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Protective Effect of Aqueous Extract of *Ocimum gratissimum* Leaf against Cadmium-Induced Toxicity in Male Wistar Rats

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ABSTRACT

Cadmium, a heavy metal, is recognized for its severe toxicity to living organisms, especially its detrimental effects on the kidneys, bones, and respiratory system. *Ocimum gratissimum* locally referred to as "scent leaf" has pharmacological properties as well as antioxidant and hepato-protective properties. This study evaluated the protective effect of aqueous extract of *O. gratissimum* leaf on cadmium-induced liver and kidney injury in male Wistar rats. In this experiment, the rats were randomly assigned to five groups. The control group received water and standard rat pellets, the negative control group was administered 50 mg/kg of $CdCl_2$ orally once every three days, and the positive control group received the same cadmium dosage along with a prior administration of 100 mg/kg of silymarin. The extract-treated groups were pretreated with 50 mg/kg of $CdCl_2$ once every three days, coupled with daily administration of 400 and 800 mg/kg body weight of the extract. Significant increases (≤ 0.05) in serum levels of ALP, AST, ALT, bilirubin, and creatinine were observed with cadmium exposure, while total protein and urea were significantly decreased (≤ 0.05). SOD, CAT, and GSH were significantly decreased (≤ 0.05), while MDA level was significantly increased (≤ 0.05). However, treatment with silymarin and varying doses of the extract substantially improved liver and renal function indices, as well as restoring the antioxidant status of the rats to near-normal levels. In conclusion, this study suggests that the extract possesses antioxidant and hepato-renal protective properties, offering promise for further investigation into its potential in mitigating cadmium-induced toxicity.

Keywords: Antioxidant Enzymes, Cadmium, Kidney, Liver, *Ocimum gratissimum*.

Introduction

Cadmium (Cd) is one of the many heavy metals that pose a health risk for living organisms and humans due to its presence in the environment.[1] Cadmium is a naturally occurring environmental pollutant derived mainly from agricultural and industrial activities.[2,3] Cd enters the body primarily, through diet (ingestion of contaminated food and water), inhalation and cigarette smoking. Cadmium exhibits an extended biological half-life of approximately 25-30 years in humans, displays a slow rate of elimination from the body, and is primarily stored in soft tissues such as the liver and kidneys.[4] It also exerts toxic effects on reproductive systems, and the development of the embryo,[5] the immune system[6] is considered as a respiratory toxicant.[7] The liver and kidneys demonstrate a high sensitivity to the toxic effects of cadmium. This sensitivity is likely attributed to the capability of these tissues to produce metallothioneins (MT), which are proteins induced by cadmium and serve to safeguard the cells by binding strongly to the harmful cadmium ions. Cd is known as a non-essential heavy metal which can cause damage and induce oxidative stress in major tissues and organs[8]. According to Ekayoda *et al*.[9] Cd was found to generate free radicals. Cadmium is considered a nephrotoxic and hepatotoxic metal[10,11]. The xenobiotic-induced oxidative stress could potentially contribute to various liver and kidney diseases. The management or treatment of cadmium-induced diseases or organ toxicities with medicinal plants involves exploring the potential therapeutic properties of certain plant extracts known for their antioxidant, anti-inflammatory, and hepatoprotective effects. While medicinal plants may not completely reverse cadmium toxicity, they could offer support by mitigating oxidative stress and inflammation associated with cadmium exposure. The aqueous extract of *Hibiscus sabdariffa calyces*, known for its antioxidant and hepatoprotective properties[12,13] has been studied for its protective effects against cadmium-induced liver and kidney injuries, potentially mitigating oxidative stress in this organ.[14] The ethanol leaf extracts of *O. gratissimum* and *Vernonia amygdalina* have also been investigated for their potential to ameliorate the lipid profile, haematology and histomorphology of cadmium-exposed rats.[15] In other related studies, the oxidative damage induced by lead acetate in lymphoid tissues and haematological parameters of adult Wistar rats was improved by administering the ethanol leaf extract of *O. gratissimum*, [16] while the methanolic leaf extract of *O. gratissimum* has also been studied for its ability to attenuates oxidative stress and liver injury in gentamicin-induced hepatotoxicity in rats.[17]

