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Original Article

Cyperus esculentus Ameliorates Cadmium Chloride Induced Testicular Toxicity in Male Rats Following Male Reproductive Hormone, Pro-Inflammatory and Anti-Inflammatory Analysis

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ABSTRACT

Background: *Cyperus esculentus* has been reported to mitigate testicular dysfunction associated with lead and salt diet. However, it is not known if it can mitigate or protect the testis from cadmium toxicity. This research seeks to ascertain the ameliorating effect of *Cyperus esculentus* on cadmium induced toxicity in male rats. **Materials and Methods:** Twenty animals divided into 4 groups were used for this research (n=5). The groups were Control (NC), Cadmium (CD), Cadmium+Quercetin (CD+QZ) and Cadmium+Extract (CD+CE). After 1 week acclimatization, animals were administered $CdCl_2$ (5 mg/kg b.w.) and/or hydro-ethanolic extract of *Cyperus esculentus* (C.E extract) (1000 mg/kg b.w.) and quercetin (2 mg/kg) once daily for 28 days. At the end of the experiment, the animals were sacrificed and serum collected for analysis. **Results:** Pro-inflammatory biomarkers (TNFα, IL-6 and IL-1β) were significantly higher in significantly higher in CD, CD+QZ and CD+CE compared to the Control (NC) group at p<0.05. However, they significantly decreased in CD+QZ and CD+CE compared to Cadmium (CD) group at levels of p<0.05. Cadmium (CD) and Cadmium+quercetin (CD+QZ) experimental groups showed significant decrease in IL-10 levels compared to Control (NC) at p<0.05. Cadmium+Quercetin (CD+QZ) and Cadmium+*Cyperus esculentus* (CD+CE) experimental groups showed significant decreased of IL-10 compared to Control (NC) at p<0.05. hormonal assessment of testosterone, FSH and LH in Cadmium group (CD) was significantly lower than the Control (NC) at p<0.05 while that of Cadmium+Quercetin (CD+QZ) and Cadmium+*Cyperus esculentus* (CD+CE) was significantly higher than FSH of Cadmium experimental group (CD) at p<0.05. **Conclusion:** Hydro ethanolic extract of *Cyperus esculentus* ameliorates cadmium toxicity on reproductive system of male rats by restoring the reproductive hormonal levels of male rats and with decrease pro-Inflammatory biomarkers (TNFα, IL-6 and IL-1β) and increased anti-Inflammatory biomarker (IL-10). *Cyperus esculentus* can therefore be recommended for patients exposed to cadmium in order to avoid cadmium toxicity on reproductive system.

Keywords: Anti-Inflammatory Biomarkers, Cadmium chloride, *Cyperus esculentus*, Male Reproductive Hormones, Pro-Inflammatory Biomarkers.

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INTRODUCTION

Cadmium chloride, a white crystalline hygroscopic salt which is highly soluble in water and slightly soluble in alcohol has a chemical formula of $CdCl_2$ with an obvious odorless smell. The use of cadmium chloride is applicable in industries as they find practical applications in photocopying, dyeing and electroplating. They are used for preparing Cadmium sulfide and are often involved in the preparation of aryl or primary alkyl organocadmium compounds.[1] Long term exposure to cadmium through air, water, soil and food leads to cancer.[2] Cadmium is seen in organ system toxicity such as skeletal, urinary, reproductive, cardiovascular, central and peripheral nervous and respiratory systems. In the reproductive system, exposure to cadmium even at very low concentrations affects human male reproductive system and deteriorate spermatogenesis, semen quality especially sperm motility and hormonal synthesis/release. In the females, it is found that cadmium impairs female reproductive system, alters hormonal balance and affects menstrual cycle.[3]

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Generally, in all systems of the body in which cadmium has been associated with poisoning and damage, there will likely be an alteration in the biomarkers of inflammation and inflammation related indexes. Lionte and colleagues showed that biomarkers of inflammation and inflammation related indexes based on Complete Blood Cell (CBC) count can identify acutely poisoned patients at increased risk for intensive care unit hospitalization and deaths.[4] Some of such inflammatory biomarkers linked with drug or chemical poisoning include Tumour Necrosis Factor (TNF) alpha, Interleukin-6 (IL-6), Interleukin-1 beta (IL-1β) and Interleukin-10 (IL-10).

The mechanism of cadmium action in causing damage to cells involves cell proliferation, differentiation and apoptosis. These activities interact with DNA repair mechanism, the generation of Reaction Oxygen Species (ROS) and the induction of apoptosis.[5] Cadmium binds to the mitochondria and can inhibit both cellular respiration and oxidative phosphorylation at low concentration.[6] In all of these activities, inflammatory biomarkers and hormonal messengers are usually altered as an increased is 1 of such pointers to a diseased or injury conditions.

