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Original Research Article

Therapeutic potential of methanol extracts of *Calocybe indica* (mushroom) on cadmium chloride-induced hepato-renal toxicity in rats

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Abstract

Purpose: *To investigate the efficacy of Calocybe indica extract (CIE) in alleviating liver and kidney toxicity caused by cadmium chloride ($CdCl_2$) in rats.*

Methods: *Six groups of five rats each were used in this study. Group A was the control while groups B to F received 3 mg/kg $CdCl_2$ subcutaneously. Group B was induced with $CdCl_2$ alone for 21 days. Orally, 100 mg/kg of vitamin C, and 200, 400, and 800 mg/kg of CIE were used to treat groups C, D, E, and F respectively. Data were analyzed using Statistical Packages for Social Sciences (SPSS), and results were presented as mean ± standard deviation (SD).*

Results: *Group B had higher liver and kidney weights, and lower body weight compared to control group ($p \geq 0.05$). Treatment with CIE increased body weight in $CdCl_2$-induced rats lowers serum levels of alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, and total bilirubin, improves liver and kidney function, and significantly increased superoxide dismutase (SOD) activity ($p < 0.05$).*

Conclusion: *Calocybe indica (CIE) extract increases body weight, lowers serum levels of liver enzymes, improves kidney function and significantly lowers SOD activity. Calocybe indica extract may serve as a potential pharmacological candidate or therapeutic alternative for managing hepato-renal injuries. Subsequent molecular studies to ascertain its bioactive compounds will pave the way for the discovery of drug candidates.*

Keywords: *Cadmium toxicity, Liver injury, Herbal medicine, Antioxidant therapy, Calocybe indica extract*

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INTRODUCTION

Cadmium (Cd), a toxic transition metal, poses significant health risks to both humans and animals due to its extensive environmental distribution and lack of a physiological role. Human activities, such as pesticide use, sewage sludge application, and phosphate fertilizer use, contribute to elevated cadmium concentrations in soils and groundwater [1]. Also, mining and using cadmium in industrial processes, such as battery manufacturing, further contaminate agrarian soils. Plants absorb cadmium from contaminated soil, leading to lethal concentrations that pose significant risks to organisms. With a prolonged physiological half-life (10–30 years), cadmium builds up in organs such as the liver, kidneys, and testes, causing cellular degeneration, necrosis, and carcinogenic effects [2].

Liver damage from cadmium exposure is characterized by a loss of hepatocyte membrane integrity, with increased expression of liver enzymes such as AST, ALT, ALP, and LDH indicating pathological lesion. Additionally, antioxidant enzymes and liver glycogen levels are reduced, indicating impaired liver function [3]. In the kidneys, cadmium exposure leads to glomerular swelling and renal injury. Cadmium also induces oxidative stress by decreasing antioxidant enzyme activity and promoting lipid peroxidation [2,4].

Herbal medicine, particularly antioxidant-rich plants has the potential to protect against environmental toxins like cadmium. Bioactive compounds in plants, such as phenolic compounds, terpenoids, and polysaccharides, neutralize free radicals, supporting antioxidant defenses [5-7]. *Calocybe indica*, a tropical mushroom, is known for its high concentration of non-enzymatic antioxidants and essential nutrients. Its extract has been shown to have anti-lipid peroxidation, anti-hyperglycemic, and immune-stimulating properties [8,9]. However, there is limited scientific literature on its effects on hepato-renal toxicity. Therefore, this study investigates the efficacy of *Calocybe indica* extract in treating hepato-renal lesions in rats.

EXPERIMENTAL

Materials

Mushroom

Samples of *Calocybe indica* used in this study were purchased from Radeagro Allied Limited (Lagos State, Nigeria (6°27′18″N 3°23′03″E)). Following identification, a voucher sample was deposited in the Department of Plant Science and Biotechnology herbarium museum at the University of Nigeria, Nsukka.

