Urtica dioca*, Cyclophosphamide, Oxidative stress, Testosterone, Antioxidant.**

1. INTRODUCTION

Cyclophosphamide (CYP) is a cancer chemotherapy agent generally utilized in cancer management to enhance the life expectancy of malignant growth patient (Olayinka et al., 2015). Cyclophosphamide, (CYP) is an alkylating agent also used as an immunosuppressant (Rehman et al., 2012). CYP is metabolized by the hepatic cytochrome-P450 enzymes to produce the metabolites-acrolein and phosphoramide (Nafees et a.l., 2012, Oyabemi et al., 2016). Phosphoramide is responsible for the CYP anticancer and immunosuppressive efficacy, while acrolein is associated with toxic effects on healthy cells (Zirak et al., 2019). Despite a restorative impact, the clinical utility of cyclophosphamide (CYP) is restrained because of several adverse effects associated with reproductive toxicity, hepatotoxicity nephrotoxicity, urotoxicity, cardiotoxicity, immunotoxicity, mutagenicity, genotoxicity, carcinogenicity, teratogenicity, and neuronal toxicity (Roy et al., 2014; Kim et al., 2014; Song et al., 2014; Kuhlen et al., 2017). CYP metabolite acrolein builds lipid peroxidation and delivers

exceptionally reactive oxygen species (ROS), experimental evidence implicates acrolein in CYP induced oxidative stress. (Singh and Kumar, 2019, Zirak et al, 2019). Acrolein induces toxicity through excessive generation of ROS capable of damaging DNA and protein adduct formation. These overabundant ROS interface with different cells and cause cellular impairments (Senthilkumar et al., 2006). A number of studies have implicated CYP to induce derangement in spermatogenesis and causes atrophy of seminiferous tubules and testosterone level depletion (Kaur et al., 1997, Masala et al., 1997). Studies have suggested that the reproductive deficits could be triggered by the generation of reactive oxygen species (ROS) and lipid peroxidation in the testis (Sulowska et al., 1998). There is a significant involvement of free radical species and inflammation caused by immune cells in pathways leading to tissue damage (Zeng et al., 2020). By lessening the abundance of ROS, it is conceivable to limit the toxicity related to cyclophosphamide. (Singh and Kumar, 2019). Several studies have shown that natural products possess potent antioxidant effect that could enhance fertility and mitigate oxidative stress-mediated toxicity. Hence, there is need to develop a practical protective model against cyclophosphamide induced-tissue injury that may preserve CYP efficacy while mitigating its side effects

Urtica dioica (stinging nettle), is a perennial plant belonging to the genus *Urtica*, of the family Urticaceae (Ahmed and Parasuraman, 2014). The plants are about 2m in height and covers with stinging hairs with hooked protrusions. The leaves are also covered with stiff hairs on both sides that produce hot sensation when touched (Manandhar, 2002). *Urtica dioica* have a long history of use in the traditional medicine and as a nutritious diet. Recently, this plant is gaining attention as the leaves are reported to be rich in many bio active compounds, such as flavonoids, phenolic acids, and amino acids (Grauso et al., 2020). The extracts from *Urtica dioica* have showed potent pharmacological activities such as anyioxidative, anti-inflammatory, anti-ulcer, anti hyperglycemic, anti-bacterial, and cardiovascular protective activities (Hari et al., 2022). The present of flavonoids and phenolic compounds makes plant become natural antioxidants (Komes et al., 2011). Several studies have shown that methanol and ethanol of extract of leaves have in vitro antioxidant activity against the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) (Khare et al., 2012; Pourmorad et al., 2006).

To this end, considering its wide spectrum of biological properties including antioxidant and anti-inflammatory effects, the current investigation was designed to study the possible attenuating effects of *Urtica dioica* on CYP-induced testicular toxicity and endocrine deficit in rats.

2. MATERIAL AND METHODS

Collection and Identification of Plant materials

Fresh leaves of stinging nettle (*Urtica dioica*) were collected from Igbere in Bende Local Government Area of Abia State. The plant was identified and authenticated by a taxonomist in the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike (MOUAU), Abia State, Nigeria. A voucher number: MOUAU/VPP/CVM/116/ 2023 was designated to the plant.

Preparation and extraction of plant material

Extraction technique described by Kalu et al., (2024) was adopted. The Fresh leaves were neatly plucked out of the stalk and then air dried at room temperature (25-27oC) for 7 days before being pulverised into fine powder. Certain quantity (350 g) of the powdered sample was weighed and dissolved in 1000ml of ethanol, stirred and kept for 48 hr. Thereafter, it was filtered using a muslin cloth. The resulting filtrate was evaporated to dryness in a water bath at 40°C until all the ethanol had been removed. The dry extract was stored in a refrigerator (4°C) until its usage. The yield of the extract (in percentage) was calculated as:

[Weight of the extract/Weight of the powdered sample] x 100.

The weight of the U. *dioica* extract was 34.04g while the percentage yield was 9.72%.

Acute toxicity study of *U. dioica*

The new Lorke's method used by Orieke et al., (2019) was adopted with little modification. At the highest tested dose of 5000 mg/kg, no mortality or obvious signs of toxicity was observed. The lethal dose of the extract was therefore obtained as >5000 mg/kg. The LD_{50} value for U. *dioica* was obtained as >5000 mg/kg body weight which was considered to be safe and on this basis, the doses of 500 and 1000 mg/kg for U. *dioica* were selected. Also LD_{50} value for was Cyclophosphamide was obtained as 1569.28mg/kg body weight, on this basis the dose 10mg/kg was selected.

Experimental Procedure

Twenty-five male rats of the wistar strain, weighing between 80 and 130 g were used. Ethical approval was obtained from the Board of the Department of Biochemistry, Rhema University, Nigeria, Abia State, Nigeria. The rats were acclimatized to their food and water for 2 weeks which they had access to *ad libitum.*

Following acclimatization, they were distributed randomly into five groups of five rats each.

The rats were randomly distributed five each per group and treated daily for 14 days: Group 1- Control Group (No treatment), Group 2- treated with Cyclophosphamide (10 mg/kg bw; ip), Group 3- oral administration of *U. dioica* extract (1000 mg/kg bw), Group 4- treated Cyclophosphamide (10 mg/kg bw; ip) and oral administration *U.dioica* (500 mg/kg bw) and Group 5- treated with Cyclophosphamide (10 mg/kg bw; ip) and oral administration *U.dioica* (1000 mg/kg bw).

The body weights of the rats were recorded on a daily basis. At the end of 14 days, the rats were fasted overnight and on the 15th day, whole blood was collected from the *retro*-orbital venous plexus, and animals were sacrificed by cervical dislocation. Sera were harvested from the clotted blood samples by centrifuging at 3000 x g for 20 min and used for biochemical analyses. The Testis, Prostates, kidneys, Liver and Heart were excised, washed with cold normal saline, blotted with filter paper, and weighed on an electronic balance (Ohaus, USA).