The treatment of Cadmium poisoning includes the use of natural and chemical decontaminant, chelating agents, combination therapy with chelating agents, used of nanoparticles and Plasma exchange-hemodialysis-plasmapheresis. In the use of natural and chemical decontaminants, medicinal plants and extract such as seeds of *Moringa oleifera*, peanuts (*Arachis hypogaea*), cowpeas (*Vigna unguiculata*), urad (*Vigna mungo*) and corn (*Zea mays*) were recommended. This is because these medicinal plants has the abilities to absorb and neutralize colloidal positive charges especially from water which appears to be 1 of such routes through which cadmium gains entrances into the body to effect damages and poisoning.[7] Since medicinal plants can be used for the treatment of cadmium poisoning, then *Cypernus esculentus*, an edible perennial grass-like plant, which propagates exclusively with underground tubers might also serve as a treatment measure. Hence, this research seeks to find out the effect of *Cyperus esculentus* on some inflammatory biomarkers of rats exposed to cadmium to ascertain if this herbal extract can ameliorate the effect of cadmium on these rats. The research will also compare the effect of *Cyperus esculentus* with quercetin, a supplement that offers anti-inflammatory and antioxidant effect with an aim of assessing to what extent the ameliorating effect of tiger nut will be mediated if it has any. Alterations of these biomarkers will be used as a pointer to the extract ability to either ameliorate or propagate the effect of cadmium on these rats.

Cadmium has been reported to cause reactive oxidative stress. In conditions of male reproductive system damages caused by cadmium, there exist an increase in Reactive Oxygen Species (ROS), a biomarker for reactive oxidative stress.[8] Inflammatory pathways are altered in cases of oxidative stress leading to increased inflammatory biomarkers as seen in damages caused by cadmium on the male reproductive system.[9] *Cyperus esculentus* has the capability of increasing the weights of the testes and epididymis, sperm count, sperm quality and testosterone level.[10] It has also been reported to mitigate testicular dysfunction associated with lead acetate, high salt diet and other substance.[11,12] However, little is known if the extract of this plant will be relevant in alleviating the inflammation caused by cadmium on male reproductive system should it be used to treat the cadmium induce damage done on male reproductive system of animals. Therefore, this research is useful as it will seek to find out the inflammation levels of animals which were induced with reproductive dysfunction following administration of cadmium when treated with *Cyperus esculentus,* an extract that has proven to be of importance in boosting fertility in male reproductive system.

Also, though Cadmium is known for its damages on various physiological system and *Cyperus esculentus* is also known for the treatment of Lead induced reproductive system damage, little information is published pertaining the effect of these extract and element (Cadmium) on inflammatory biomarkers of the body. This research will further seek to find out the possible ameliorative or curative effect that *Cyperus esculentus* will mediate on Cadmium-induced animals following the changes that will be seen on the inflammatory biomarkers of such animals.

Again, there exist possible alterations in hormonal level following cadmium toxicity and in many infertilities disorder.[13,14] It is therefore essential to investigate the effect of *Cyperus esculentus* on the male reproductive hormonal levels of these rats exposed to cadmium with the aim of ascertaining if the extract will have ameliorating effect of any disorder caused by cadmium on hormonal balance.

MATERIALS AND METHODS

Chemicals

Cadmium Chloride ($CdCl_2$), quercetin, thiobarbituric acid, hydrogen peroxide, Isoflurane and glutathione were purchased from Sigma-Aldrich, St Louis, MO, USA. All other chemicals used were of analytical grade.

Plant material and hydro-ethanolic extract preparation

Dried tubers of *C. esculentus* were authenticated in the Herbarium Unit of Department of Botany in our institution after purchase from Bogobiri market, Calabar, Nigeria. The extract was prepared according to a method previously described by Costa and colleagues.[13] Bad tubers and dirt were removed. The good ones were thereafter washed and air-dried. A blender (Kenwood 1.6 L, BL480 Prestons, Australia) was then used to crush the dried tubers into fi A powder for 10 min. Five hundred grams of the dried powder was then extracted by percolation at room temperature using 5 L of hydroethanolic solution (ethanol:water, 70:30 v/v) for 48 hr and fi and fil using Whatman No. 1 filter paper. The filtrate

was concentrated using a rotary evaporator (Searl Instruments Ltd. England) under reduced pressure in a vacuum at 45°C into a colloid form and stored at 4°C. The yield of the extract was 165 g.

Laboratory animals

Approval to conduct the study was sought from the Animal Research Ethics Committee of Faculty of Basic Medical Sciences, University of Calabar, Cross River State. Twenty male Wistar rats (190-220 g) were bought from the Department of Agriculture, University of Calabar, Cross River State and kept in the animal house of Department of Physiology, University of Calabar. The rats were housed in well-ventilated wooden cages having wood dust as bedding. They were acclimatized for 7 days. All rats freely assessed rat chow and water and were exposed to 12/12-hr light/dark cycle. The rats were handled following the guidelines provided in the Animal Ethics handbook of Faculty of Basic Medical Sciences, University of Calabar.