Animals

A total of 30 adult rats, weighing 180 ± 20 g and aged 10 to 12 weeks, were obtained from the Animal House rat colony at the University of Nigeria, Nsukka, managed by the Faculty of Veterinary Medicine. Each rat was maintained under standard conditions (12-hour light/dark cycle with unrestricted access to food and water) for acclimatization. Ethical approval was obtained from the Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Nigeria Nsukka (approval no. FVM/UN2021/1/22) and complied with the recommendations of the National Research Council (US) for the Care and Use of Laboratory Animals [10].

Hydro-methanol extraction of mushrooms

Hydro-methanol extraction of the mushrooms was conducted following the method of Boonsong *et al* [11] with modifications. The mushrooms were air-dried for 10 days and ground into fine powder using a mill. Thereafter, 70 % methanol solution was used to soak 500 g of the powdered material, and the mixture was manually shaken every 2 h for 72 h. The extract was filtered using Whatman No. 1 filter paper, and a rotary evaporator was employed to concentrate the filtrate. Thereafter, the dehydrated extracts were kept at 4 °C in a refrigerator until needed.

Investigation of acute toxicity of *Calocybe indica* extract

The Organization for Economic Cooperation and Development (OECD) [12] protocol 423 for evaluating chemicals was followed in the assessment of the acute toxicity of the extract from *Calocybe indica*. Adult male rats were given oral doses of the extract (200, 400, and 800 mg/kg), and their acute morbidity and mortality were monitored. Rats given 800 mg/kg dose of the extract exhibited no symptoms of pathological lesion or death. As a result, the median lethal dose (LD_{50}) exceeded 800 mg/kg body weight.

Treatments

After a brief acclimation period of 14 days, the rats were divided into 6 equal groups of five rats each, ensuring similar body weights across the groups: Group A was the control, Group B received $CdCl_2$ (3 mg/kg only), Group C received

3 mg/kg each of $CdCl_2$ and vitamin C, Group D received 3 mg/kg $CdCl_2$ and treated with 200 mg/kg CIE, Group E received 3 mg/kg $CdCl_2$ and treated with 400 mg/kg CIE, Group F received 3 mg/kg $CdCl_2$ and treated with 1000 mg/kg CIE). Cadmium chloride ($CdCl_2$) was delivered through the subcutaneous route into the rats at a concentration of 3 mg/kg body weight per day for four weeks to induce hepato-renal lesions. The rats were simultaneously treated with different mushroom extracts every day for 2 weeks. The optimal dose of vitamin C for treating hepato-renal lesions and the cadmium chloride dose used to induce the lesions were applied as previously recommended [13].

Animal sacrifice and sampling

After 24 h, 4 mL of blood was drawn via the orbital plexus and collected into two specimen bottles one containing EDTA and the other without any reagent, for serum extraction. The rats were fasted overnight [14]. Serum was obtained for biochemical analyses by centrifuging the blood samples at 1000 g for 10 min. After dissection, the liver and kidneys were excised for lipid peroxidation assays and antioxidant enzyme activity tests.

Evaluation of parameters/indices

Levels of malondialdehyde (MDA)

A spectrophotometric method was used to measure malondialdehyde (MDA), a biomarker of lipid peroxidation, by reacting it with thiobarbituric acid to form a pink-colored MDA-TBA adduct, which was quantified at 532 nm [15].

Serum protein

The Bromocresol Green technique was used to quantify albumin levels, while the direct biuret method was used to evaluate serum total protein *in vitro* [16]. Serum creatinine was assessed *in vitro* with the Quimica Clinica Applicada (QCA) Creatinine test kit (QCA, Spain), following a modified Jaffe technique [17]. Randox enzyme test kits were used to measure the activity of the enzymes alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Thereafter, 500 µL of the reagent and 10 µL of the sample were mixed in a cuvette, and absorbance was measured at 405 nm at the beginning and 3 min later. Enzyme activity was calculated based on the mean absorbance per minute [16].

Antioxidant assay

Determination of superoxide dismutase and catalase activities was conducted following the method of Hadwan *et al* [18], involving the measurement of individual tissue samples using a spectrophotometer and cuvette.