After measurements of the Testis weights, two Testis were selected from each group and processed for histology. The remaining three Testis from each group were homogenized in ice-cold phosphate buffered saline and centrifuged at 10 000 xg for 15 minutes and the supernatants were analyzed for lipid peroxidation, catalase, reduced glutathione (GSH), superoxide disthmutase (SOD) and glutathione peroxidase (GPx) activities.

The percentage change in the body weights of the rats was calculated as:

{Final body weight -Initial body weight}/Final body weight} -100.

Similarly, the relative organ weights of the rats were calculated as follows:

Relative Testis weight (g/100 g) = {Total Testis weight/Final body weight} - 100

Relative Prostate weight (g/100 g) = {Total Prostate weight/Final body weight} - 100

Relative Kidney weight (g/100 g) = {Total Kidney weight/Final body weight} - 100

Relative Liver weight (g/100 g) = {Total liver weight/Final body weight} - 100

Relative Heart weight (g/100 g) = {Total heart weight/Final body weight} -100

Determination of Haematological Parameters.

Harmatological analysis of the blood samples was performed in an auomated haematology analyzer (BC-2300 model, Minday Medical Co., China) with the procedure carried as specified by the producer. Packed cell volume (PCV), white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), mean corpuscular volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were determined using a hematology analyzer.

Analysis of serum

Assay of markers of oxidative stress in the Kidney

Catalase activity was determined in the prostates of the rats using the method of Sinha. SOD activity was determined using the method of Misra and Fridovich. Glutathione peroxidase (GPx) was estimated using the method of Paglia and Valentine. GSH was determined using the method of Beutler et al., 1963. Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substance (TBARS) using the method of Varshney and Kale (1990).

Assessment of reproductive hormones

The serum was used to analyse luteinising hormone (LH), follicle stimulating hormone (FSH) and testosterone concentrations. The serum concentrations of LH, FSH and testosterone were estimated by ELISA assay kits (DRG Diagnostics Marburg, Germany), according to the kit manufacturer's instructions

Assay for inflammatory markers levels

Interleukin 6 (IL-6), interleukin 1 Beta (IL-1b) concentrations of the rats were determined using ELISA Kit (ELabscience, USA) following the instructions of the manufacturer. Data that were obtained are expressed as pg/ml of protein.

TNF-α concentrations of the rats were determined using Rat Tumor Necrosis Factor Alpha ELISA Kit (ELabscience, USA) following the instructions of the manufacturer. Data that were obtained are expressed as pg/ml of protein.

Determination of semen parameters

Semen collection

The sperm cells were harvested from the epididymal reserve. The rats were anaesthetized with chloroform (inhalation), and their epididymedes extracted. The caudal portion of each epididymis was incised and a smear made on the preheated glass slides for evaluation.

Macroscopic examination

The semen colour and consistency was evaluated macroscopically and recorded. The consistency scale (1-4), adopted by Orieke et al. (2019) was used.

Microscopic examination

Determination of sperm mass motility

The method described by El- Sherbiny (1987) was adopted for sperm mass motility, sperm morphologyand sperm cell viability. Sperm concentration was determined by the methodology described byHerbert (1992).

Evaluation of hepatic-renal functionality assays

AST, ALP, ALT, Bilirubin, Albumin, Globulin, Total Protein concentrations were determined using spectrophotometric techniques using RANDOX Kits and following standard procedure outlined by the manufacturer (Randox Laboratories Limited).

Urea, Creatinine, and Electrolytes concentrations were determined using spectrophotometric techniques using RANDOX Kits and following standard procedure outlined by the manufacturer (Randox Laboratories Limited).

Determination of lipid profile in the sera

Total cholesterol, TAG and HDL concentrations were determined using spectrophotometric techniques using RANDOX Kits and following standard procedure outlined by the manufacturer (Randox Laboratories Limited). LDL and VLDL concentrations were derived from HDL, TAG and Cholesterol.

Testis histology

Sections of the the fixed Testis tissues were dehydrated with alcohol, cleared with xylene and embedded in molten paraffin wax. On solidifying, the paraffin blocks were subsequently sectioned at 5 µm using a microtome. The sections were subsequently stained with infiltrated Ehrlich hematoxylin for 15 minutes for viewing under a microscope (Ajonuma et al., 2005). Photomicrographs of the tissues were taken with a light microscope at x100 magnification.

Statistical analysis

Data were analyzed statistically using Graph pad prism and the statistical package for social sciences (SPSS) version 26.0. One-way analysis of variance (ANOVA) was used for comparison of means. Differences between means were considered to be significant when $P < 0.05$.

3. RESULTS

The LD_{50} of *U. dioica* leaf ethanol extract was 5000 mg/kg.

Table 1 below shows the result of percentage weight gain in CYP treated rats. Values shown in the table indicated no significant ($P>0.05$) difference in the following parameters: initial animal weight (g), final animal weight (g), weight gain (g) and % weight gain (g) in cyclophosphamide- induced rats compared to the normal control.

Table 1.0: Result of percentage weight gain in CYP treated rats

Treatment groups	Initial animal weight (g)	Final animal Weight (g)	Weight gain (g)	%Weight gain (g)
Control	73.80±12.58[a]	109.37±15.36[a]	35.57±4.29[a]	48.70±6.57[a]
Cyclophosphamide (10 mg/kg) only	86.96±32.28[a]	119.20±32.57[a]	32.24±13.54[a]	43.14±31.75[a]
Extract only (1000 mg/kg)	103.37±10.09[a]	120.08±27.39[a]	16.71±35.90[a]	18.23±34.86[a]
Cyclophosphamide (10 mg/kg) + Extract (500 mg/kg)	106.33±7.71[a]	124.09±2.97[a]	17.77±9.01[a]	17.18±10.07[a]
Cyclophosphamide (10 mg/kg) + Extract (1000 mg/kg)	100.68±16.88[a]	108.64±35.01[a]	7.96±19.78[a]	6.16±20.36[a]

Values are presented as mean ± standard deviation (n = 5), values with different letter superscripts within each of columns are significantly (P<0.05) different.

Table 2.0 below presents the effect of UD on rat body weight, testis and prostate weight. At sacrifice, there was no significant different in body weight of rats in all the groups. On the other hand, CYP reduced the relative weight of rat testis and prostate significantly (p > 0.05) compared to normal group in this study. However, treatment with UD at 500mg/kg and 1000mg/kg increased the testicular weight compare to disease control, although it was not significant (p > 0.05) in comparison with normal control group.