O. gratissimum is a perennial and herbaceous plant. It belongs to the family *Labiatae*, genus *Ocimum* and species *gratissimum*.[18] The plant is an erect small plumb with many barnacles usually not more than 1 m high.[19] The plant is native to tropical regions such as India and West Africa. In Nigeria, this plant is found in the savannah and coastal areas of the country. In Nigeria, the plant is called Effinrin-nla by the Yorubas, Daidoya by the Hausas and Ahuji by the Igbos.[20] *O. gratissimum* is used for a variety of reasons. It is used to prepare

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salads, soups, pepper soups, pastas, vinegars and jellies in many parts of the world. In traditional medicine, the leaves are used in the treatment of fever, diarrhoea, abdominal pain, convulsion, ear infection, conjunctivitis, epilepsy and regulation of menstruation.[20-22] The dried leaves are sniffed to alleviate headaches and fever among other uses.[18] *O. gratissimum* has antimicrobial, anticarcinogenic, diuretic, hepatoprotective, antioxidant, and other medicinal properties. These properties may be a result of its phytochemical constituents such as alkaloids, tannins, flavonoids, phenolics, saponins, glycosides, cardiac glycosides, resins, steroids, phlobatannins, anthraquinones, and terpenoids.[23-26] Silymarin is an extract from the plant *Silybum marianum* (milk thistle). The plant contains various flavonolignans with silybin being the major one.[27] Silymarin contributes to the antioxidant defenses firstly, by direct free radical scavenging. Secondly, it achieves this by either preventing the formation of free radicals through the inhibition of specific enzymes responsible for their production or by preserving the integrity of the electron-transport chain in mitochondria during stressful conditions. Thirdly, it plays a role in maintaining the optimal redox status of the cell by activating various antioxidant enzymes and non-enzymatic antioxidants, primarily through transcription factors such as Nrf2 and NF-κB. Lastly, it activates a range of vitagenes that oversee the synthesis of protective molecules, including heat shock proteins (HSPs), thioredoxin (Trx), sirtuins, etc., offering additional protection during stressful conditions. Silymarin's protective actions are attributed to its antioxidant properties. The primary objective of this study is to provide significant insights into the therapeutic possibilities of natural remedies for addressing injuries induced by cadmium. Specifically, the focus is directed towards evaluating the potential protective advantages offered by the aqueous leaf extract of *O. gratissimum* against the detrimental effects of cadmium on the liver and kidneys. Through a thorough examination of liver and kidney function indices, coupled with an investigation of oxidative stress markers, our research aims to clarify the extent to which *O. gratissimum* could act as a mitigating agent. This exploration presents a promising avenue for the development of strategies to counteract the harmful impact of cadmium exposure on these vital organs.

Materials and Methods

Chemicals
Cadmium and silymarin capsules were procured from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade.

Plant material and extraction
Fresh leaves of *O. gratissimum* were collected from a garden in May 2018 and identified by Dr Garuba Omosun of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike as *O. gratissimum*. A herbarium number MOUAU/COLNAS/PSB/17/217 was obtained. The leaves were gently rinsed to remove dust and dirt, and then air dried at room temperature. The dried leaves were subsequently ground into fine powder using an electric dry mill blender. The extract was obtained by extracting the milled plant material (100 g) with 500 ml of distilled water. The mixture was thoroughly shaken and allowed to stand for 48 hours with intermittent shaking to increase the rate of extraction. The extract was filtered through Whatman filter paper No. 1 and concentrated at low temperature (30-40ºC) under reduced pressure (40-45 Psi) using a rotary evaporator.

Determination of lethal dose (LD_{50})
An acute toxicity study was carried out on the aqueous extract according to the method described by Lorke. [28]

Experimental animals
Twenty-five adult male Wistar rats weighing 150-200 g were used for this experiment. They were obtained from the animal house of the Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. The animals were housed in clean plastic cages under normal environmental conditions of a light/dark cycle and had free access to standard rat pellets and clean water. They were allowed to acclimatize in the Laboratory for a week before the commencement of the study. The rats were maintained by the recommendation of the guide for the care and use of Laboratory animals.[29] All the experimental handling procedures were performed in strict accordance with protocols approved by the Animal Care and Ethical Committee of Michael Okpara University of Agriculture, Umudike with the approval number COLNAS18106.

