https://doi.org/10.51483/AFJPS.4.1.2024.89-97 ISSN: 2789-5092

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Research Paper Open Access

Assessment of Testicular Recovery of Ethanolic Extracts of Calocybe Indica Following Cadmium Chloride-Induced Testicular Toxicity in Rats

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Article Info

Volume 4, Issue 1, March 2024
Received : 16 December 2023
Accepted : 17 February 2024
Published : 05 March 2024
doi: 10.51483/AFJPS.4.1.2024.89-97

Abstract

Occupational and environmental exposure to cadmium poses a harmful consequence inherent to its toxicity. In addition to harming target cells to varied degrees, it primarily affects the liver, kidney, and testes. It may be found in food, the atmosphere, and possibly even drinking water. In this study, the effect of the mushroom *Calocybe indica* (CLE) on rats' testicular toxicity caused by cadmium chloride ($CdCl_2$) is being evaluated. The six groups of experimental animals, each with five animals, were utilized. Groups B, C, D, E, and F received subcutaneous injections of (3 mg/kg $CdCl_2$), while Group A served as the experiment's control group. For 21 days, group C received an oral dose of 100 mg/kg of vitamin C. For 21 days, Groups D, E, and F received CLE treatments at doses of 200 mg/kg, 400 mg/kg, and 800 mg/kg respectively. It was evident that exposure to 3 mg/kg of cadmium chloride for three weeks resulted in substantial harm to the reproductive system. In this study, it has been established that administering the mushroom extract (*Calocybe indica*) to the rats induced with cadmium chloride testicular toxicity for three weeks resulted in to increase in antioxidant activity as well as a minor reduction in the toxicity of cadmium to the testicles.

Keywords: *Antioxidants, Cadmium, infertility, Calocybe indica, Testes, Toxicity*