Table 2.0: Result of effect of UD on relative testicular and prostate weight in CYP-treated rats

Parameters	Control	Cyclophosphamide only (10 mg/kg)	Extract only (1000 mg/kg)	Cyclophosphamide (10 mg/kg) + Extract (500 mg/kg)	Cyclophosphamide only (10 mg/kg) + Extract (1000 mg/kg)
Animal live weight (g)	114.73±4.59[a,b]	126.08±3.59[c]	121.96±2.33[b,c]	125.67±2.39[c]	111.06±6.16[a]
Paired testicular weight (g)	1.39±0.03[a]	1.71±0.02[b]	1.95±0.03[c]	2.32±0.08[d]	2.82±0.01[e]
Weight of prostate (g)	0.08±0.01[a]	0.07±0.01[a]	0.12±0.01[b]	0.11±0.01[b]	0.11±0.01[b]
ROW of Testes (%)	1.21±0.03[a]	1.35±0.06[b]	1.60±0.01[c]	1.85±0.08[d]	2.54±0.14[e]
ROW Prostate (%)	0.08±0.01[b]	0.06±0.00[a]	0.10±0.01[c]	0.09±0.01[b]	0.10±0.00[b,c]

Values are presented as mean ± standard deviation (n = 5), values with different letter superscripts within each of row are significantly (P<0.05) different.

Table 3.0 below shows the data on the organo-somatic indices of the liver, kidney and heart of the experimental animal. In comparison with the control, animals exposed to CYP alone demonstrated significant (p < 0.05) decrease organo-somatic indices of the liver. On the other hand, this indices were efficiently augmented close to control in rats following co-treatment with UD at 500 and 1000 mg/kg body weight.

Table 3.0: Result of effects of UD on Relative Body, liver, heart and kidney weight in CYP treated rats

Treatment groups	Animal live body weight (g)	Weight of liver (g)	Weight of right kidney (g)	Weight of left kidney (g)	Total weight of kidney (g)	Weight of heart (g)
Control	109.37±15.36[a]	4.22±0.68[a]	0.44±0.08[a]	0.41±0.08[a]	0.85±0.15[a]	0.46±0.05[b]
Cyclophosphamide (10 mg/kg) only	130.998±25.67[a]	3.96±0.09[a]	0.52±0.02[b]	0.51±0.02[b]	1.03±0.01[b]	0.46±0.01[b]
Extract only (1000 mg/kg)	120.08±27.39[a]	5.06±0.07[b]	0.60±0.02[c]	0.54±0.05[b,c]	1.15±0.07[b,c]	0.40±0.01[a]
Cyclophosphamide (10 mg/kg)+Extract (500 mg/kg)	123.83±2.84[a]	5.20±0.14[b]	0.56±0.01[b,c]	0.55±0.02[b,c]	1.11±0.02[b,c]	0.65±0.01[d]
Cyclophosphamide (10 mg/kg)+Extract (1000 mg/kg)	108.64±35.02[a]	5.52±0.09[b]	0.58±0.02[b,c]	0.60±0.02[c]	1.18±0.03[c]	0.55±0.02[c]

Values are presented as mean ± standard deviation (n = 5), values with different letter superscripts within each of columns are significantly (P<0.05) different.

As shown in table 3.0 above, there were no significant (P>0.05) difference in the animal live weights of all the groups. The weight of the liver did not differ significantly (P>0.05) in the control and cyclophosphamide alone treated groups but were significantly (P<0.05) different from the other groups.

UD enhanced antioxidant status and inhibited oxidative damage in CYP-treated rats.

The effects of UD on antioxidant status in the serum of CYP-exposed rats are illustrated in Fig. 1. Compared with the control; activities of various antioxidant enzymes such as SOD, CAT, GSH-Px, and the GSH level, were markedly ($p < 0.0001$) lower whereas the levels of MDA produced were significantly higher in seruim of rats administered CYP alone. On the other hand, the co-administration of UD at 500 and 1000 mg/kg significantly enhanced the activities of these enzymes. Correspondingly, the levels of MDA in the serum of UD co-treated rats were significantly ($p < 0.0001$) lower in comparison with rats exposed to CYP alone.

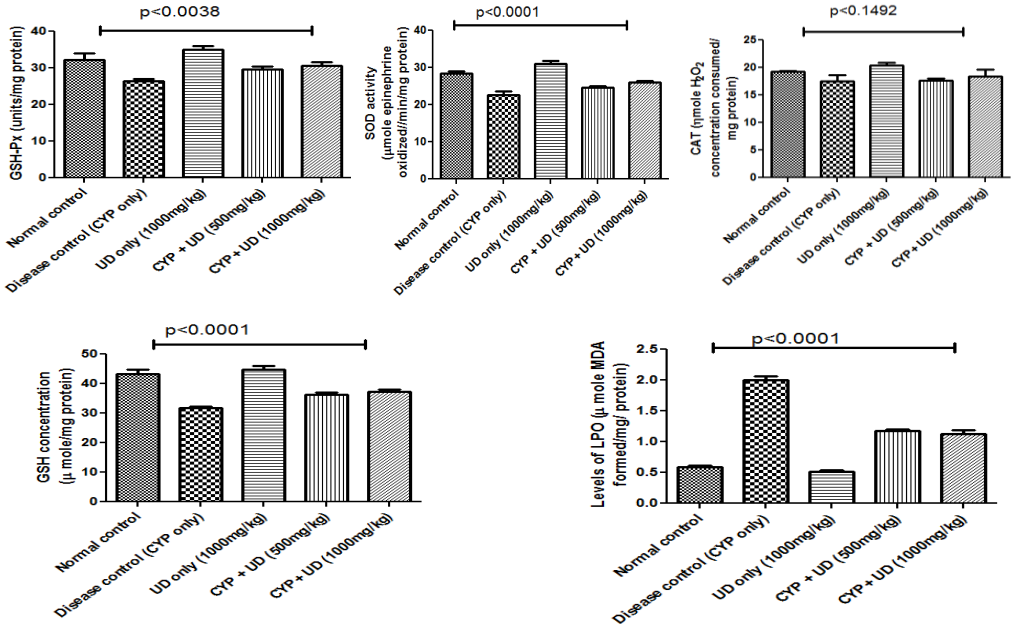

Fig 1. Effect of UD on antioxidant parameters of cyclophosphamide treated rats

Fig 2. shows the effect of UD on LH, FSH and testosterone in rats exposed to CYP. The results show that CYP significantly ($p < 0.0001$) reduced the serum levels of LH, FSH and testosterone compared to normal control. However, treatment with UD at both doses improved the levels of LH, FSH and testosterone in the serum significantly ($p < 0.0001$) in comparison to CYP group.