Experimental design
The animals were randomly divided into five groups of five animals each, and treated as follows: Group 1, designated as the control group, received a standard diet of distilled water and rat pellets. Group 2, the negative control, received an oral administration of 50 mg/kg body weight of $CdCl_2$ every three days via a gavage. Group 3, the positive control, received the same cadmium dosage every three days, preceded by a daily administration of 100 mg/kg body weight of silymarin. For Groups 4 and 5, the rats were pre-treated with 50 mg/kg of $CdCl_2$ every three days. Subsequently, Group 4 received a daily administration of the aqueous leaf extract at a dose of 400 mg/kg body weight, while Group 5 received a daily administration of 800 mg/kg body weight of the extract. The experiment lasted for 28 days and the animals were sacrificed afterwards.

Biochemical assay
Blood sample and tissue collection
At the end of the experiment, the animals were fasted overnight and anaesthetized by chloroform inhalation in a closed chamber. Liver and kidney were excised while blood sample was collected through cardiac puncture into plain sample bottles, and centrifuged at 4000 rpm for 10 minutes to obtain serum which was used for liver and kidney function tests. CAT and SOD activities as well as GSH and MDA levels were evaluated in liver and kidney homogenates.

Preparation of liver and kidney homogenates
The tissues (liver and kidney) were homogenized in cold phosphate buffer (0.05M, pH 7.0) with a Teflon homogenizer. Little quantity (1g) of the tissue was homogenized in 9ml of phosphate buffer to give 10% homogenate. The homogenate was centrifuged at 4000rpm for 10 minutes. The supernatant obtained was stored frozen at -20^0C until required for the analyses of CAT and SOD activities as well as GSH and MDA levels.

Liver and kidney function tests
The activities of AST, ALT, and ALP, as well as the concentrations of total bilirubin and total protein (TP) were determined in sera using their respective Randox kits. Creatinine and urea concentrations were determined using Fawcet and Scott[30] and Bartels and Bohmer[31] methods respectively.

Antioxidant assays
Catalase (CAT) activity in liver and kidney homogenates was determined using the modified method described by Atawodi.[32]
Superoxide dismutase (SOD) activity in liver and kidney homogenates was determined using the method described by Sun *et al.* [33]
Reduced glutathione (GSH) levels in liver and kidney homogenates were determined using the method described by Tietze. [34]
The concentration of MDA in liver and kidney homogenates was determined using the method described by Draper and Hadley. [35]

Statistical analysis
Data are presented as means ± SEM of 5 independent determinations. Statistical analysis was performed using SPSS (22.0). Means were compared using Duncan's multiple-test range. Values with $p \leq 0.05$ were considered statistically significant.

Results and Discussion

LD_{50} of Aqueous Extract of O. gratissimum Leaf
Acute toxicity test (LD_{50}) of aqueous leaf extract of *O. gratissimum* in the experimental animals didn't record any death. The LD_{50} of the extract was greater than 5000 mg/kg. The LD_{50} (lethal dose for 50% of the test subjects) is a measure used to assess the acute toxicity of a

substance.[28] In this case, the LD_{50} of the aqueous leaf extract of *O. gratissimum* was found to be greater than 5000 mg/kg. This means that a dose of over 5000 mg/kg of body weight did not result in the death of half of the experimental animals. That no deaths were recorded in the experimental animals indicates that, at the doses tested, the extract did not cause lethal effects within the observation period of the study. The conclusion drawn from the LD_{50} value being greater than 5000 mg/kg is that the extract could be considered relatively safe for consumption. This suggests that, at the tested doses, the extract does not pose an immediate and severe risk of toxicity.