Experimental design and study protocol

The twenty rats were randomly divided into 4 groups (n=5) and administered $CdCl_2$ (5 mg/kg b.w.) and/or hydro-ethanolic extract of *Cyperus esculentus* (C.E extract) (1000 mg/kg b.w.) orally once daily for -28 days (Table 1) according to the method of Udefa and colleagues.[11] The doses for $CdCl_2$ and quercetin were chosen from previous study based on the fact that this dose has been reported to cause impairment in male reproductive functions.[11] The dose of 1000 mg/kg of extract was chosen based on the reports of previous studies where this dose was effective in mitigating male reproductive impairment associated with lead acetate and high salt diet.[11,12] All the animals were given rat feed and water throughout the study duration. After the 28-day study duration, all the rats were anesthetized with isoflurane and sacrificed. Blood samples were collected analyses. The process of blood collection was via cardiac puncture with 5 mL syringes attached to 21 G needles. The blood samples were used to determine the levels of Tumour Necrosis Factor alpha (TNF-α), Interleukin-6 (IL-6), Interleukin-1 beta (IL-1 β), Interleukin -10 (IL-10).

Collection of serum for hormonal assessment

Blood was collected from the animals through Cardiac Puncture after an Isoflurane anesthesia administered on the animals. The blood was introduced into a sample bottle which was further spun in a hematocrit centrifuge at 3000 revolution per minute (3000 rpm) to get the serum.

Collection of semen for pro- and anti-inflammatory biomarkers in the testis

Isofluorane anesthesia was used for animals. The animals were made to inhale isofluorane from a dessicator and later removed after losing consciousness. Subsequent dislocation on the cervical region of vertebrate was carried out on the animals before sacrifice. Orchidectomy was done on the animals, precisely the open method castration where there was an incision made on the midline of the scrotom. Semen was collected by softly milking out the scrotom. The tunica vaginalis was cut, exposing the testicles. The spermatic cord was then cut, ligated and exposed. After that, the cauda epididymis was identified and the semen samples were taken from it.[15]

Determination of selected pro- and anti-inflammatory cytokines in the testis

The levels of Tumor Necrosis Factor-α (TNF-α), Interleukin (IL)-6, IL-1β and IL-10 in the testes were determined by ELISA analysis (Bioassay Systems, Hayward, CA, USA) following the manufacturer's protocol.

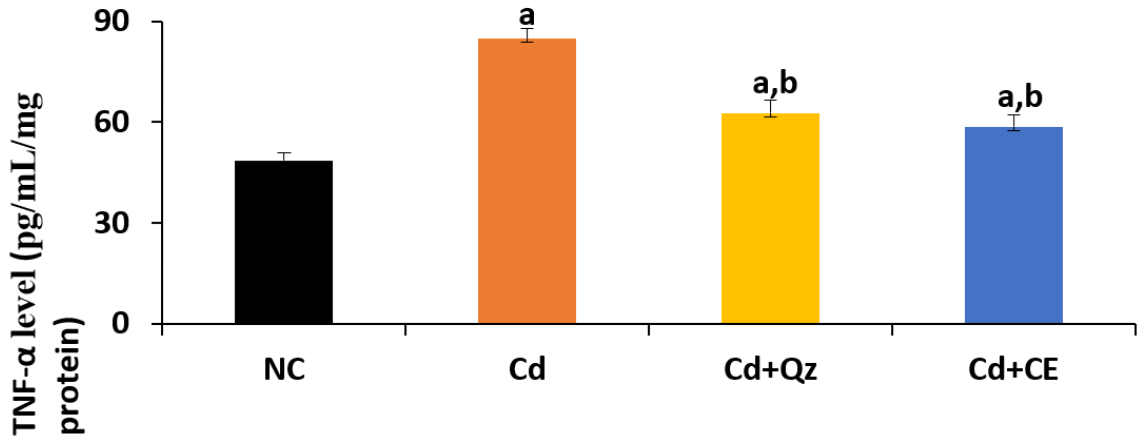

Figure 1: TNF - α level in the testis of rats in the different experimental groups. Values are expressed as mean±SD, n=5. a=p<0.05 vs NC, b=p<0.05 vs Cd.

Table 1: **Experimental design and animal groups treated with cadmium chloride and hydroethanolic extract of *C. esculentus*.**

Sl. No.	Group	Treatment
1	Control (NC)	0.5 mL of 2% DMSO in distilled water for 28 days.
2	Cadmium (CD)	5 mg/kg b.w. of $CdCl_2$ in 2% DMSO for 28 days.
3	Cadmium+Quercetin(CD +QZ)	5 mg/kg b.w. of $CdCl_2$+20 mg/kg b.w. of Quercetin in 2% DMSO in distilled water.
4	Cadmium+Extract(CD+CE)	5 mg/kg b.w. of $CdCl_2$ plus 1000 mg/kg b.w. of *Cyperus esculentus* extract in 2% DMSO in distilled water for 28 days.