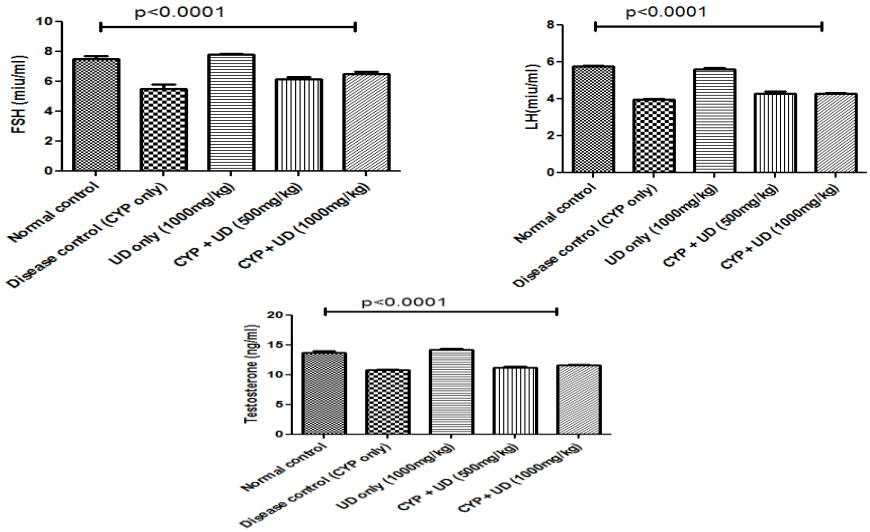

Fig 2. Effects of UD on hormone of Cyclophosphamide treated rats

UD co-treatment suppressed inflammatory markers in the serum of CYP-treated rats as shown in Fig. 3. CYP treatment significantly ($p < 0.0001$) elevated the levels of IL-6, IL-1 and TNF-α respectively compared with the control. UD co-treatment exhibited significant lower levels of IL-6, IL-1 and TNF-α in the serum of rats.

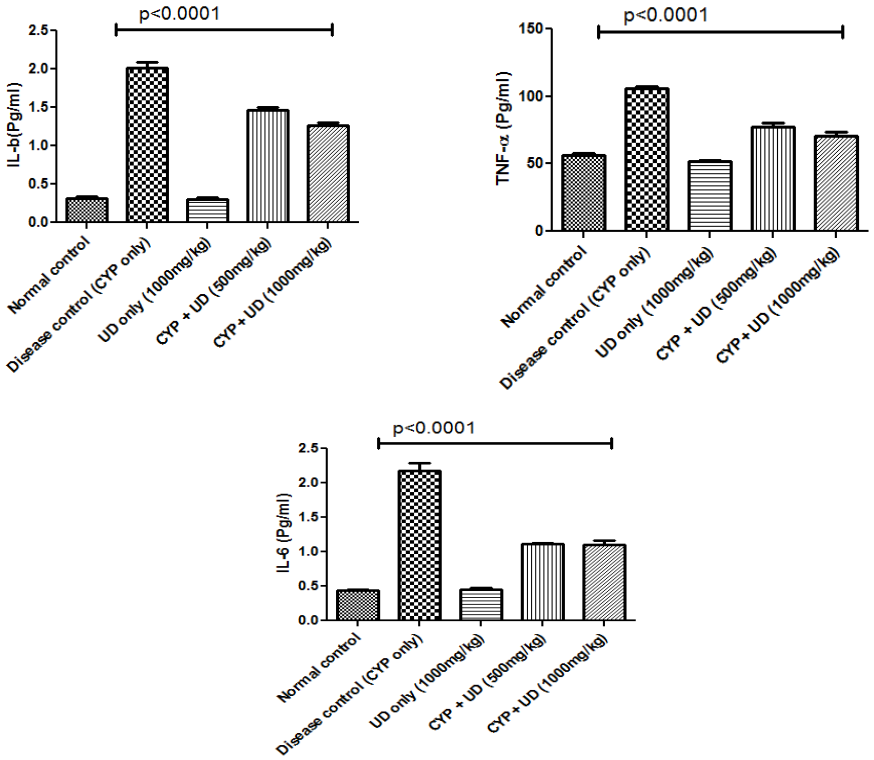

Fig 3.Effect of UD on Inflammatory markers of Cyclophosphamide treated rats

UD co-treatment improves hematological indices in CYP-treated rats. Cyclophosphamide treatments elicited significant decrease ($p<0.0001$) in RBC, PVC and HGB levels respectively compared to the Control group (Table 4). However, UD co-treated group exhibited marked significant increase ($p<0.0001$) in the hematological indices compared to the CYP-treated group. Cyclophosphamide treatments elicited significant increase ($p<0.0001$) in TWBC, PLT, MCV.MCH and MCHC levels compared to the Control group (Table 4). However, UD co-treated group exhibited marked significant decrease ($p<0.0001$) in the TWBC, PLT, MCV.MCH and MCHC compared to the CYP-treated group.

Table 4.0: Result of effects of UD on Heamatological parameters of cyclophosphamide treated rats

Parameters	Control	Cyclophosphamide only (10 mg/kg)	Extract only (1000 mg/kg)	Cyclophosphamide (10 mg/kg) + Extract (500 mg/kg)	Cyclophosphamide only (10 mg/kg) + Extract (1000 mg/kg)
RBC ($x10^6/mm^3$)	7.12 ± 0.22^{d}	4.09 ± 0.04^{a}	7.34 ± 0.13^{d}	4.53 ± 0.18^{b}	4.94 ± 0.29^{c}
PCV (%)	45.33 ± 0.58^{c}	31.33 ± 1.16^{a}	46.67 ± 1.16^{c}	38.67 ± 1.16^{b}	39.67 ± 2.52^{b}
Hb (g/dl)	15.23 ± 0.25^{c}	10.67 ± 0.78^{a}	15.73 ± 0.25^{c}	13.30 ± 0.26^{b}	13.40 ± 0.53^{b}
TWBC ($x10^3/mm^3$)	8.65 ± 0.22^{a}	13.03 ± 1.55^{c}	8.41 ± 0.37^{a}	$12.23\pm0.50^{b,c}$	10.73 ± 1.01^{b}
PLT ($x10^3/mm^3$)	234.33 ± 5.69^{a}	264.00 ± 14.53^{b}	234.00 ± 4.58^{a}	264.00 ± 6.25^{b}	258.33 ± 6.66^{b}
MCV (fl)	63.73 ± 1.31^{a}	76.54 ± 2.36^{b}	63.60 ± 0.63^{a}	85.43 ± 5.54^{b}	$80.33\pm3.61^{a,b}$
MCH (pg)	21.41 ± 0.53^{a}	26.05 ± 1.79^{b}	21.45 ± 0.05^{a}	29.37 ± 1.30^{c}	$27.18\pm1.68^{b,c}$
MCHC (g/dl)	33.60 ± 0.24^{a}	34.01 ± 1.32^{a}	33.72 ± 0.40^{a}	34.41 ± 0.78^{a}	33.83 ± 1.25^{a}

Values are presented as mean ± standard deviation (n = 5), values with different letter superscripts across each row are significantly ($P<0.05$) different.

