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Nephroprotective Effects of Aqueous Extract of *Loranthus micranthus* Linn Leaf Against Cadmium-Induced Kidney Toxicity in Male Rats

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ABSTRACT
Cadmium is a heavy metal widely distributed in the environment due to anthropogenic activities, such as industrial processes, mining, and agricultural practices. Exposure to cadmium has been associated with various adverse health effects, particularly on the kidneys. The kidneys are major target organs for cadmium toxicity, as the metal tends to accumulate in renal tissues, leading to nephrotoxicity and impaired renal function.This study aims to evaluate the potential nephroprotective effects of the aqueous extract of *Loranthus micranthus* Linn leaf against cadmium-induced kidney toxicity in a rat model. Twenty-four male rats were divided into four groups: Group 1 served as the normal control and received no treatment. Group 2 was exposed to cadmium toxicity, receiving 2 mg/kg body weight of cadmium intraperitoneally daily. Group 3 received 150 mg/kg body weight of the extract daily. Group 4 received 2 mg/kg body weight of cadmium intraperitoneally, followed by a daily administration of 150mg/kg body weight of the extract. This experiment was carried out for a period of 28 days. Biochemical alterations in serum creatinine, blood urea nitrogen, total protein, superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and malondialdehyde (MDA) were assessed as markers of kidney function and oxidative stress. Additionally, ultrastructural changes in the kidneys of the experimental animals were evaluated using electron microscopy. The study revealed significant biochemical alterations in markers of kidney function and oxidative stress in rats exposed to cadmium-induced kidney toxicity. Treatment with the extract attenuated these biochemical changes, indicating potential nephroprotective effects. Furthermore, ultrastructural changes observed in the kidneys of cadmium-exposed rats were ameliorated by treatment with the extract. The findings of this study suggest that the extract possesses potential nephroprotective effects against cadmium-induced kidney toxicity. Further research is necessary to elucidate the mechanisms of action and explore the therapeutic potential of these natural compounds in managing kidney-related disorders.

Keywords:
Cadmium,
Kidney,
Loranthus micranthus Linn,
Nephroprotective,
Oxidative stress,
Wistar rats.

INTRODUCTION

Cadmium is a non-essential toxic heavy metal found naturally in the earth's crust and released into the environment through industrial processes, mining, and agricultural activities. Chronic exposure to cadmium primarily occurs through contaminated foods, air, and water sources (Mitra et al., 2022). The kidneys are the major target organ for cadmium toxicity, with long-term exposure leading to severe renal damage (Satarug, 2018). The kidneys play a vital role in maintaining homeostasis, filtering waste products, and regulating electrolyte balance within the body. Their intricate structure and essential functions make them susceptible to damage from environmental toxins, including cadmium (Rennke & Denker, 2020). Once absorbed into the body, cadmium accumulates in the kidneys, where it can persist for long periods due to the organ's limited ability to excrete the metal efficiently (Genchi et al., 2020; Hernández-Cruz et al., 2022). Over the past decades, numerous studies have shed light on the detrimental impact of cadmium on renal

health. Chronic exposure to cadmium has been linked to the development of severe renal pathologies, including chronic kidney disease (CKD), proximal tubular dysfunction, and glomerular impairment (Orr & Bridges, 2017; Satarug et al., 2018; Yimthiang et al., 2023). Numerous studies have also investigated the molecular mechanisms underlying cadmium-induced kidney damage. Cadmium is taken up by renal tubular cells, where it disrupts cellular functions and induces oxidative stress, leading to the generation of reactive oxygen species (ROS). These ROS overwhelm the antioxidant defence systems, causing oxidative damage to cellular components, such as lipids, proteins, and DNA (Yan & Allen, 2021). Additionally, cadmium can directly impair various cellular processes, including apoptosis, autophagy, and DNA repair, further exacerbating renal injury (Cui et al., 2021; Wang et al., 2020). Human epidemiological studies have demonstrated a strong association between cadmium exposure and renal dysfunction. Occupational exposure, particularly in industries involving cadmium processing, battery manufacturing, and welding, has been linked to an increased risk of chronic kidney disease (Baloch et al., 2020). Additionally, cadmium-contaminated diets, such as rice and shellfish from polluted areas, have been shown to contribute to elevated cadmium levels in the kidneys and an increased risk of kidney dysfunction (Arao et al., 2019; Simmons et al., 2005; Wang et al., 2019). Several biomarkers have been identified and utilized to assess cadmium-induced renal damage. These include urinary markers such as beta-2 microglobulin, N-acetyl-beta-D-glucosaminidase, and metallothionein, as well as blood markers like creatinine, cystatin C, and kidney injury molecule-1 (KIM-1). Monitoring these biomarkers helps in the early detection and management of cadmium nephrotoxicity (Prozialeck et al., 2016; Prozialeck & Edwards, 2010). Efforts to mitigate cadmium-induced renal toxicity involve reducing exposure through strict regulations, occupational safety measures, and improved waste disposal practices (Sripada & Lager, 2022).