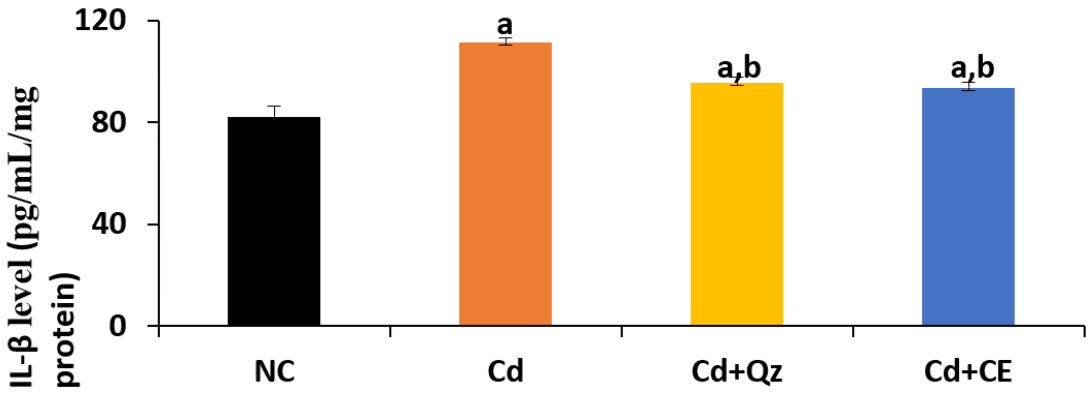

Figure 2: IL - 6 level in the testis of rats in the different experimental groups. Values are expressed as mean±SD, *n*=5. a=*p*<0.05 vs NC, b=*p*<0.05 vs Cd.

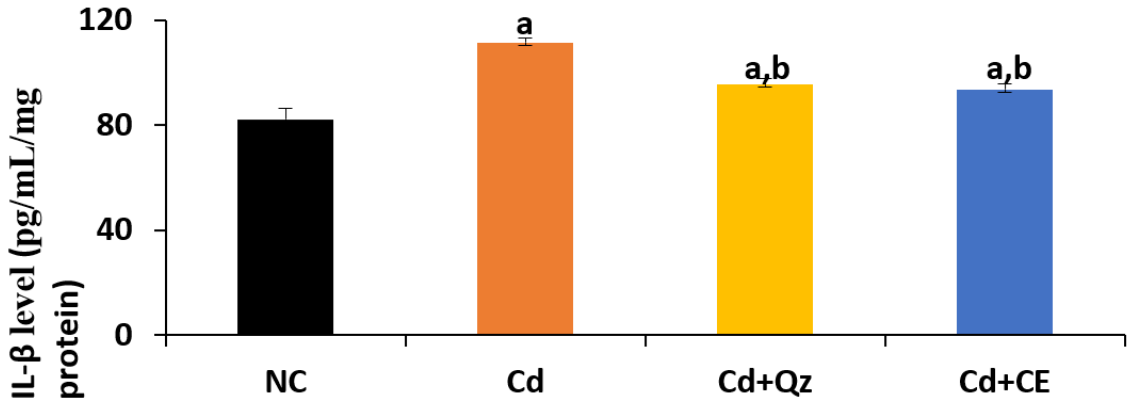

Figure 3: IL-β level in the testis of rats in the different experimental groups. Values are expressed as mean±SD, *n*=5. a=*p*<0.05 vs NC,b=*p*<0.05 vs Cd.

Hormonal Assessment

Testosterone, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) were determined by Elisa method

Statistical analysis

Results are presented as mean±Standard Deviation (SD). All data were analyzed using Statistical Package for Social Science (SPSS) version 20. To test for homogeneity of variance, Levene's test was used. Assessment of distribution of the data was carried out using Shapiro Wilk and Kolmogorov Smirnov normality tests. Results of these tests revealed that the data were normally distributed and the variance was homogenous. Consequently, the data were analyzed by one-way Analysis of Variance (ANOVA) while Tukey *post hoc* test was performed to make comparisons.

Ethical Approval

All experiments were performed in accordance with the guideline for care and use of laboratory animal of the Faculty of Basic Medical Science Animal Research Ethics Committee, University of Calabar,

Cross River State. Ethical approval was granted by the committee with approval number UNICAL-FAREC-FBMS-02285.

RESULTS

Tumour Necrosis Factor-alpha (TNF-α) among experimental groups

The Mean±SD of TNF-α among the experimental groups were 48.5±2.4368 pg/mL/mg protein, 84.686±3.0184 pg/mL/mg protein, 62.644±4.0015 pg/mL/mg protein and 58.58±3.4629 pg/mL/mg protein for Control (NC), Cadmium (CD), Cadmium+quercetin (CD+QZ) and Cadmium+*Cyperus esculentus* (CD+CE) experimental groups respectively. TNF-α was significantly higher in CD, CD+QZ and CD+CE compared to the Control (NC) group at $p<0.05$. However, TNF-α was significantly decreased in CD+QZ and CD+CE compared to Cadmium (CD) group at levels of $p<0.05$ (Figure 1).