Cyclophosphamide treatments elicited a non-significant effects in Neutrophils, Lymphocytes, Monocytes, Eosinophils levels respectively compared to the Control group (Table 5).

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Table 5.0 Result of effects of UD on differential WBC counts of cyclophosphamide treated rats

Treatments	Control	Cyclophosphamide only (10 mg/kg)	Extract only (1000 mg/kg)	Cyclophosphamide (10 mg/kg) + Extract (500 mg/kg)	Cyclophosphamide only (10 mg/kg) + Extract (1000 mg/kg)
Neutrophils (%)	38.33 ± 1.53^{b}	26.33 ± 2.08^{a}	38.33 ± 3.06^{b}	27.33 ± 2.08^{a}	29.00 ± 1.00^{a}
Lymphocytes (%)	54.33 ± 2.08^{a}	65.00 ± 1.73^{c}	55.00 ± 3.00^{a}	$57.67\pm5.69^{a,b}$	$62.67\pm0.58^{b,c}$
Monocytes (%)	4.67 ± 0.58^{a}	5.33 ± 0.58^{a}	4.33 ± 0.58^{a}	5.33 ± 1.16^{a}	4.67 ± 0.58^{a}
Eosinophils (%)	2.67 ± 0.58^{a}	3.00 ± 0.00^{a}	2.33 ± 0.58^{a}	2.67 ± 0.58^{a}	3.00 ± 0.00^{a}
Basophils (%)	0.00 ± 0.00^{a}	0.33 ± 0.58^{a}	0.00 ± 0.00^{a}	0.33 ± 0.58^{a}	0.67 ± 0.58^{a}

Values are presented as mean ± standard deviation (n = 5), values with different letter superscripts across each row are significantly (P<0.05) different.

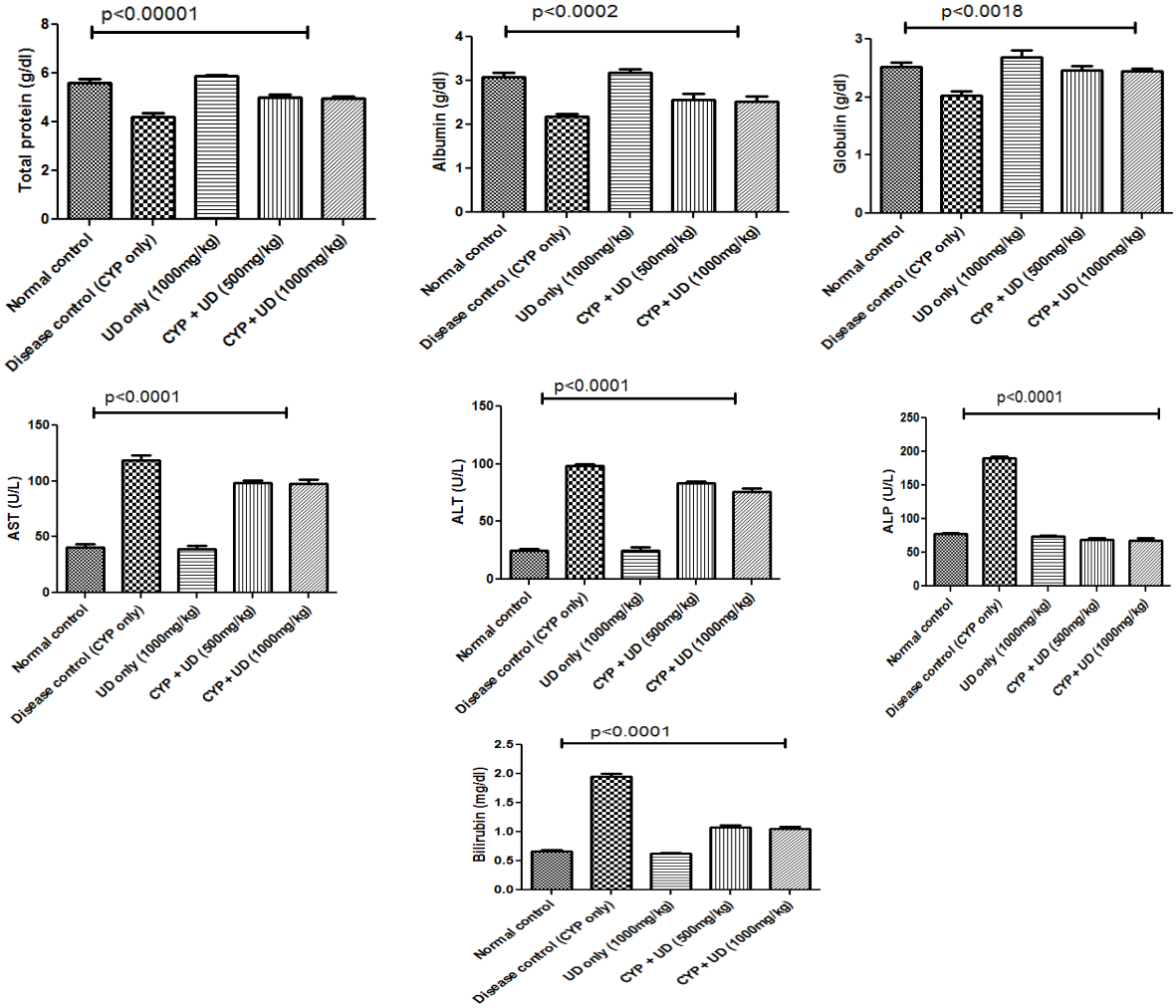

Fig 4. Effect of UD on liver function parameters of Cyclophosphamide treated rats

UD prevented enhanced hepatic function bio-markers concentrations in CYP-exposed rats. The influence of UD on hepatic function assays in CYP-exposed rats are illustrated in Fig. 4. In comparison with the control, the serum activities of ALT, AST, and ALP were significantly ($P < 0.0001$) higher in CYP exposed rats alone. Conversely, the administration of UD at 500 and 1000 mg/kg resulted to a noticeable lower level of liver function tests indistinguishable to control values. UD inhibited decreased protein, albumin and globulin in serum of CYP-exposed rats. Compared to the control treatment with UD significantly ($p<0.0001$) increased the protein, albumin and globulin in serum of CYP-exposed rat Fig 4.