Statistical analysis

Data were analyzed using Statistical Packages for Social Sciences, (SPSS version 22.0, IBM, Armonk, NY, USA) and GraphPad Prism 7.0 (GraphPad Software, Inc. San Diego, California, USA). Measurement data was compared using one-way analysis of variance (ANOVA).

RESULTS

Effect of *Calocybe indica* extract on body weight, organ weight, and organismal indices of cadmium chloride-induced rats

Body weight and liver index were higher in rats treated with CIE compared to control group ($p > 0.05$; Table 1).

Effect of CIE on rat serum biochemistry after cadmium chloride intoxication

Treatment with vitamin C (Group C) and CIE significantly reduced levels of ALT, AST, ALP, total bilirubin, creatinine, and urea ($p < 0.05$).

Table 1: Effect of *Calocybe indica* extract on body weight, liver weight, kidney weight and organosomatic indices of rats intoxicated with cadmium chloride

Group	Body weight (g)	Liver weight (g)	Liver index (%)	Kidney weight (g)	Kidney index (%)
A-Control	200.33±26.77	8.50±0.21	1.55±0.19	4.19±0.40	0.75±0.03
B-3mg/kg $CdCl_2$	197.33±7.31	8.56±0.28	1.34±0.06	4.3 ±0.28	0.69±0.06
C-3mg/kg $CdCl_2$+100mg/kg Vit C	210.67±10.68	8.71±0.15	1.37±0.07	4.18±0.28	0.65±0.02
D-3mg/kg $CdCl_2$+200mg/kg CIE	216.67±16.76	8.56±0.22	1.40±0.08	3.99±0.23	0.65±0.03
E-3mg/kg $CdCl_2$+400mg/kg CIE	219.00±6.08	8.67±0.23	1.45±0.06	3.96±0.10	0.66±0.03
F-3mg/kg $CdCl_2$+800mg/kg CIE	180.00±22.91	8.31±0.12	1.35±0.05	4.24±0.15	0.69±0.04

Table 2: Effect of *Calocybe indica* extract on serum biochemistry of rats intoxicated with cadmium chloride

Parameter	Group					
	A	***B***	***C***	***D***	***E***	***F***
AST (IU/L)	66.88±1.8*	91.84±2.45$^{\#}$	76.22±7.51*$^{\&}$	82.92±3.39$^{\#\&}$	83.90±3.39$^{\#\&}$	75.20±6.09*$^{\&}$
ALT (IU/L)	26.56±0.66*	35.50±4.57$^{\#}$	29.90±2.08*$^{\#}$	30.10±2.04*$^{\#}$	29.88±2.90*$^{\#}$	29.09±1.48*$^{\#}$
ALP (IU/L)	99.31±13.89*	159.52±16.15$^{\#}$	23.29±15.47*$^{\#}$	119.86±7.41*	127.0±10.80*$^{\#}$	124.83±1.99*$^{\#}$
Bilirubin (mg/dL)	0.15±0.01	0.84±0.24	0.66±0.32	0.79±0.33	0.66±0.35	0.53±0.37
Total Protection (mg/dL)	10.27±0.10*	8.53±0.70$^{\#}$	10.46±0.30*	9.37±0.11*$^{\#}$	9.58±0.51*$^{\#}$	9.92±0.24*
Albumin (g/dL)	5.05±0.05*	3.72±0.16$^{\#}$	4.09±0.22*$^{\#}$	3.96±0.09$^{\#}$c	3.96±0.14*$^{\#}$	3.96±0.14*$^{\#}$
Globulin (g/dL)	5.22±0.15*$^{\&}$	4.50±0.52$^{\#}$	6.38±0.14$^{\&}$	5.42±0.12*$^{\#\&}$	5.64±0.41*$^{\&}$	5.63±.41*$^{\&}$
Creatinine (g/dL)	1.46±0.22*	2.35±0.26$^{\#}$	2.17±0.37$^{\#}$	1.91±0.08*$^{\#}$	1.75±0.01*$^{\#}$	1.76±0.01*$^{\#}$
Urea (g/dL)	7.63±2.56*	26.50±1.53$^{\#}$	23.79±2.87*$^{\#}$	23.67±3.22*$^{\#}$	22.43±1.84*$^{\#}$	22.43±4.12*$^{\#}$