Liver function indices

The effect of the aqueous extract of *O. gratissimum* leaf (Scent leaf) on serum ALT, AST, ALP and total bilirubin of rats treated with cadmium is shown in Table 1. Animals treated with 50 mg/kg of cadmium had significant increases ($p \leq 0.05$) in serum ALP, AST, ALT and total bilirubin compared to control and other groups. This elevation suggests that cadmium exposure had a detrimental impact on liver function, as reflected in the heightened levels of these enzymes and bilirubin. This finding corresponds with the outcomes documented in the research study titled "Aqueous Extract of *Hibiscus sabdariffa* Alleviates Cadmium-Induced Liver and Kidney Injuries in Male Wistar Rats" [14] and the research on "*Oligochaeta ramosa* (Roxb.) Extract Regulating Lipid Metabolism and Exerting Hepatoprotective Effects in Cadmium-Induced Hepatic Injury in Rats.[36] However, administration of silymarin and the varying doses of the extract significantly decreased ($p \leq 0.05$) ALP, AST, ALT and total bilirubin in the serum of the experimental rats when compared to the untreated animals. This observation is highly significant, indicating that both silymarin and the extract possess hepatoprotective properties, mitigating the adverse effects induced by cadmium on liver function. This finding corresponds with the outcomes documented in both the study titled "Aqueous Extract of *Hibiscus Sabdariffa* Ameliorates Cadmium-Induced Liver and Kidney Injuries in Male Wistar Rats"[14] and that of "*Oligochaeta ramosa* (Roxb.) Extract Regulating Lipid Metabolism and Exerting Hepatoprotective Effects in Cadmium-Induced Hepatic Injury in Rats."[36] The reduction in ALP, AST, ALT and total bilirubin by the extract was dose-dependent. This finding underscores the importance of dosage considerations in harnessing the therapeutic benefits of *O. gratissimum* against cadmium-induced liver damage. In this study, silymarin demonstrated superior efficacy in enhancing liver function indices as compared to the studied extract. Cadmium is one of several toxic metals capable of disrupting certain enzymatic systems and metabolic activities in humans and animals.[37] The toxic effect of cadmium has been linked with numerous clinical conditions such as renal dysfunction, bone diseases, as well as hepatic dysfunction.[38] The liver principally assists in the filtration of blood from the digestive tract before it is circulated to other parts of the body, it also helps to metabolize and detoxify potentially harmful chemicals and drugs. The liver primarily takes up the greatest quantity of cadmium during the initial hours after exposure. Studies have shown that approximately 60% of absorbed Cd is deposited in the liver (30%) and kidney (30%), while the rest is distributed throughout the other parts of the body.[39, 40, 41] Previous report indicates that acute Cd poisoning causes increased levels of liver marker enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in the blood and also increases the incidence of non-alcoholic hepatitis and fatty liver.[42] In this study, the activities of liver marker enzymes (ALP, AST and ALT) were elevated in the serum of rats exposed to cadmium treatment. This may be indicative of hepatic injury or hepatocellular necrosis which may have increased the permeability of the hepatic cell membrane resulting in the release of transaminases into the bloodstream. Treatment with the extract reduced serum levels of ALP, AST and ALT, suggesting that the extract had hepatoprotective properties. Bilirubin is a yellow pigment that is made during the normal breakdown of erythrocytes. Bilirubin passes through the liver and is eventually excreted out of the body. Higher than normal levels of bilirubin may indicate different types of liver or bile duct problems.[43] Elevated levels of bilirubin in rats exposed to cadmium treatment in this study, may indicate liver damage or disease. However, the administration of the extract lowered the elevated level of bilirubin in the serum of the experimental rats.

Renal function indices

The effect of the aqueous extract of *O. gratissimum* leaf (Scent leaf) on serum total protein, urea, and creatinine concentrations of rats treated with cadmium is shown in Table 2. Total protein was significantly decreased ($p \leq 0.05$) in rats treated with cadmium compared to control and the treatment groups (silymarin and the varying doses of the plant extract). This discovery aligns with the results documented by Ebhohon *et al.*[14] The significant decrease ($p \leq 0.05$) in total protein observed in rats treated with cadmium, as opposed to the control and treatment groups (silymarin and varying doses of the plant extract), suggests a potential disruption in protein metabolism induced by cadmium toxicity.[44] This decline underscores the adverse impact of cadmium on overall protein balance in the serum. However, concentrations of urea and creatinine in the serum of rats administered cadmium were significantly increased ($p \leq 0.05$) compared to control and the treatment groups. These findings are also similar to those reported by Ebhohon *et al.*[14] Elevated levels of urea and creatinine are indicative of compromised kidney function, reflecting the detrimental impact of cadmium on renal health. [45, 46]
The administration of silymarin and the plant extract at varying doses demonstrated a noteworthy reversal of these anomalies. Total protein levels were significantly increased ($p \leq 0.05$), indicating a restoration of protein homeostasis, while urea and creatinine concentrations were significantly decreased ($p \leq 0.05$), signifying an amelioration of kidney function. The therapeutic impact of silymarin exhibited a comparable effectiveness to that of the extract. This discovery is also consistent with the findings previously documented by Ebhohon *et al.*[14] In this research, the efficacy of silymarin in improving renal function indices was found to be comparable to that of the extract. Importantly, the dose-dependent increase in total protein and the dose-dependent decrease in urea and creatinine concentrations by the plant extract underscore its potential as a protective agent against cadmium-induced kidney injury. The observed dose-dependent trends further emphasize the potential dose-related therapeutic benefits of the plant extract in addressing cadmium-induced disturbances in serum markers related to renal health. These findings contribute to the growing body of evidence supporting the protective properties of natural remedies against heavy metal-induced organ toxicity.