Fig 5.shows the lipid profile in the sera of the rats that were studied. As shown in the Fig 5, there were significant increases ($p<0.0001$) in the total cholesterol, TAG, LDL and VLDL but significant decreases ($p<0.0001$) in the HDL concentrations of the CYP only group in comparison with the control. On the other hand, there were significant decreases ($p<0.05$) in the total cholesterol, TAG, LDL and VLDL but significant increases ($p<0.0001$) in the HDL concentrations of UD alone 1000mg and both doses of UD (500mg/kg and 1000mg/kg) as compared to the CYP only group.

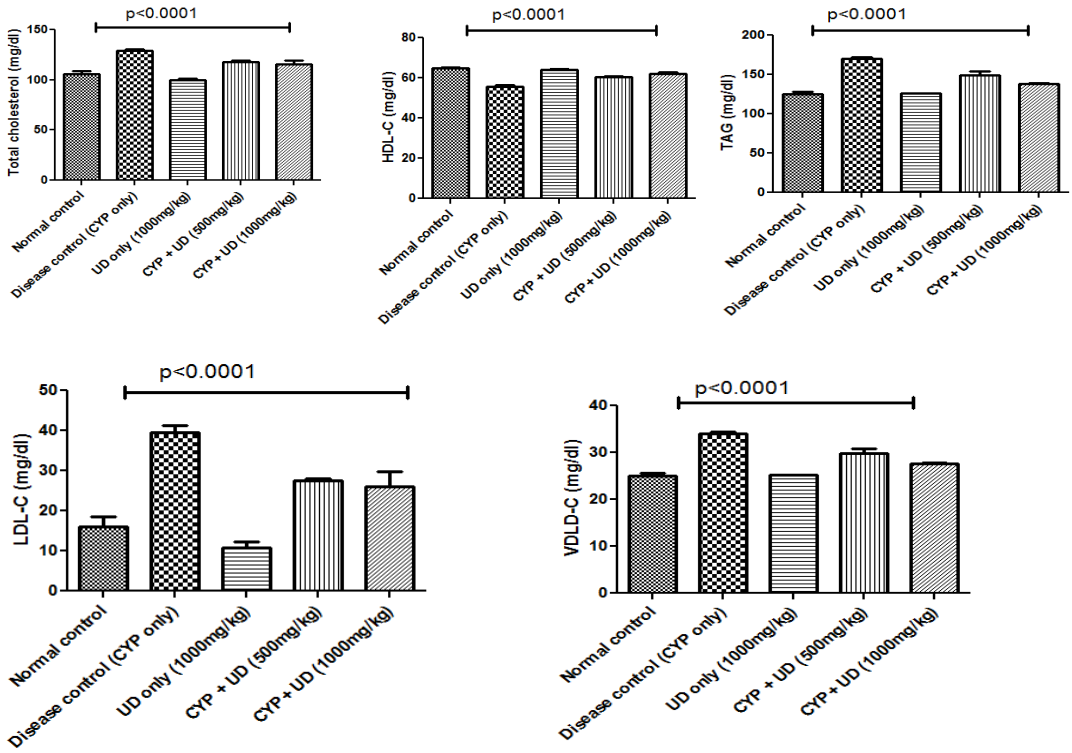

Fig 5. Effect of UD on lipid profile parameters of Cyclophosphamide treated rats

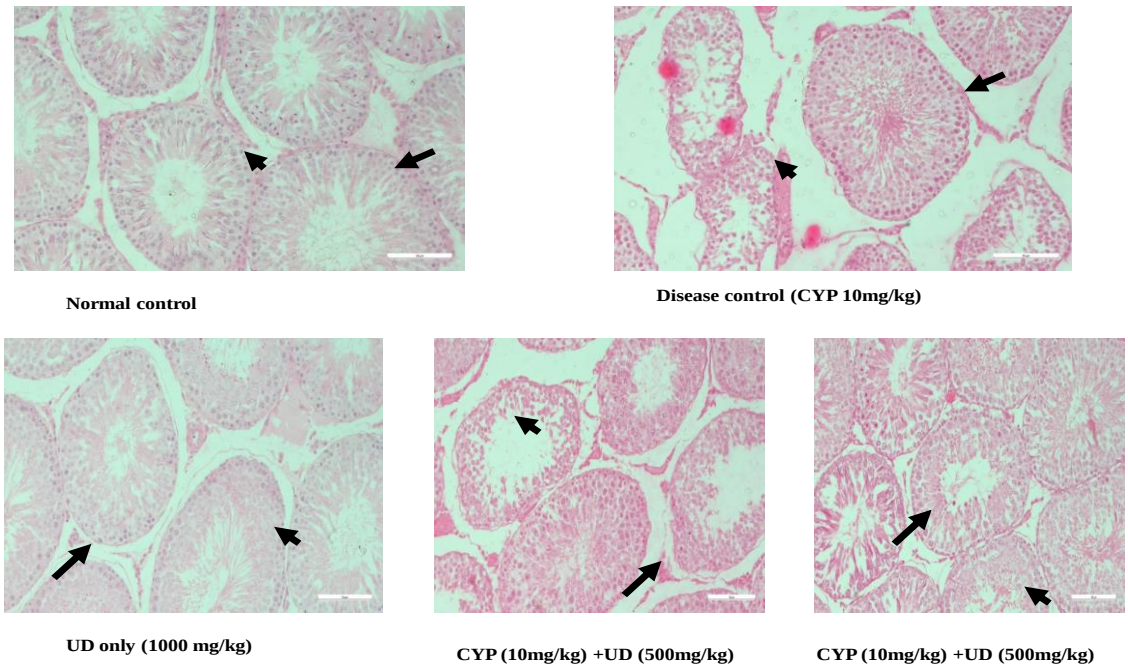

Fig 6. Effect of UD on Histology of the Testes of CYP treated rat

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Effects of UD on histological changes of Testes.

Fig. 6 shows the histological observations of the effect of UD on CYP-injected rats. The testis histology of rats in normal control and UD only group show normal architecture of the testis with normal seminiferous tubules (arrow) lined by stratified epithelium of germ cells and normal testicular interstitium.. In rats injected with cyclophosphamide (CYP) only), mutifocal widespread areas of degeneration of the seminiferous tubules (arrow) was observed. The affected tubules appear shrunken and showed dyspasia of the seminiferous epithelia lacking mature spermatocytes in their lumens. Treatment of UD to CYP injected rats markedly recovered the adverse effects inflicted on the tissue by CYP. Histology of the testes mid degeneration of the seminiferous tubules (arrow) was observed.