A – control, B – 3 mg/kg $CdCl_2$, C – 3 mg/kg $CdCl_2$ + 100 mg/kg vitamin C, D – 3 mg/kg $CdCl_2$ + 200 mg/kg CIE, E – 3 mg/kg $CdCl_2$ + 400 mg/kg CIE, F – 3 mg/kg $CdCl_2$ + 800 mg/kg CIE. *$^{\#\&}$*P* < 0.05

Table 3: Effect of *Calocybe indica* extract on lipid peroxidation and antioxidant enzyme activity in the liver and kidney of cadmium chloride intoxicated rat

Group	Tissues	MDA (nmol /g. protein)	SOD (U/g. protein)	CAT (U/g. protein)
A-Control	Liver	44.92±1.10	68.08±1.65*	90.38±1.17*
	Kidney	126.65±0.62	102.15±2.59*	62.02±1.24*
B-3mg/kg $CdCl_2$	Liver	62.35±1.71	43.83±1.00$^{\#}$	63.44±1.00$^{\#\&}$
	Kidney	209.20±5.86	63.81±1.10$^{\#}$	38.41±0.87$^{\#}$
C-3mg/kg $CdCl_2$+100mg/kg Vit C	Liver	33.28±1.99	52.48±2.85$^{\&}$	79.27±4.68$^{\&}$
	Kidney	138.17±6.18	85.16±2.52$^{\&}$	39.27±4.68$^{\&}$
D-3mg/kg $CdCl_2$+200mg/kg CIE	Liver	53.34±4.96	51.87±0.49	77.55±3.27
	Kidney	160.02±4.73	84.60±6.79	50.66±3.27
E-3mg/kg $CdCl_2$+400mg/kg CIE	Liver	50.18±2.87	82.91±0.81$^{\&}$	57.47±3.88$^{\&}$
	Kidney	162.52±3.15	54.66±3.27$^{\&}$	90.14±4.96*
F-3mg/kg $CdCl_2$+800mg/kg CIE	Liver	46.60±2.83	54.66±3.27$^{\&}$	90.14±4.96*
	Kidney	151.01±1.46	88.87±0.49*$^{\&}$	41.60±3.5$^{\#\&}$

*$^{\#\&}$*p* < 0.05 significantly different with varying treatment. g = gram; $CdCl_2$ = cadmium chloride, CIE = *Calocybe indica*, mg = milligrams; kg = kilograms

Effect of *Calocybe indica* extract on lipid peroxidation and antioxidant enzyme function in hepatic and renal tissues of rats induced with cadmium chloride

When compared to untreated cadmium chloride-induced group B, Treatment with *Calocybe indica* extract (CIE) caused a non-significant ($p > 0.05$) decrease in MDA levels. Furthermore, cadmium-chloride induction resulted in significantly lower CAT and SOD levels compared to control and treated groups ($p < 0.05$).

DISCUSSION

Cadmium-induced oxidative stress has been shown to cause damage to organs, particularly the liver and kidneys [3]. This study assessed the potential of a hydro-methanolic extract of *Calocybe indica* to reduce the effects of cadmium-induced hepato-renal toxicity in rats. The findings revealed that acute exposure to cadmium led to oxidative damage to both the liver and kidneys. Hepatocellular necrosis, which alters cell membrane permeability, leads to decreased levels of albumin and total protein, as well as increased serum levels of AST, ALT, ALP, and bilirubin. These demonstrate the hepatotoxic effects of Cadmium (Cd) which is in tandem with prior findings [1,3].