Interleukin-6 (IL-6) among experimental groups

The Mean±SD of IL-6 among the experimental groups were 23.184±4.33762 pg/mL/mg, 61.644±1.61125 pg/mL/mg, 42.266±2.19205 pg/mL/mg and 39.01±2.14237 pg/mL/mg for Control (NC), Cadmium (CD), Cadmium+Quercetin (CD+QZ) and Cadmium+*Cyperus esculentus* (CD+CE) experimental groups respectively. IL-6 was significantly higher in CD, CD+QZ and CD+CE compared to the Control (NC) group at $p<0.05$. However, IL-6 significantly decreased in CD+QZ and CD+CE compared to Cadmium (CD) group at levels of $p<0.05$ (Figure 2).

Interleukin-1 beta (IL-1β) among experimental groups

The Mean±SD of IL-1β among the experimental groups were 82.086±4.62847 pg/mL/mg, 111.444±5.24924 pg/mL/mg, 95.392±4.81032 pg/mL/mg and 93.436±3.92308 pg/mL/mg for Control (NC), Cadmium (CD), Cadmium+Quercetin (CD+QZ) and Cadmium+*Cyperus esculentus* (CD+CE) experimental groups respectively. IL-1β was significantly higher in CD, CD+QZ and CD+CE compared to the Control (NC) group at $p<0.05$. However, IL-1β was significantly lower in CD+QZ and CD+CE compared to Cadmium (CD) group at levels of $p<0.05$ (Figure 3).

Interleukin-10 (IL-10) among experimental groups

The result of IL-10 in various experimental groups presented as Mean±SD were 32.634±4.22892 pg/mL/mg, 14.852±3.93904 pg/mL/mg, 24.64±3.54044 pg/mL/mg and 29.972±4.11912 pg/mL/mg for Control (NC), Cadmium (CD), Cadmium+Quercetin (CD+QZ) and Cadmium+*Cyperus esculentus* (CD+CE) experimental groups respectively. Cadmium (CD) and Cadmium+Quercetin (CD+QZ) experimental groups showed significant decrease in IL-10 levels compared to Control (NC) at $p<0.05$. Cadmium+Quercetin (CD+QZ) and Cadmium+*Cyperus esculentus* (CD+CE) experimental groups showed significant decreased of IL-10 compared to Control (NC) at $p<0.05$ (Figure 4).

Testosterone level among experimental groups

The result of Testosterone in various experimental groups presented as Mean±SD were 4.12±0.37683 ng/mL, 1.3±0.47434 ng/mL, 3.98±0.36332 ng/mL and 3.68±0.36469 ng/mL for Control (NC), Cadmium (CD), Cadmium+Quercetin (CD+QZ) and Cadmium+*Cyperus esculentus* (CD+CE) experimental groups respectively. Testosterone level in Cadmium (CD) group was significantly lower than the Control (NC) at i<0.05. Testosterone level in Cadmium+Quercetin (CD+QZ) and Cadmium+*Cyperus esculentus* (CD+CE) experimental groups were significantly

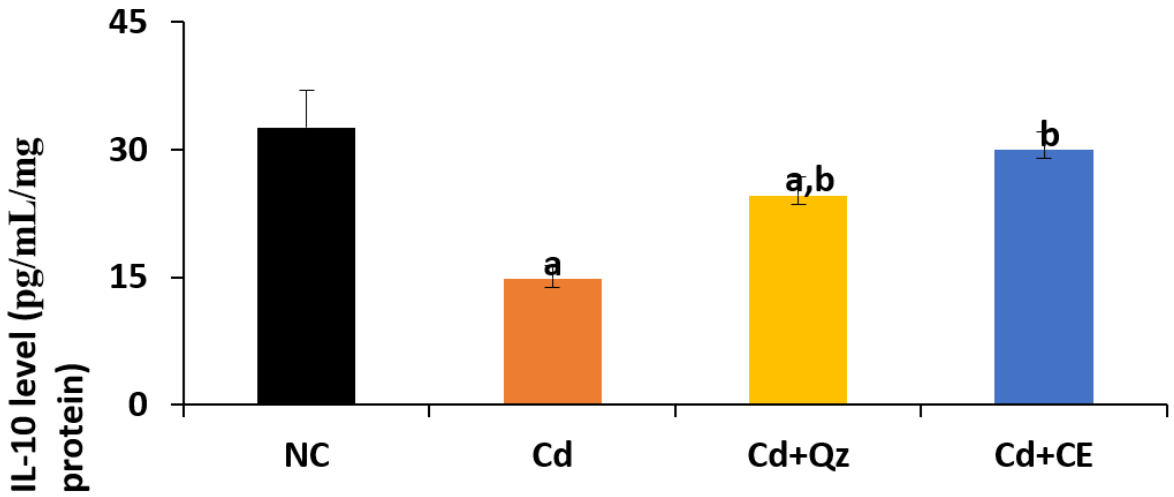

Figure 4: IL-10 level in the testis of rats in the different experimental groups. Values are expressed as mean±SD, $n=5$. a=$p<0.05$ vs NC, b=$p<0.05$ vs Cd.

higher than the Cadmium group (CD) experimental animals (p<0.05) (Figure 5).