Loranthus micranthus, commonly known as mistletoe, is a hemiparasitic plant that belongs to the Loranthaceae family. This intriguing plant is widely distributed in tropical and subtropical regions, often found growing on the branches or trunks of various host trees, including fruit trees, shrubs, and forest trees (Moghadamtousi et al., 2013). Historically, mistletoe has been held in high regard by traditional medicine practitioners for its diverse medicinal properties and therapeutic potential (Szurpnicka et al., 2020). Various indigenous cultures have used *Loranthus micranthus* as a valuable medicinal plant for centuries. Its traditional uses span a wide range of ailments and conditions, including cardiovascular disorders, respiratory problems, gastrointestinal issues, and reproductive disorders (Moghadamtousi et al., 2013). The plant's healing properties have been attributed to its rich array of bioactive compounds (Hlophe & Bassey, 2023). *Loranthus micranthus* contains potent antioxidants, such as polyphenols and flavonoids, which help neutralize harmful free radicals and protect cells from oxidative stress (Hlophe and Bassey, 2023). The plant's antioxidant properties may contribute to its traditional use in combating oxidative damage and age-related diseases (Nicoletti, 2023). *Loranthus micranthus* has been employed for managing kidney-related ailments, including urinary tract infections, kidney stones, and renal insufficiency (Ebokaiwe et al., 2019). Traditional practitioners have used the plant's extracts and decoctions to promote diuresis and cleanse the kidneys. However, the traditional use of mistletoe for kidney health lacks robust scientific validation and requires further investigation. Some studies have explored the potential nephroprotective effects of *Loranthus micranthus* (Ebokaiwe et al., 2019). The plant's antioxidant properties have been suggested to counteract oxidative stress-induced renal damage (Ebokaiwe et al., 2018). Preclinical studies on animal models have demonstrated that mistletoe extracts could attenuate kidney injury caused by various nephrotoxic agents (Imade et al., 2014). These findings indicate the possibility of mistletoe as a complementary therapeutic option for protecting the kidneys against certain insults. Cadmium's toxic effects on the kidneys are a pressing public health issue. Understanding the underlying mechanisms and risk factors associated with cadmium nephrotoxicity is crucial for implementing effective preventive measures and targeted interventions to safeguard vulnerable populations. Further research is needed to explore new therapeutic strategies and assess cadmium exposure's long-term consequences on renal health. This study aims to provide comprehensive insights into the potential protective effects of aqueous extract of *Loranthus micranthus* Linn leaf against cadmium-induced kidney toxicity, contributing to the understanding of its pharmacological properties and potential applications in preventing or ameliorating heavy metal-induced renal damage.

Figure 1: *Loranthus micranthus* Linn on *Alstonia boonei* (popularly known as God's tree or "Onyame dua" within West Africa.)

MATERIALS AND METHODS

Plant Collection and Authentication

Fresh leaves of mistletoe (*Loranthus micranthus*. *L*) harvested from the host plant Oil Bean (*Pentacletra macrophylla*) were obtained in May 2016 from a plantation at Amaoba community in Ikwuano Local Government Area, Abia State, Nigeria. It was identified and authenticated by Mr PiPi Okey in the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. A voucher number MOUAU/COLNAS/PSB/16/A266 was obtained.

Plant Extraction

Fresh leaves of the mistletoe plant (*Loranthus micranthus*. *L*) were air-dried at room temperature and pulverized using a glass electric blender. A known amount (100 g) of the pulverized sample was weighed into a glass beaker and a known volume of distilled water (1000 ml) was added. It was stirred vigorously for 20 minutes and allowed to stand for 24 hours. The filtrate was concentrated via lyophilisation to obtain a coffee brown residue. The concentrated extract was stored at 4ºC in an air-tight bottle until further use.

Qualitative phytochemical analyses of *Loranthus micranthus Linn*

Phytochemical screening of the aqueous extract was carried out to identify secondary metabolites- alkaloids, flavonoids, saponins, and tannins using standard phytochemical methods (Harborne, 1984; Sofowora, 1993; Trease and Evans, 2002).

Determination of Lethal Dose (LD_{50})

An acute toxicity study was carried out on the aqueous leaf extract according to the method described by Lorke (1983).