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1. Introduction

Cadmium poisoning cases are reported from many regions of the world. It is a common health problem that can, under certain conditions, result in chronic illness as it impacts several organs. Chronic exposure to cadmium in the form of air, water, soil, and food can cause toxicity to the organ systems, including damage to the reproductive, cardiovascular, skeletal, urinary, central and peripheral nervous, and respiratory systems, as well as cancer (Rahimzadeh *et al.*, 2017). Heavy metal cadmium (Cd) is usually considered toxic and has no physiological use (Sinicropi *et al.*, 2010; Genchi *et al.*, 2020). Cadmium exposure has been observed in many different contexts over the past century, and it is widely present in the environment due to a variety of human activities (Rahimzadeh *et al.*, 2017). Cadmium contamination has persistent sources, including its use as a corrosive reagent in industry, its stabilizing qualities in color pigments, Nickel-Cadmium (Ni-Cd) batteries, and Polyvinyl Chloride (PVC) products (Genchi *et al.*, 2020). In areas with contaminated soils, exposure to house dust can also expose individuals to cadmium (Hogervost *et al.*, 2007). Anthropogenic sources of cadmium in the environment include the burning of fossil fuels, the smelting and refining of copper and nickel, and the use of phosphate fertilizers. Smelters of non-ferrous metals and recycling of electronic waste both contain cadmium as a contaminant. Volcanic activity, the slow process of rock and soil erosion and abrasion, and forest fires are some of the factors contributing to the rise in the amounts of Cd in the soil, water, and atmosphere where people live. This metal is also released into the atmosphere by lead, zinc, and copper mines, contaminating soil (Casado *et al.*, 2008). The gastrointestinal tract absorbs cadmium to a lesser extent than the respiratory system, and the skin absorbs it less frequently. Cadmium enters the body by complexing with blood constituents; erythrocytes and albumin, where it is then deposited in the kidneys, liver, and intestines (Satarug, 2018; Tinkov *et al.*, 2018). During lactation, cadmium is gradually removed from the body by the kidneys, urine, saliva, and milk. Humans suffer from kidney and liver problems, pulmonary oedema, testicular damage, osteomalacia, adrenal and hematopoietic system damage, and other conditions because of exposure to Cd. Furthermore, peripheral artery disease, coronary heart disease, stroke, and abnormalities in the atherogenic lipid profile were associated with blood and urine Cd exposure markers. In terms of pathophysiology, reactive oxygen species are produced when cadmium is present, which is what causes toxicity. According to Stohs *et al.* (2000), cadmium generates reactive oxygen species which damages cellular organelles. Although the precise molecular process through which cadmium produces free radicals is still unknown, reports suggest that it does so indirectly (Watkin *et al.*, 2003). It has been demonstrated that the interactions of reactive oxygen species with cellular biomolecules cause lipid peroxidation, damage to membrane proteins, changes to the antioxidant system, damage to DNA, gene expression alteration, and apoptosis (Stohs *et al.*, 2000). Moreover, the onset of harmful oxidative stress caused by cadmium may be considerably aided by the depletion of endogenous antioxidants such as glutathione (Bagchi *et al.*, 1996) including catalase, superoxide dismutase, and copper-zinc superoxide dismutase. Cells that suffer damage experience necrosis or apoptosis if repair mechanisms fail to counteract these ROS-mediated stress events (Thevenod, 2003). In another perspective, metallothionein is a zinc-concentrating protein with free radical-scavenging properties. According to Han *et al.* (2015), cells that do not produce metallothionein are susceptible to the harmful effects of cadmium, while those that do are resistant to those effects. The expression of metallothionein influences the occurrence of necrosis or apoptosis in Cd-induced toxicity (Renugadevi and Prabu, 2010). It is also imperative to know that apart from the diseases associated with cadmium poisoning those who are affected may also stand the chance to be exposed to drug-induced injuries associated with such medications usable in treatment of cadmium-induced toxicity. Also, the cost of the treatment of cadmium toxicity is highly exorbitant. As a result of this, there is a need for inexpensive complementary medicine. Recently, research has been conducted on the potential medicinal benefits of alternative medicines, which include extracts from higher fungi such as mushrooms. *Calocybe indica* (*C. indica*) is a good target for alternative and complementary medicine owing to numerous bioactive polysaccharides, including glucans, and polyphenols, alkaloids that are extracted from them. Other phytochemicals include flavonoids, saponins, tannins, phenols, coumarins, quinones, thiols, terpenoids, cardiac glycosides, proteins, and steroids (Wu *et al.*, 2021; Niego *et al.*, 2021) with substantial health benefits such as antioxidant, anticancer, immunological stimulant and the protective effects on the liver (Kumar *et al.*, 2021; Akor *et al.*, 2024; Odu *et al.*, 2023). By scavenging free radicals or reactive oxygen species, bioactive compounds present in *Calocybe indica* reduce the risk of tissue damage which is associated with testicular toxicity (Lobo *et al.*, 2010). Keeping the foregoing

in mind, the goal of this study investigates the effect of *Calocybe indica* on oxidative damage caused by Cd in the testes of rats.

2. Materials and Methods

2.1. Materials

2.1.1. Plant Material

The *Calocybe indica* fruiting bodies used in this study came from Radeagro Allied Limited, a commercial farm in Lagos State, Nigeria (6°272 182 2 N 3°232 032 2 E). Following identification, a voucher sample was maintained in the herbarium museum in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

2.1.2 Experimental Animals

The rat colony maintained at the Animal House in the University of Nigeria, Nsukka's Faculty of Veterinary Medicine, provided the thirty adult male Sprague-Dawley rats utilized in this investigation, which weighed 180 ± 20 g and were 10-12 weeks old. Every animal was housed in facilities featuring a light/dark cycle lasting twelve hours, unrestricted access to tap water, and free access to standard lab food. The animals had a week to acclimate before the trial started. Animal Care and Use Committee of the Faculty of Veterinary Medicine of our university granted ethical permission for this study following the experimental procedures recommended by the National Institute of Health Guide for Care and Use of Laboratory Animals (NRC, 2011).