Table 6.0: Result of Effects of UD on renal function parameters in cyclophosphamide treated rats

Treatment groups	Control	Cyclophosphamide only (10 mg/kg)	Extract only (1000 mg/kg)	Cyclophosphamide (10 mg/kg) + Extract (500 mg/kg)	Cyclophosphamide only (10 mg/kg) + Extract (1000 mg/kg)
Urea (mg/dl)	17.52 ± 0.36^{a}	46.35 ± 5.01^{c}	20.27 ± 3.33^{a}	29.43 ± 2.17^{b}	29.22 ± 1.49^{b}
Creatinine (mg/dl)	0.81 ± 0.04^{a}	2.42 ± 0.18^{c}	0.80 ± 0.09^{a}	1.18 ± 0.08^{b}	1.14 ± 0.06^{b}
Uric acid (mg/dl)	4.48 ± 0.21^{a}	7.82 ± 0.28^{c}	4.32 ± 0.08^{a}	5.93 ± 0.14^{b}	5.57 ± 0.48^{b}
Na^{+} (mEq/L)	130.83 ± 2.26^{c}	107.70 ± 3.51^{a}	131.57 ± 3.35^{c}	112.93 ± 3.02^{b}	115.83 ± 1.46^{b}
K^{+} (mEq/L)	4.42 ± 0.15^{b}	4.05 ± 0.15^{a}	4.47 ± 0.08^{b}	4.12 ± 0.07^{a}	4.22 ± 0.05^{a}
Cl^{-} (mEq/L)	89.83 ± 0.91^{b}	78.50 ± 2.05^{a}	89.97 ± 5.56^{b}	80.43 ± 1.56^{a}	83.03 ± 0.61^{a}
HCO_3^{-} (mEq/L)	19.67 ± 0.25^{a}	20.30 ± 0.26^{b}	19.73 ± 0.32^{a}	$20.13\pm0.25^{a,b}$	$20.13\pm0.29^{a,b}$

Values are presented as mean ± standard deviation (n = 5), values with different letter superscripts across each row are significantly (P<0.05) different.

The administration of UD at 500 and 1000 mg/kg resulted to a noticeable lower level of kidney function tests parameters indistinguishable to control values Table 6. Conversely, we observed a significant (P < 0.05) lower levels of kidney function tests parameters in the CYP-exposed rats alone.

Table 7.0: Result of effect of extract on semen quality in cyclophosphamide treated rats

Treatments	**Control**	**Cyclophosphamide only (10 mg/kg)**	**Extract only (1000 mg/kg)**	**Cyclophosphamide (10 mg/kg) + Extract (500 mg/kg)**	**Cyclophosphamide only (10 mg/kg) + Extract (1000 mg/kg)**
Semen colour (1-2)	2.00 ± 0.00^{b}	1.00 ± 0.00^{a}	2.00 ± 0.00^{b}	$1.33\pm0.58^{a,b}$	$1.67\pm0.58^{a,b}$
Semen consistency (1-4)	3.67 ± 0.58^{c}	1.00 ± 0.00^{a}	4.00 ± 0.00^{c}	2.00 ± 0.00^{b}	2.33 ± 0.58^{b}
Semen pH	$6.95\pm0.05^{b,c}$	6.33 ± 0.04^{a}	6.99 ± 0.05^{c}	6.84 ± 0.05^{b}	$6.91\pm0.12^{b,c}$
Motile sperm cells (%)	89.66 ± 0.91^{d}	59.90 ± 1.61^{a}	91.91 ± 3.03^{c}	76.36 ± 3.66^{b}	84.25 ± 2.44^{c}
Non motile sperm cells (%)	11.66 ± 0.43^{b}	17.04 ± 1.24^{c}	6.71 ± 0.45^{a}	12.31 ± 1.24^{b}	12.56 ± 0.21^{b}
Sperm cells with abberant movement (%)	10.36 ± 0.54^{b}	25.81 ± 1.83^{d}	5.49 ± 0.37^{a}	13.83 ± 0.70^{c}	13.18 ± 0.28^{c}
Sperm count (x10^{6}/CEp)	115.63 ± 2.69^{d}	67.62 ± 0.16^{a}	126.10 ± 4.87^{e}	99.82 ± 2.38^{b}	107.99 ± 1.92^{c}
Live proportion (%)					
Normal sperm proportion (%)	88.11 ± 3.14^{c}	76.03 ± 2.51^{a}	91.97 ± 1.15^{d}	$79.72\pm2.86^{a,b}$	83.38 ± 0.92^{b}
Total abnormal sperm (%)	97.22 ± 0.38^{c}	87.12 ± 1.98^{a}	92.20 ± 0.627^{b}	91.76 ± 2.33^{b}	92.75 ± 0.28^{b}
	2.78 ± 00.38^{a}	12.88 ± 01.97^{c}	7.00 ± 00.62^{b}	8.24 ± 02.32^{b}	7.25 ± 00.28^{b}

Values are presented as mean ± standard deviation (n=5), values with different letter superscripts across each row are significantly (P<0.05) different.

Table 8.0: Result of effect of extract on sperm morphology in cyclophosphamide treated rats

Treatments	Control	Cyclophosphamide only (10 mg/kg)	Extract only (1000 mg/kg)	Cyclophosphamide (10 mg/kg) + Extract (500 mg/kg)	Cyclophosphamide only (10 mg/kg) + Extract (1000 mg/kg)
Total head abnormalities (%)	0.70 ± 0.02^{a}	2.12 ± 0.25^{c}	$1.25\pm0.09^{a,b}$	$1.83\pm0.71^{b,c}$	$1.22\pm0.05^{a,b}$
Total tail abnormalities (%)	0.65 ± 0.07^{a}	3.93 ± 0.26^{c}	2.09 ± 0.07^{b}	2.41 ± 0.61^{b}	2.13 ± 0.07^{b}
Mid-piece head (%)	0.18 ± 0.06^{a}	1.03 ± 0.14^{d}	0.54 ± 0.05^{b}	0.44 ± 0.08^{b}	0.83 ± 0.02^{c}
Cytoplasmic droplets (%)	1.25 ± 0.27^{a}	5.80 ± 1.32^{c}	3.13 ± 0.43^{b}	3.56 ± 0.95^{b}	3.07 ± 0.15^{b}

Values are presented as mean ± standard deviation (n=5), values with different letter superscripts across each row are significantly ($P<0.05$) different.