Follicle stimulating hormone (FSH) among experimental groups

The Mean±SD of FSH among the experimental groups were 4.18±0.32711 ng/mL, 2.08±0.27749 ng/mL, 3.92±0.78867 ng/mL and 3.94±0.51284 ng/mL for Control (NC), Cadmium (CD), Cadmium+Quercetin (CD+QZ) and Cadmium+*Cyperus esculentus* (CD+CE) experimental groups respectively. FSH of Cadmium group (CD) was significantly lower than the control (NC) at p<0.05 while that of Cadmium+Quercetin (CD+QZ) and Cadmium+*Cyperus esculentus* (CD+CE) was significantly higher than FSH of cadmium experimental group (CD) at p<0.05 (Figure 6).

Luteinizing hormone (LH) among experimental groups

The Mean±SD of FSH among the experimental groups were 3.98±0.27749, 1.98±0.44385, 3.5±0.35355 and 3.62±0.44385 for Control (NC), Cadmium (CD), Cadmium+Quercetin (CD+QZ) and Cadmium+*Cyperus esculentus* (CD+CE)

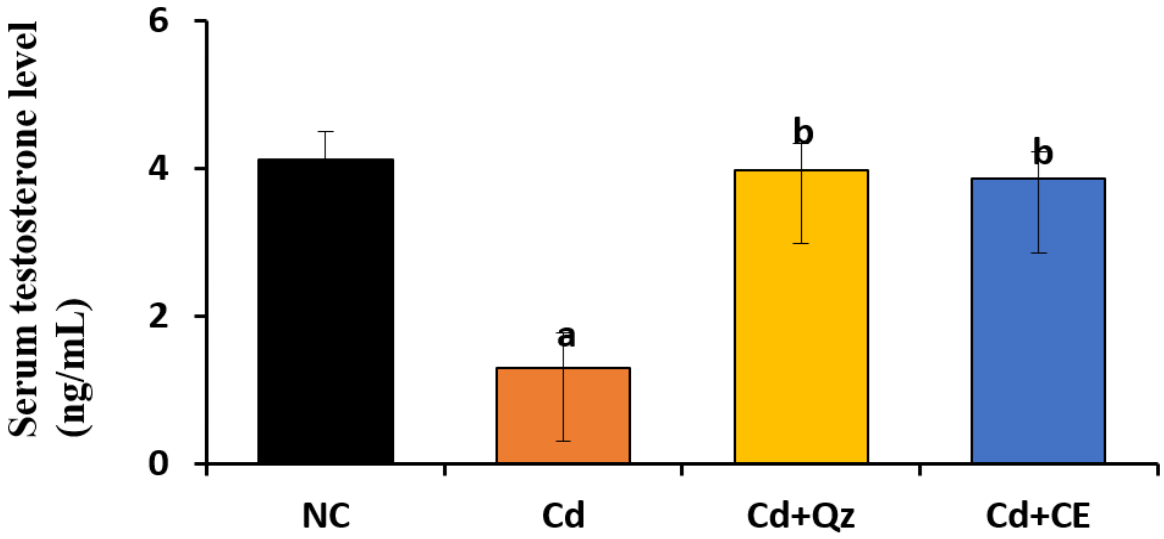

Figure 5: Serum testosterone level in the different experimental groups. Values are expressed as mean±SD, n=5. a=p<0.05 vs NC, b=p<0.05 vs Cd.

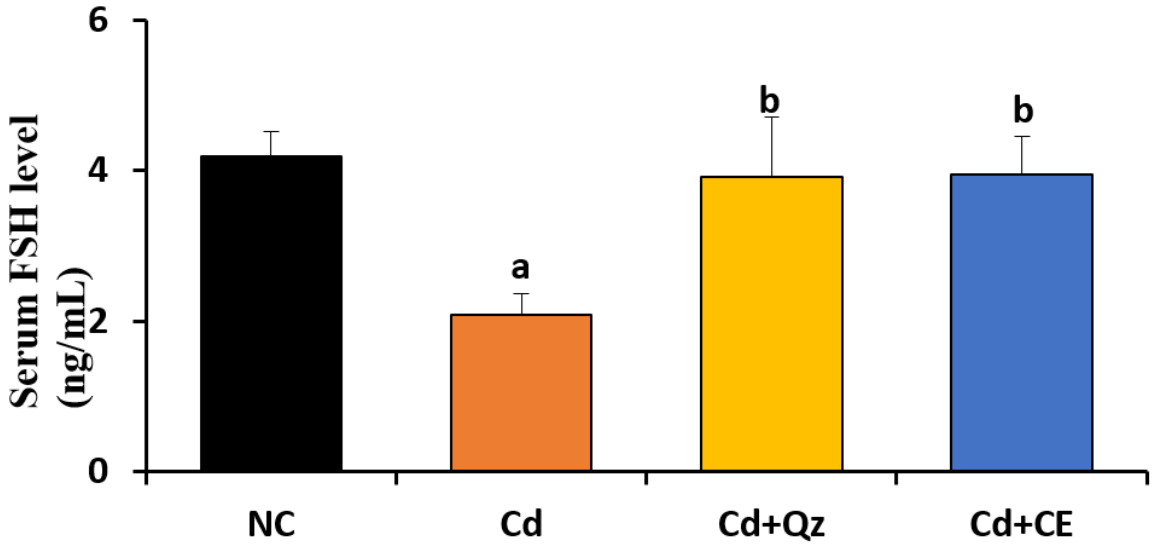

Figure 6: Serum FSH level in the different experimental groups. Values are expressed as mean±SD, n=5. a=p<0.05 vs NC, b=p<0.05 vs Cd.