2.2. Methods

2.2.1 Hydro-Ethanol Extract of Mushrooms

Minor modifications were made to the methods of Boonsong *et al.* (2015) and Sudha *et al.* (2016) for the hydro-ethanol extraction of mushrooms. After ten days of shade drying, the mushroom was ground into a powder using a grinding machine. After being manually shaken every two hours for 72 hours, a solution of 70% ethanol was used to soak 500 g of the powdered material. Whatman paper (No. 1) was then used to filter the mixture, and a rotary evaporator was used to concentrate it. Until they were needed, the dried extracts of both mushrooms were kept in a refrigerator at 4 °C.

2.2.2. Investigation of Acute Toxicity of Mushroom Extracts

The Organization for Economic Cooperation and Development (OECD) guideline 423 for evaluating chemicals was followed in the assessment of the acute toxicity of extracts from *Calocybe indica* (OECD, 2003).

2.2.3. Experimental Design

Each group of six animals contained five rats. The group given Cd alone treatment got one subcutaneous injection of 3 mg/kg $CdCl_2$, while the control group received a standard saline injection. As the conventional medication, Vitamin C was used daily to treat one group injected with cadmium; the other groups received low, medium, and high dosages of CLE. The grouping of the animals was as follows:

Group A: - Control (distilled water)

Group B: - 3 mg/ kg $CdCl_2$

Group C: - 3 mg/ kg $CdCl_2$ + vitamin C

Group D: - 3 mg/ kg $CdCl_2$ + 200 mg/kg CLE

Group E: - 3 mg/ kg $CdCl_2$ + 400 mg/kg CLE

Group F: - 3 mg/ kg $CdCl_2$ + 800 mg/kg CLE

2.2.4. Sample Collection

Following intraperitoneal injections of ketamine hydrochloride dosage of 90 mg/kg body weight and xylazine dosage of 5 mg/kg body weight for euthanasia, the rats were allowed to fast for the entire night before 2 mL of blood was drawn via the retroorbital plexus and placed into straightforward sample vials (Zarei and Shahrooz,

2019). Testes that were paired off were quickly separated, cleaned in 0.9% cold normal saline, and weighed. After allowing the blood in the unadorned sample bottles to coagulate, the 3000 g sample was centrifuged.

2.2.5. Spermatogenic Activity

The hemocytometric method was used to determine the epididymal sperm count, and a sensitive Mettler weighing balance (produced by Mettler Toledo, Switzerland) was utilized to measure the testicular and epididymal weights (Obembe and Ige, 2016).

2.2.6. Hormonal Assay

The amount of testosterone was quantified using an ELISA kit in compliance with the guidelines provided by the manufacturer.

2.2.7. Lipid Peroxidation and Antioxidant Assay

As previously reported (Ohkawa *et al.*, 1979), malondialdehyde (MDA), a lipid peroxidation biomarker, was assessed using a spectrophotometric technique, and the activity of superoxide dismutase (SOD) and catalase (CAT) was measured using conventional methods (Nishikimi *et al.*, 1972; Hadwan, 2018).

2.2.8. Statistical Analysis

A one-way analysis of variance (ANOVA) was performed on the data using SPSS version 22.0, the Statistical Package for Social Sciences. At $p \leq 0.05$, the mean differences were deemed statistically significant. The statistical program used for the analysis was Prism 6 GraphPad (GraphPad Software, Inc. San Diego, California).

3. Results

3.1. Effect of Calocybe Indica Extract on Body Weight, Testis Weight and Testicular Index on Cadmium Chloride Intoxicated Rats

Table 1 shows the effects of *Calocybe indica* extract on rats' body weight, testicular weight, and testicular index after they were given cadmium chloride. Rats given cadmium chloride alone (group B) had a lower body weight compared to the control group, however, there was no statistically significant difference ($p > 0.05$). The rats intoxicated with cadmium chloride did not gain as much body weight as the control group, but the group given 800 mg/kg of *Calocybe indica* had a mean body weight that was considerably ($p \leq 0.05$) lower than that of the control and untreated cadmium chloride group B. Additionally, the testicular weight and index of the intoxicated rats were lower than the control.