4. DISCUSSION

Cyclophosphamide induces a broad spectrum of toxicities and tissue dysfunctions constituting a serious health hazards to cancer patients (Mohammadi et al., 2014, Oyabemi et al., 2016, Olayinka et al., 2015). The current study disclosed the valuable health effect of UD against the adverse effects of CYP induced toxicity in rats. It is pertinent to underscore that multiple side effects underlined chiefly by oxidative stress, including testicular toxicity are a major challenge that limits the use of CYP for cancer patients. Studies show that antioxidant agents can modulate chemotherapy that not only destroy the cancer growth but also mitigate and or prevent side effects (Mehta et al., 2018). Animals treated with CYP alone exhibited a marked decrease in the body weight gain and organo-somatic indices of the testes, liver, and kidney, consequently indicating general toxicity and disturbance of metabolic functions in the exposed rats. The decrease in the testes, liver, and kidney weights in rats exposed to CYP alone implies degeneration resulting from the toxic impact of CYP in the studied tissues. The restoration of the body and organ weights gain following co-treatment with UD demonstrated the beneficial effects of UD on the metabolism and detoxification potentials of liver and kidney in the experimental animals. Oxidative stress is a condition produced by the imbalance between oxidants and antioxidants in a biological system. The imbalance occurs as a result of the surplus level of reactive oxygen species or malfunctioning of the antioxidant system (Chiurchiu et al., 2016). Studies revealed that CYP is able to induce oxidative-inflammatory stress and multi organs injuries in the testes, liver, lung and kidneys (Rehman et al., 2012; Shokrzadeh et al., 2015, Doustimotlagh et al., 2020). Oxidative stress is one of the principal causes of CYP-induced testicular toxicity and is mediated by the production of CYP metabolites (Doustimotlagh et al., 2020). Antioxidant defense mechanisms protect cellular macromolecules from the damaging effect of ROS, such as hydroxyl radical, superoxide anion, and peroxides that have direct link with oxidative damage of testes, lung, liver and kidney (Ebokaiwe et al., 2020). Studies suggests that CYP interferes with antioxidant defense mechanisms by ROS generation associated with its toxic hepatic metabolite, acrolein, known to promote oxidative stress (Olayinka et al., 2015, Shahana et al., 2016). In the current study, CYP treatment resulted in heightened MDA levels with decreasing SOD, CAT, GSH-Px activities, and GSH concentration. Whereas, UD co-treatment prevented the CYP-induced depletion of antioxidants activities, and heightened MDA levels in the serum. These results indicate clearly that UD co-treatment protected against oxidative stress induced by CYP. Several studies revealed that CYP induced toxicity induces disturbances in testosterone levels leading to testicular dysfunction (Farombi et al., 2012, llbey et al., 2009). The current study reveal that CYP significantly decreased LH, FSH and testosterone levels in rats. It is known that alterations in LH and FSH secretion could affect quality and quantity of sperm and testosterone regulation (Okon and Utuk, 2016). The oxidative testicular damage by CYP may be related to the depletion in testosterone level and feedback on the hypothalamus and the pituitary that control the secretion of LH and FSH. The decreased testosterone level could be attributed to impaired Leydig cells as the decreased testosterone induced arrest of spermatogenic tissues as observed in the histopathological results. Our result show that treatment of CYP adversely affects testicular function by decreasing pituitary LH and FSH secretion and reducing testosterone level and is consistent with the previous findings showing the toxicity of CYP by reducing the levels of testosterone, LH and FSH (IIbey et al., 2009). Co- treatment with UD is attributed to have markedly improved the levels of LH, FSH and testosterone. This was further confirmed by the histology result of the testicular architecture, which demonstrated multifocal areas of degeneration of semi-ferous tubules following CYP treatment. These histological alterations were abated by UD co-treatment which could be predicated on its anti-oxidant, anti-inflammatory, and anti-lipid

peroxidation activities, hence protecting against tissue damage. This study corroborates earlier reports by Qayyum et al., 2016, Khare et al., 2012 and Grauso et al., 2020 on the hepato-protective and testicular-protective influence of UD against CYP induced histological alterations.

The current study further accentuates induction of inflammatory response following CYP treatment via an increase in the inflammatory cytokines IL-6, IL-b and TNF-α. UD co-treatment inhibited oxidative stress and lowered the proinflammatory cytokines thereby preventing CYP-induced injury in the testes. The decreasing of these inflammatory indices following co-treatment with UD revealed the involvement of the anti-inflammatory mechanism of UD in attenuating testicular toxicity associated with CYP exposure.

Cyclophosphamide is a known immunosuppressive drug and acts on the cells by suppressing the activity of the bone marrow, spleen, and innate immune responses (Ahlman and Hempel, 2016). Our result showed that cyclophosphamide may have increased bone marrow suppressive effect causing aplastic anemia as shown in the significant reduction of the RBC level of the rats. The immunosuppressive effect of cyclophosphamide is shown in the significant reduction of the WBC, LYM, Neut, P-LCR, RBC, and HGB levels as compared to the control group. Treatment with UD reversed the altered hematological condition induced by CYP and stimulated the nonspecific immune response. This study has shown that UD could stimulate the hemopoietic system and could restore the production of immune cells that were depleted by CYP treatment.

CYP-treated animals, in the current study showed significant elevation of liver and kidney function indices in the serum. This is an indication of liver and kidney injury since their leakage into serum defines the severity of damage (Yogalakshmi et al., 2010; Hamsa and Kuttan, 2011). Hepatic dysfunction was the most common toxicity reported in patients treated with CYP (McDonald and Frieze, 2008). Hepatic tissues were the primary sites for the microsomal activation of the drugs. Hence hepatic metabolism of CYP elicited formation of toxic metabolite that compromised the architecture of the liver and kidney tissues as demonstrated by elevated liver enzymes and kidney function indices in serum. The decrease activities of serum AST, ALT and ALP and lower levels of creatinine and urea in animals co-dosed with UD demonstrate the protective activity of UD against CYP-induced liver and kidney injury. Thus, UD prevents CYP-induced liver and kidney injury via attenuation of oxidative-inflammatory stress and restoration of antioxidant defenses.

Dyslipidemia refers to an elevation in blood concentrations of total cholesterol or LDL or decreased concentrations of HDL (Fodor, 2011). In this study, we found increased concentrations of cholesterol, TAG, LDL, VDL but decreased HDL concentration in the sera of the disease control group, establishing the development of dyslipidemia in this group of rats. Although how CYP leads to dyslipidemia has not been fully elucidated, attenuation of TAG, total cholesterol, LDL, VLDL, with corresponding increase in HDL in the *U. dioica* co-treatment groups suggest the capacity of *U. dioica* to ameliorate dyslipidemia.

5. CONCLUSION

In conclusion, this study suggests that UD protect morphological structure of sperms and testicular tissue against CP toxicity. UD abated altered parameters due to its biological activities, such as antioxidant and anti-inflammatory properties, modulation of serum testosterone LH and FSH levels and amelioration of induced histopathological changes in the CYP-treated testes of Wistar rats as well as its ability to attenuate lipid peroxidation, platelet aggregation, and a potent stimulant of the immunological system via increasing the levels of and white blood cells which are important for immunological system self-defense. Therefore, UD may be used with CP in cancer patients to improve CP-induced injuries in sperm morphology and oxidative stress parameters.

Ethical approval

Ethical approval was obtained from the Board of the Department of Biochemistry, Rhema University, Nigeria, Abia State, Nigeria.

Disclaimer (Artificial intelligence)

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

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