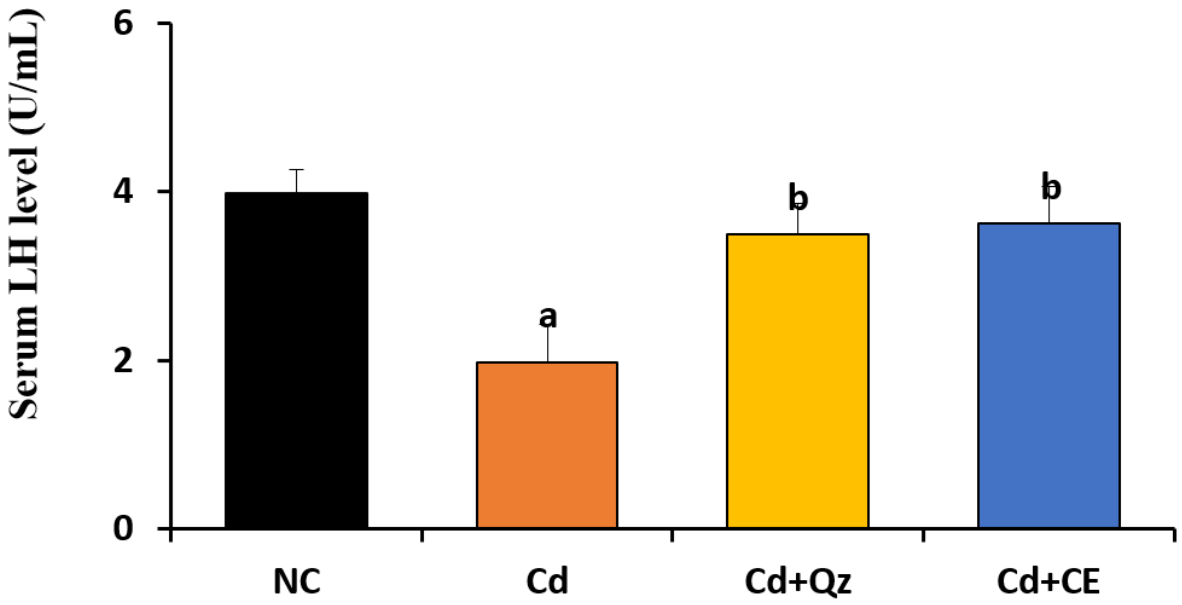

Figure 7: Serum LH level in the different experimental groups. Values are expressed as mean±SD, *n*=5. a=*p*<0.05 vs NC, b=*p*<0.05 vs Cd.

experimental groups respectively. LH of Cadmium group (CD) was significantly lower than the control (NC) at $p<0.05$ while that of Cadmium+Quercetin (CD+QZ) and Cadmium+*Cyperus esculentus* (CD+CE) was significantly higher than FSH of cadmium experimental group (CD) at $p<0.05$ (Figure 7).