3.2. Effect of Calocybe Indica Extract on the Mean Epididymal Sperm Count and Mean Serum Testosterone Concentration of Rats Intoxicated with Cadmium Chloride

The mean epididymal sperm count as indicated in Table 2 below for rats intoxicated with cadmium chloride when compared to the control was significantly ($P < 0.05$) lower, whereas the mean epididymal sperm count of groups treated with *Calocybe indica* extract was considerably ($P < 0.05$) less than the control and not considerably ($P > 0.05$) higher compared to the mean epididymal sperm count of untreated rats intoxicated with cadmium chloride. Table 2 also illustrates that the mean serum testosterone concentration of rats intoxicated with cadmium chloride was significantly ($P < 0.05$) lower than that of the control, while the mean serum testosterone concentration of groups treated with *Calocybe indica* extract was lower than that of the control but higher than that of those rats intoxicated with cadmium chloride without treatment.

3.3. Effect of Calocybe indica Extract on Antioxidant Enzyme Activity and Lipid Peroxidation in the Testis of Rats Intoxicated with Cadmium Chloride

Treatment with *Calocybe indica* extract (CLE) resulted in a non-significant ($p > 0.05$) reduction in MDA concentration compared to the untreated cadmium chloride intoxicated group B, while the untreated group B revealed a non-significant ($p < 0.05$) increase in MDA compared to the control group. Table 3 also demonstrates that the untreated cadmium chloride intoxicated group B's CAT and SOD activity compared to the control group were significantly ($p < 0.05$) lower, while SOD activity increased significantly ($p > 0.05$) after receiving CLE treatment.

Table 1: Effect of *Calocybe indica* Extract on Rats given Cadmium Chloride Intoxication in Terms of Body Weight, Testis Weight, and Testicular Index

Groups/Parameters	Body Weight (g)	Testis Weight (g)	Testicular Index (%)
A – Control	200.33 ± 26.77	2.49 ± 0.43^{a}	1.27 ± 0.24^{a}
B – 3 mg/kg $CdCl_2$	197.33 ± 7.31	0.93 ± 0.04^{b}	0.93 ± 0.04^{b}
C – 3 mg/kg $CdCl_2$ + 100 mg/kg vitamin C	210.67 ± 10.68	1.63 ± 0.08^{c}	0.78 ± 0.07^{b}
D – 3 mg/kg $CdCl_2$ + 200 mg/kg CLE	216.67 ± 16.76	1.40 ± 0.08^{bc}	0.66 ± 0.09^{b}
E – 3 mg/kg $CdCl_2$ + 400 mg/kg CLE	219.00 ± 6.08	1.44 ± 0.11^{bc}	0.66 ± 0.05^{b}
F – 3 mg/kg $CdCl_2$ + 800 mg/kg CLE	180.00 ± 22.91	0.98 ± 0.08^{b}	0.55 ± 0.03^{b}

Note: A significant difference ($p \leq 0.05$) is observed between mean values in the same column that have distinct superscripts. g = gram; $CdCl_2$ = cadmium chloride; CLE = *Calocybe indica*; mg = milligrams; kg = kilograms.