Experimental Animals

Thirty (30) male Wistar rats, weighing 150 ± 10 g were obtained from the animal house of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State. The animals were allowed to acclimatize for one week in the animal house of the Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike before the study commenced. They were housed in aluminium cages with six rats per cage and were fed *ad libitum* with standard commercial pelleted growers' feed (Vital, Nigeria). The animals had free access to clean drinking water and were kept at normal light/dark daily cycles. They were maintained in accordance with the recommendation of the guide for the care and use of laboratory animals (NIH, 2002).

Experimental Design

Twenty-four male rats were divided into four groups, with each group consisting of six rats:

Group 1: Normal Control. Rats in this group received no treatment and served as the control group to establish baseline parameters.

Group 2: Cadmium Only. Rats in this group received an intraperitoneal administration of cadmium at a dose of 2 mg/kg body weight daily to induce kidney toxicity. Cadmium chloride was dissolved in normal saline at a concentration of 2 mg/kg body weight.
Group 3: *Loranthus micranthus* Extract Only. Rats in this group received a daily oral administration of 150 mg/kg body weight of the aqueous leaf extract of *Loranthus micranthus* Linn.
Group 4: Cadmium + *Loranthus micranthus* Extract. Rats in this group were first exposed to cadmium toxicity by receiving an intraperitoneal administration of 2 mg/kg body weight of cadmium daily for two (2) weeks. Subsequently, they received a daily oral administration of 150 mg/kg body weight of the aqueous leaf extract of *Loranthus micranthus* Linn for another two (2) weeks. This experiment was carried out for 28 days mirroring real-world scenarios where individuals are exposed to environmental toxins over an extended period. This timeframe aimed to provide a more comprehensive understanding of the potential therapeutic advantages of the plant extract in safeguarding against cadmium-induced kidney toxicity in male rats.

Biochemical Analysis

Blood Sample and Tissue Collection

At the end of the experiment, all the rats were anesthetized with an intraperitoneal injection of urethane and sacrificed immediately. Blood samples were drawn using a 5 ml syringe via cardiac puncture (Arunachalam & Sasidharan, 2021) into sample tubes, and centrifuged at 4000 rpm for 10 minutes to obtain serum which was used for biochemical assays in this study. The kidneys were excised, trimmed of connective tissue, and washed thoroughly in ice-cold saline.

Preparation of Kidney Homogenates

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

Kidney Function Tests

The concentrations of total protein (TP), creatinine, and urea were determined in plasma using their respective Randox (Antrim, U.K) assay kits,

Antioxidant Assays

Estimation of Catalase Activity. Catalase activity in liver and kidney homogenates was determined using the modified method described by Atawodi (2011).

Estimation of SOD Activity. Superoxide dismutase (SOD) activity in liver and kidney homogenates was determined using the method described by Sun et al. (1988).

Estimation of GSH Levels. GSH levels in liver and kidney homogenates were determined using the method described by Tietze (1969).

Assessment of Lipid Peroxidation. The concentration of MDA in liver and kidney homogenates was determined using the method described by Draper & Hadley (1960).

Ultrastructural Investigations of Rat Kidney

The right kidneys of the rats were preserved in 10% formalin for a minimum of 24 hours, underwent standard dehydration procedures, and were subsequently encased in paraffin. The paraffin-embedded tissues were sectioned at a thickness of approximately 4-5 µm using a microtome, which was later stained with hematoxylin and eosin (H&E). These stained sections were then observed using a light microscope (Leica DM 500, Leica Biosystems, Germany) at x 400 magnification and multiple microscopic fields were assessed to obtain representative images of each tissue sample.

Statistical Analysis

Data are expressed as mean ± SEM. Significant differences were determined using one-way analysis of variance (ANOVA). Differences between means were analyzed for significance using Dunnett's multiple-range tests. ANOVA and Dunnett's multiple range tests were based on the computer software, graph pad prism, version 7. Values of $p \leq 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

LD_{50} of Aqueous Extract of *Loranthus micranthus. Linn* Leaf

The LD_{50} of aqueous extract of *Loranthus micranthus. Linn* leaf was greater than 5000 mg/kg.