Table 1: Outcome of Acute Toxicity Study of Aqueous Extract of *O. gratissimum* leaf (Scent leaf)

Dose (mg/kg b.w.t)	No. of rats	No. of deaths	Survival	Mortality ratio
10	3	0	3	0/3*
100	3	0	3	0/3*
1000	3	0	3	0/3*
1600	1	0	1	0/1*
2900	1	0	1	0/1*
5000	1	0	1	0/1*

*Number of deaths/surviving animals.

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Table 2: Effect of aqueous extract of *O. gratissimum* leaf (Scent leaf) on serum ALT, AST, ALP and total bilirubin of rats treated with cadmium

Treatment	ALP (IU/L)	AST(IU/L)	ALT (IU/L)	Total Bilirubin (mg/dl)
Control	51.55 ±0.53^{c}	18.96 ±1.13^{c}	19.18 ±0.29^{d}	0.21 ±0.02^{c}
Cadmium (50 mg/kg bwt)	62.06 ±1.50^{a}	32.78 ±0.91^{a}	30.87 ± 0.46^{c}	0.84 ±0.05^{a}
Cadmium (50 mg/kg bwt) and Silymarin (100 mg/kg bwt)	53.20 ±1.93^{bc}	23.39 ±2.00^{b}	19.81± 0.38^{b}	0.38 ± 0.65^{b}
Cadmium (50 mg/kg bwt) and *O. gratissimum* (400 mg/kg bwt)	56.40 ±0.31^{b}	22.47 ±1.20^{bc}	24.69 ± 1.19^{ab}	0.51 ±0.03^{b}
Cadmium (50 mg/kg bwt) and *O. gratissimum* (800 mg/kg bwt)	54.89 ± 1.84^{bc}	21.86 ±1.08^{bc}	23.10 ± 1.96^{a}	0.43 ± 0.44^{b}

Values are expressed as mean ± SEM. Means with homogeneous superscripts in each column are statistically non-significant ($p \geq 0.05$), while means with heterogeneous superscripts are statistically significant ($p \leq 0.05$), when compared to the untreated group. n=5.

Table 3: Effect of aqueous extract of *O. gratissimum* leaf (Scent leaf) on sera total protein, urea, and creatinine concentrations of rats treated with cadmium

Treatment	Total protein (g/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Control	5.06 ± 0.03^{c}	31.95 ± 0.80^{a}	0.87 ± 0.03^{b}
Cadmium (50 mg/kg bwt)	4.75 ± 0.02^{d}	47.09 ± 1.68^{b}	0.98 ± 0.02^{a}
Cadmium (50 mg/kg bwt) and Silymarin (100 mg/kg bwt)	5.44 ± 0.17^{b}	32.27 ±1.09^{a}	0.88 ± 0.02^{b}
Cadmium (50 mg/kg bwt) and *O. gratissimum* (400 mg/kg bwt)	5.68 ± 0.41^{ab}	30.46 ± 0.29^{a}	0.84±0.03^{b}
Cadmium (50 mg/kg bwt) and *O. gratissimum* (800 mg/kg bwt)	5.70 ± 0.35^{a}	31.86 ± 0.49^{a}	0.89 ± 0.02^{b}

Values are expressed as mean ± SEM. Means with homogeneous superscripts in each column are statistically non-significant ($p \geq 0.05$), while means with heterogeneous superscripts are statistically significant ($p \leq 0.05$), when compared to the untreated group. n=5.