Table 2: Effect of *Calocybe indica* Extract on Epididymal Sperm Count and Serum Testosterone Concentration of Rats Intoxicated with Cadmium Chloride

Groups/Parameters	Sperm Count ×10^6/ml	Serum Testosterone (ng/ml)
A – Control	171.03 ± 3.38^{a}	2.68 ± 0.46^{a}
B – 3 mg/kg $CdCl_2$	60.27 ± 3.49^{b}	0.55 ± 0.06^{b}
C – 3 mg/kg $CdCl_2$ + 100 mg/kg vitamin C	73.13 ± 1.96^{c}	0.54 ± 0.04^{b}
D – 3 mg/kg $CdCl_2$ + 200 mg/kg CLE	69.33 ± 0.71^{c}	0.59 ± 0.07^{b}
E – 3 mg/kg $CdCl_2$ + 400 mg/kg CLE	71.20 ± 1.41^{c}	0.93 ± 0.03^{b}
F – 3 mg/kg $CdCl_2$ + 800 mg/kg CLE	76.00 ± 2.66^{c}	0.66 ± 0.02^{b}

Note: A significant difference ($p \leq 0.05$) is observed between mean values in the same column that have distinct superscripts. g = gram; $CdCl_2$ = cadmium chloride; CLE = *Calocybe indica*; mg = milligrams; kg = kilograms

Table 3: Effect of *Calocybe indica* Extract on Antioxidant Enzyme Activity and Lipid Peroxidation in the Testis of Rats Intoxicated with Cadmium Chloride

Groups/Parameters	MDA (IU/mg Protein)	CAT (IU/mg Protein)	SOD (IU/mg Protein)
A – Control	50.63 ± 3.82	52.40 ± 8.05^{ac}	81.85 ± 3.48^{a}
B – 3 mg/kg $CdCl_2$	91.85 ± 36.63	35.92 ± 1.87^{ab}	51.81 ± 1.20^{b}
C – 3 mg/kg $CdCl_2$ + 100 mg/kg vitamin C	62.28 ± 1.99	41.60 ± 3.53^{ac}	65.49 ± 3.83^{c}
D – 3 mg/kg $CdCl_2$ + 200 mg/kg CLE	82.28 ± 4.16	39.27 ± 4.68^{ac}	66.48 ± 2.85^{c}
E – 3 mg/kg $CdCl_2$ + 400 mg/kg CLE	86.01 ± 1.46	47.34 ± 5.00^{ac}	58.61 ± 2.52^{bc}
F – 3 mg/kg $CdCl_2$ + 800 mg/kg CLE	75.60 ± 2.83	54.89 ± 6.75^{c}	67.82 ± 5.24^{c}

Note: A significant difference ($p \leq 0.05$) is observed between mean values in the same column that have distinct superscripts. g = gram; $CdCl_2$ = cadmium chloride; CLE = *Calocybe indica*; mg = milligrams; kg = kilograms.

4. Discussion

One heavy metal that is commonly found in the atmosphere is cadmium, which is also very toxic and lethal. According to Jaishankar *et al.* (2014), cigarette smoke, tainted food, water, and air are the main ways that people are exposed to cadmium. By upsetting the blood testis barrier, causing germ cell death, testicular edema, haemorrhage, and necrosis, cadmium can harm the physiology and anatomy of reproductive organs and could trigger infertility that may not be reversed (Massányi *et al.*, 2020). Table 1 data analysis reveals that the groups receiving CLE and vitamin C reduced the negative effects of Cd; however, unlike Group F, this effect is not dose dependent. Higher doses of the CLE were found to be toxic to rats, resulting in a significant