DISCUSSION

Common pro-inflammatory biomarkers include TNF-α, IFm, IL-6 and IL-8. Reproductive system toxicity and many other chronic disorders such as arterial disease, cancer, obstructive pulmonary diseases and many others are usually linked to inflammation.[16] This inflammation can only be assessed following the levels of pro-inflammatory biomarkers. Oxidative stress is usually casual or secondary to inflammation. Oxidative stress results from the production of oxygen radicals more than the antioxidant capacity of the stressed tissue. Many conditions or events associated with male infertility are inducers of oxidative stress. X-irradiation, for example, or exposure to environmental toxicants and the physical conditions of varicocele and cryptorchidism have been demonstrated to increase testicular oxidative stress, which leads to an increase in germ cell apoptosis and subsequent hypospermatogenesis.[17] Therefore, an increase in pro-inflammatory biomarkers is often 1 of the methods of ascertaining inflammation as applicable in a physiological organ or system. There also exists relationship between anti-inflammatory biomarkers and pro-inflammatory biomarkers. Certain research has shown this relationship in some diseases such as Type 2 Diabetes Mellitus.[18] In the said research, it was concluded that there exists a strong relationship between TNFα, IL-6, CRP, IL-10 and T2DM patients of Kashmiri ethnicity, treated at SMHS Hospital. Thus, supporting other studies and showing that cytokines may be good markers for T2DM development. In this research, all pro-inflammatory biomarkers assessed (TNFα, IL-6 and IL-1β) were significantly higher in cadmium group compared to control. Though not significant, these same pro-inflammatory biomarkers were also higher in cadmium group compared to groups administered with Cadmium+Quercetin and Cadmium+*Cyperus esculentus*. This indicates possible alterations and inflammation effect of cadmium on testis and reproductive system as earlier reported by other authors.[3,6] This effect of cadmium was further seen in reproductive hormonal levels as there was a decrease in testosterone, FSH and LH levels of Cadmium groups compared to others. Though groups administered with quercetin and cadmium as well as cadmium and *Cyperus esculentus* showed higher levels of pro-inflammatory biomarkers compared to control, the increase was nothing compared to that of cadmium. The result of anti-inflammatory markers further ascertains the ability of both quercetin and *Cyperus esculentus* to ameliorate the toxicity effect of cadmium as there was an increase of IL-10 in groups of Cadmium+Quercetin and Cadmium+*Cyperus esculentus* compared to cadmium. *Cyperus esculentus* tends to offer a better anti-inflammatory effect following the significant increase recorded by Cadmium+*Cyperus esculentus* group compared to the control. Extract is known to mediate similar effect compared to those of orthodox drugs. In some cases, even better effect. Cases of such has been reported in liver functions and cytoprotections of the stomach.[19,20] Again, there are previous record of *Cyperus esculentus's* ability in ameliorating and correcting reproductive dysfunction or toxicity caused by high salt diet and lead.[11,12] This research agrees with these records and further confirms the effectiveness of the extract in ameliorating toxicity caused by cadmium. *Cyperus esculentus* was further found to correct the hormonal imbalance mediated by cadmium

as there was no significant different of testosterone, FSH and LH levels between Cadmium+Quercetin and Cadmium+*Cyperus esculentus* compared to control. As previously recorded, nutrient from dietary supplement and even herbal extract may be better remedy for ameliorating hormonal imbalance. Research on Tom brown weaning meal shows it ability to enhance reproductive function sequel to its enhancing and maintenance effect on reproductive hormone.[21] *Cyperus esculentus* also ameliorate cadmium toxicity on hormonal assay by restoring the levels of testosterone, FSH and LH.

CONCLUSION

Hydro ethanolic extract of *Cyperus esculentus* ameliorates cadmium toxicity on reproductive system of male rats by restoring the reproductive hormonal levels of male rats and with decrease pro-inflammatory biomarkers (TNFα, IL-6 and IL-1β) and increased anti-inflammatory biomarker (IL-10). *Cyperus esculentus* can therefore be recommended for patients exposed to cadmium in order to avoid cadmium toxicity on reproductive system.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CD: Cadmium; **QZ:** Quercetin; **$CdCl_2$:** Cadmium chloride; **CE:** *Cyperus esculentus;* **TNFα:** Tumor Necrotic Factor alpha; **IL-6:** Interleukin- 6; **IL-1β:** Interleukin-1 Beta; **IL-10:** Interleukin-10; **mg/kg b.w:** Milligram per kilogram body weight.

SUMMARY

Cyperus esculentus was found to ameliorates cadmium chloride induced testicular toxicity in male rats following male reproductive hormone, pro-inflammatory and anti-inflammatory analysis. The groups of animals used for this study were Control (NC), Cadmium (CD), Cadmium+Quercetin (CD+QZ) and Cadmium+Extract (CD+CE). $CdCl_2$ (5 mg/kg b.w.) and/or hydro-ethanolic extract of *Cyperus esculentus* (C.E extract) (1000 mg/kg b.w.) and quercetin (2 mg/kg) were given to the animals once daily for -28 days. Following laboratory findings, hydro ethanolic extract of *Cyperus esculentus* ameliorates cadmium toxicity on reproductive system of male rats by restoring the reproductive hormonal levels of male rats, decreasing pro-inflammatory biomarkers (TNFα, IL-6 and IL-1β) and increasing anti-inflammatory biomarker (IL-10). *Cyperus esculentus* can therefore be recommended for patients exposed to cadmium in order to avoid cadmium toxicity on reproductive system.

LIMITATION OF THE STUDY

This study was limited to male animals as assessment were done on parameters of male fertility hormone only and pro-inflammatory and anti-inflammatory biomarkers. More research should be carried out on female fertility parameters using *Cyrus esculentus* to ascertain its effect of female fertility. Also, molecular docking and Gas Chromatography Mass Spectrometry (GC-MS) should be carried out the extract to expose the phytochemical ligands and possible docking procedures done to throw more light on the signaling pathways through which *Cyperus esculentus* mediates these effects.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All experiments were performed in accordance with the guideline for care and use of laboratory animal of the Faculty of Basic Medical Science Animal Research Ethics Committee, University of Calabar, Cross River State. Ethical approval was granted by the committee with approval number UNICAL-FAREC-FBMS-02285.

AUTHOR CONTRIBUTION STATEMENT

Justina Nwandimma Nwangwa: Supervision of the work. Ekementeabasi Aniebo Umoh: manuscript writing. Esu Ukpai Enene: Laboratory work. Augustine L Udefa: Supervision.

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