Reduction in total protein of cadmium-treated rats suggests hepatic injury. However, treatment with the extract increased serum total protein, which suggests that the extract has a protective effect against hepatic injury. Urea is the main nitrogenous waste product of metabolism and is generated from protein breakdown. It is the main nitrogen-containing compound in the urine of mammals and is usually excreted through the kidney. Creatinine is a chemical waste product of creatine. Creatine supplies energy mainly to muscles, and it is filtered from the blood mainly by the kidneys.[47] If kidney function is abnormal, creatinine level increases in the blood because less creatinine is excreted through the urine. Therefore, creatinine levels in the blood and urine may be used to calculate the creatinine clearance, which corresponds with the glomerular filtration rate (GFR) of the kidney's filtering units. In this study, an increase in serum urea and creatinine concentrations of rats exposed to cadmium could be a result of decreased renal excretion of urea and creatinine, suggesting impaired renal function or damage. Treatment with extract decreased raised levels of serum urea and creatinine in cadmium-treated rats suggesting that the extract could have a protective effect against cadmium-induced renal impairment.

Antioxidant activity of aqueous extract of O. gratissimum leaf (Scent leaf) in cadmium treated rats.

The effect of aqueous extract of *O. gratissimum* leaf (Scent leaf) on the antioxidant status of rats exposed to cadmium treatment is shown in Table 3. The activities of the enzymes SOD and CAT as well as GSH level were significantly decreased($p \leq 0.05$), whereas MDA level in kidney homogenate was significantly increased ($p \leq 0.05$), upon administration of cadmium. Upon cadmium administration, a notable reduction ($p \leq 0.05$) in the activities of SOD and CAT enzymes, along with a decrease in GSH levels, signifies a compromised antioxidant defense system in the kidneys. [48] Concurrently, the significant increase ($p \leq 0.05$) in MDA levels indicates elevated lipid peroxidation, reflective of heightened oxidative stress in the kidney tissue. [49]. This discovery aligns with results documented in studies conducted by Ebhohon *et al.*[14] and Oluwadare *et al.*[17] Administration of the antioxidant silymarin and varying doses of the extract restored SOD, CAT, GSH and MDA to near-normal levels, and were statistically significant ($p \leq 0.05$) when compared to the untreated group. However, the administration of the antioxidant silymarin and varying doses of the *O. gratissimum* extract remarkably restored the activities of SOD, CAT, and GSH, while reducing MDA levels to near-normal values. These observations indicate a robust protective effect of both silymarin and the extract against cadmium-induced oxidative damage, emphasizing their potential in maintaining a balanced antioxidant status in the kidney tissue. [50,17] The mitigating impact of silymarin on cadmium-induced oxidative stress (SOD, CAT, and GSH) was similar to that observed with the *O. gratissimum* extract. This finding is in line with the results recorded in studies carried out by Ebhohon *et al.*[14] and Farjad and Momeni. Additionally, the extract demonstrated a dose-dependent amelioration of cadmium's impact on oxidative stress and the antioxidant status of kidney homogenate. Furthermore, the dose-dependent amelioration of the extract on oxidative stress markers and antioxidant status underscores its potential therapeutic efficacy. This suggests that higher doses of the *O. gratissimum* extract correspond to more pronounced protective effects against cadmium-induced oxidative stress in kidney tissues. The observed dose-dependent response further accentuates the potential of the extract as a valuable resource in combating oxidative stress induced by heavy metal exposure, providing a foundation for future research on natural remedies for oxidative damage. Oxidative stress arises when there is an imbalance between the concentrations of reactive oxygen species (ROS) and the antioxidant defense mechanism of the body. Cadmium (Cd) has been widely reported to induce oxidative stress in both *in vitro* and *in vivo* studies. In *in vivo* studies, cadmium induces lipid peroxidation and also deranges the prooxidant-antioxidant balance indirectly by damaging the antioxidant barrier. Cd decreases the level of non-enzymatic antioxidants, including GSH and the total sulfhydryl groups, and inactivates antioxidants enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx).[51] Oxidative stress induced by the administration of cadmium to the rats in this study, was ameliorated by the extract, subsequently reinstating the activities of these antioxidant enzymes (SOD, CAT and GSH) in the kidney of the rats to near normal levels.