reduction in testicular index, body weight, and testis weight. The significant seminiferous tubule necrosis and testicular interstitial inflammation demonstrated that the overdose of CLE extract and the acute toxicity of cadmium on the testes were probably the causes of the decrease in testicular weights seen in animals receiving cadmium (Group B and F). Though previous studies have reported rapid weight loss (Massányi *et al.*, 2020). As a result, it was determined that 400 mg/kg would be the maximum CLE dose that would be safe for this investigation. The results of this study show that the sperm count and serum testosterone levels of the $CdCl_2$ treatment group were significantly lower than those of the control group. Research has previously demonstrated that male fertility is negatively impacted by Cd exposure due to pathology that reduces sperm counts (López-Botella *et al.*, 2021; Zhao *et al.*, 2017). In comparison to the untreated group, Table 2 demonstrates a significant difference in the reduction of CdCl2 toxicity in the treated groups (D to F). Previous research has shown that the testis is more susceptible to cadmium than other body organs. Additionally, sperm count is one of the most important factors to look at when evaluating spermatogenesis and fertility (Kumar *et al.*, 2021; Omu, 2019). Cadmium can also affect how well the testicles' function (Skolarczyk *et al.*, 2018). The results of this study also indicated that cadmium treatment decreased the number, motility, and percentage of live sperm in rats, which is consistent with previous research showing that cadmium inhibits testicular function (López-Botella *et al.*, 2021; Zhao *et al.*, 2017; Zhu *et al.*, 2020; Ali *et al.*, 2022). According to the current study, decreased sperm production in the testes may be associated with lower serum concentrations of testosterone, luteinizing hormone (LH), and/or follicle stimulating hormone (FSH). Thus, at higher dosages and/or for longer study periods, it can be claimed that CLE can reverse or mitigate the testicular damage caused by Cd. One biomarker for oxidative stress that naturally exists in living cells is MDA (Nair *et al.*, 2008). In comparison to the control group, there was a rise in the protein level after the $CdCl_2$ was administered. This suggests that oxidative stress is induced by $CdCl_2$ toxicity, as observed in earlier research (Jahangir *et al.*, 2006), but that CLE treatment reduced oxidative stress in comparison to the untreated group (Group B). The MDA levels in the treated groups were accompanied by a corresponding decrease in CAT and SOD levels when compared to the control group. However, when treated with a higher dose of CLE extract than the control group, an increase in MDA and CAT activity was observed. It can be argued that CLE is somewhat ameliorative and that the toxic effect increases with dose, except for MDA and CAT, where lower levels were observed than in other groups treated with CLE. There could be multiple functional factors causing this. Oxidative stress, which lowers antioxidant capacity and causes significant metabolic changes, including weight loss, is what causes the toxic effects of cadmium (Genchi *et al.*, 2020).

5. Conclusion

It has been demonstrated from this study beyond doubt that three weeks of exposure to 3 mg/kg of cadmium chloride caused a considerable degree of reproductive injuries. However, despite that this study was limited by the absence of histopathological examination, *Calocybe indica* extract showed positive effects on restoring physiological parameters that indicate testicular damage. Enzyme activities and other assay parameter concentrations were able to be reinstated to levels like the control. Furthermore, it was discovered that administering the mushroom (*Calocybe indica*) to rats given cadmium chloride for three weeks resulted in a notable increase in antioxidant activity as well as a slight improvement in the toxicity of the cadmium-induced testicles. The potential protective mechanism of *Calocybe indica* may stem, at least in part, from its antioxidant properties. Subsequent molecular research may validate *Calocybe indica* to have contained bioactive compounds useful in treating testicular lesions and possibly a pathway for discovery of drug candidate.

Conflicts of Interest

The authors alone are responsible for the content and writing of the paper and hereby declare no conflict of interest.

Funding

No funding was received.

Availability of Data and Materials

All data related to this study are included herein, otherwise available on request. Ethical Approval and Consent to Participate Animal Care and Use Committee of the Faculty of Veterinary Medicine of our university granted ethical permission for this study following the experimental procedures recommended by the National Institute of Health Guide for Care and Use of Laboratory Animals.

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Cite this article as: Joseph Akor, Faith Lebechi Akor, Esther Ifunanya Chude, Nwamaka Maureen Odu, Angela Ifeanyi Odenigbo and Ebuka Leonard Onyeyilim (2024). Assessment of Testicular Recovery of Ethanolic Extracts of Calocybe Indica Following Cadmium Chloride-Induced Testicular Toxicity in Rats. *African Journal of Pharmaceutical Sciences,* 4(1), 89-97. doi: 10.51483/AFJPS.4.1.2024.89-97.