Hepatocellular diseases are indicated by elevated ALT and AST that are out of proportion to ALP and bilirubin. An abnormally high level of ALP and bilirubin in ALT and AST indicates cholestasis [1]. The function and condition of liver cells are closely connected to serum ALP levels. Elevated synthesis in the context of rising biliary pressure is the reason for the rise in serum ALP [4]. Treatment with CIE (200, 400, and 800 mg/kg) reduced the high concentrations of ALP, AST, and ALT enzymes in rats following induction with Cd. Furthermore, it has been observed that rats induced with cadmium exhibited elevated serum levels of bilirubin. This rise in bilirubin is a definitive indicator of hepatic dysfunction [1,4]. Also, cadmium causes heme catabolism, which raises blood serum bilirubin levels [14,15]. Treatment with CIE (200, 400, and 800 mg/kg body weight) significantly reduced serum bilirubin levels in cadmium-induced rats. This reduction indicates that CIE effectively mitigates hepatocyte damage induced by

cadmium exposure. Albumin and total protein serve as key biomarkers of liver function, and a decrease in their levels reflects impaired hepatic biosynthetic activity and potential loss due to cadmium-induced glomerular and tubular injury [3]. Following CIE treatment, serum albumin, and total protein levels significantly increased, demonstrating the hepatoprotective effect of CIE in restoring hepatic synthetic function. In rats induced with cadmium, serum globulin levels were reduced, consistent with findings by Genchi *et al* [1], which attribute low globulin levels to compromised antibody production. Conversely, all CIE-treated groups exhibited elevated serum globulin levels, indicating that CIE supports the recovery of protein synthesis and immune function.

Cadmium exposure primarily targets the kidneys, where it accumulates in the proximal tubules following glomerular filtration, leading to nephrotoxicity and renal failure [4,3]. This process results in elevated serum levels of creatinine and urea, which are metabolic waste products normally excreted via renal filtration. Treatment with CIE significantly reduced serum creatinine and urea concentrations, indicating that CIE alleviates cadmium-induced renal toxicity and minimizes structural damage to renal tubules and glomeruli. Furthermore, cadmium induction increases malondialdehyde (MDA) concentration in liver and kidney tissues, indicating lipid peroxidation and oxidative stress. Treatment with CIE significantly decreased MDA concentrations, demonstrating the efficacy of the extract in reducing oxidative damage to tissues. Given that MDA is a biomarker for oxidative tissue injury [9], its reduction underscores the antioxidant properties of CIE.

Antioxidant enzymes, such as catalase (CAT) and superoxide dismutase (SOD), are essential for shielding cells from oxidative damage. In this investigation, SOD and CAT activities in liver and kidney tissues were significantly inhibited by cadmium toxicity. However, treatment with CIE restored the enzymatic functions of these antioxidants, reinforcing its role in enhancing the antioxidant defense system and mitigating oxidative stress caused by cadmium exposure [1,2]. Although this study lacked histopathological examination, *Calocybe indica* extract demonstrated significant potential in restoring physiological biomarkers of liver function. Enzyme activity and other metabolic parameters demonstrated the restoration of normal liver function. Additionally, administering *Calocybe indica* to cadmium chloride-induced rats for two weeks led to a significant increase in antioxidant activity and normalization of metabolic processes. This indicates that the protective mechanism of *Calocybe indica* may be due, at least in part, to its antioxidant properties.

Limitations of the study

There is the absence of histopathological analysis which would have provided a more detailed and direct examination of tissue damage caused by cadmium chloride and the protective effects of *Calocybe indica* extract. Histopathological assessments, such as tissue sectioning and microscopic examination, could have unveiled more cellular alterations, inflammation, and structural damage at the microscopic level, complementing the biochemical and metabolic findings. Incorporating histological analysis in future studies would help strengthen the understanding of underlying mechanisms and provide a more comprehensive evaluation of the therapeutic potentials of *Calocybe indica* against hepato-renal injuries.

CONCLUSION

Calocybe indica extract effectively reduces cadmium-induced hepato-renal toxicity by improving liver and kidney function, reducing oxidative damage, and enhancing antioxidant enzyme activity. Therefore, *Calocybe indica* extract may serve as a potential pharmacological candidate or therapeutic alternative for managing hepato-renal injuries. Subsequent molecular studies may validate *Calocybe indica* to have contained bioactive compounds useful in treating hepatotoxicity and liver lesions and possibly pave the way for the discovery of drug candidates.

DECLARATIONS

Acknowledgement/Funding

None.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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