Cadmium induces the production of metallothioneins and reactive oxygen species, leading to oxidative damage in various tissues such as the liver, kidneys, and testes.[52] This, in turn, results in the impairment of membrane functions.[53] This toxic effect has been linked to lipid peroxidation. The elevation in lipid peroxidation resulting from exposure to cadmium and its toxicity is linked to changes in both enzymatic and non-enzymatic antioxidant defence systems. These systems typically protect against the harmful effects of free radicals.[54, 55] The level of Malondialdehyde (MDA) serves as a biomarker for

oxidative stress in living organisms,[56] and it is produced as a byproduct of lipid peroxidation. An elevation in free radicals results in the excessive production of MDA. Administration of extract to cadmium-treated rats in this study reduced MDA levels in their kidneys.

Research indicates that the extract derived from *O. gratissimum* leaves possesses significant potential for cell reinforcement, likely due to its phytochemical components, including phenolic-eugenols, thymols, flavonoids, phenylpropanoids, linalool, and citral.[57,58] These bioactive agents confer on it, its antioxidative, chemotherapeutic, antispasmodic, and analgesic properties.[59,60] The presence of phenolic compounds in *O. gratissimum* may have allowed it to act as a reducing agent, hydrogen contributor and singlet oxygen quencher.[61] Phenolic compounds have high antioxidant activity.[62] The antioxidant effects of the extract may also be due to the presence of flavonoids. Flavonoids have been reported to exhibit antioxidant activity [63] and are effective scavengers of superoxide anions.[64] This ameliorative effect of *O. gratissimum* might be through a decrease in lipid peroxidation (LPO) and an increase in SOD, CAT and GSH (as studies have shown that Cd exposure leads to an increase in LPO which concomitantly leads to a decrease in the activities of SOD, CAT, GPx and GSH levels as well as an increase in free radical generation), indicating that the extract possesses antioxidant and hepato-renal protective properties.

Conclusion

This experimental investigation affirmed that exposure to cadmium (Cd) resulted in toxicity and damage to the liver and kidneys, as evidenced by alterations in the biochemical indices assessed for these organs. Notably, the adverse effects were mitigated in the treated group through the administration of the extract. In summary, the study outcomes emphasize the potential of *O. gratissimum* extract in ameliorating the detrimental impacts of cadmium on liver and kidney function indices, as well as mitigating the adverse effects on the antioxidant status of kidney tissue.

Table 4: Effect of aqueous extract of *O. gratissimum* leaf (Scent leaf) on SOD, CAT, GSH, and MDA of kidney homogenate in rats exposed to cadmium.

Treatment	SOD (U/mL)	CAT (U/mL)	GSH (U/mL)	MDA x 10^{-3} (mmole/mL)
Control	12.76 ± 0.36^{ab}	14.35 ± 0.20^{a}	6.42 ± 0.01^{a}	1.72 ± 0.27^{b}
Cadmium (50 mg/kg bwt)	10.69 ± 0.87^{b}	10.92 ± 0.19^{c}	4.02 ± 0.15^{c}	3.53 ± 0.27^{a}
Cadmium (50 mg/kg bwt) and Silymarin (100 mg/kg bwt)	12.76 ± 0.35^{ab}	13.97 ± 0.30^{ab}	5.68 ± 0.01^{ab}	2.05 ± 0.03^{b}
Cadmium (50 mg/kg bwt) and *O. gratissimum* (400 mg/kg bwt)	12.67 ± 0.33^{ab}	13.36 ± 0.33^{b}	5.32 ± 0.01^{b}	2.59 ± 0.29^{b}
Cadmium (50 mg/kg bwt) and *O. gratissimum* (800 mg/kg bwt)	12.95 ± 0.90^{a}	14.21 ± 0.20^{a}	5.48 ± 0.01^{b}	2.35 ± 0.33^{b}

Values are expressed as mean ± SEM. Means with homogeneous superscripts in each column are statistically non-significant ($p \geq 0.05$), while means with heterogeneous superscripts are statistically significant ($p \leq 0.05$), when compared to the untreated group. n=5.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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