Table 1: Phytochemical Constituents of Crude Aqueous Extract of *Loranthus micranthus Linn* Leaf

Phytochemical Constituents	*Loranthus micranthus Linn*
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Glycosides	+

Key: + = detected

Table 2: Effect of Cadmium-induced toxicity and aqueous leaf extract of *Loranthus micranthus*. L on serum Urea, Creatinine, and Total Protein

GROUP	TREATMENT	Urea (mg/dl)	Creatinine (mg/dl)	Total Protein (g/dl)
1	Control	31.36 ± 0.97^b	1.85 ± 0.01^b	6.31 ± 0.17^b
2	2 mg/kg body weight of Cadmium	38.27 ± 3.01	3.78 ± 0.01	9.38 ± 0.26
3	150 mg/kg body weight of *Loranthus micranthus*. L	29.35 ± 0.97^b	2.11 ± 0.18^b	7.91 ± 0.43^b
4	2 mg/kg body weight of Cadmium and 150 mg/kg body weight of *Loranthus micranthus*. L	35.78 ± 0.24^b	2.70 ± 0.43^b	6.61 ± 0.84^b

Values are expressed as mean ± SEM, n=6. Values with superscript letter 'b' are statistically significant ($p \leq 0.05$) relative to the untreated cadmium group of rats

The results indicate that exposure to cadmium at a dose of 2 mg/kg body weight led to significant kidney toxicity, as evidenced by the notable increase in serum concentrations of urea, creatinine, and total protein compared to the control group. Elevated levels of urea and creatinine are indicative of impaired kidney function and reduced glomerular filtration rate (Brookes & Power, 2022; Norris et al., 2018), while the increased total protein in serum may indicate kidney damage and protein leakage (Cravedi & Remuzzi, 2013; Haider & Aslam, 2022). However, the administration of the aqueous leaf extract of *Loranthus micranthus* to the cadmium-exposed rats showed a protective effect on the kidneys. The extract significantly lowered the serum concentrations of urea, creatinine, and total protein compared to the cadmium-treated group. This suggests that *Loranthus micranthus* extract has potential nephroprotective properties, helping to mitigate the harmful effects of cadmium-induced kidney toxicity. By reducing urea and creatinine levels, the extract may support renal function and prevent kidney damage. Interestingly, when the *Loranthus micranthus* extract was administered alone (without cadmium exposure), it still had an impact on serum urea, creatinine, and total protein levels. The extract significantly decreased serum urea concentration and increased the concentration of total protein when compared to the cadmium plus extract treated and the control group. The concentration of creatinine was also significantly increased when compared to the control group.

Table 3: Effect of Cadmium-induced toxicity and aqueous leaf extract of *Loranthus micranthus*. L on superoxide dismutase (SOD), Catalase (CAT), reduced glutathione (GSH) and Malondialdehyde (MDA) of Kidney homogenate in experimental animals

GROUP	TREATMENT	SOD (U/ml)	CAT (U/ml)	GSH (U/ml)	MDA x 10^{-3} (mmole/ml)
1	Control	0.75 ± 0.01^b	0.38 ± 0.01^b	0.48 ± 0.01^b	0.58 ± 0.01^b
2	2 mg/kg body weight of Cadmium	0.48 ± 0.01	0.27 ± 0.01	0.39 ± 0.01	0.78 ± 0.01
3	150 mg/kg body weight of *Loranthus micranthus*. L	0.88 ± 0.01^b	0.64 ± 0.01^b	0.81 ± 0.01^b	0.48 ± 0.01^b
4	2 mg/kg body weight of Cadmium and 150 mg/kg body weight of *Loranthus micranthus*. L	0.57 ± 0.01^b	0.34 ± 0.01^b	0.44 ± 0.01^b	0.68 ± 0.01^b

Values are expressed as mean ± SEM, n=6. Values with superscript letter 'b' are statistically significant ($p \leq 0.05$) relative to the untreated cadmium group of rats

The results indicate that exposure to cadmium at a dose of 2 mg/kg body weight caused significant alterations in antioxidant and oxidative stress markers in the Wistar rats' serum, reflecting oxidative damage to the cells and tissues. There were significant decreases in the activities of superoxide dismutase (SOD) and catalase (CAT),

which are important antioxidant enzymes responsible for neutralizing harmful reactive oxygen species (Ighodaro & Akinloye, 2018). The reduced levels of glutathione (GSH), an essential intracellular antioxidant, suggest a compromised antioxidant defence system in the kidneys, as GSH plays a vital role in the detoxification and neutralization of ROS. The significant increase in malondialdehyde (MDA) levels, a marker of lipid peroxidation (Gawel et al., 2004), indicates oxidative damage to lipids in the kidney tissues. This suggests that cadmium exposure induced oxidative stress, resulting in cellular damage and lipid peroxidation (Branca et al., 2020). This study's findings align with those documented by Dkhil et al. (2020). The administration of the aqueous leaf extract of *Loranthus micranthus* to the cadmium-exposed rats led to a restoration of antioxidant defences.

Effect of Cadmium-induced toxicity and aqueous leaf extract of *Loranthus micranthus*. L on Kidney Ultrastructure in Male Wistar Rats

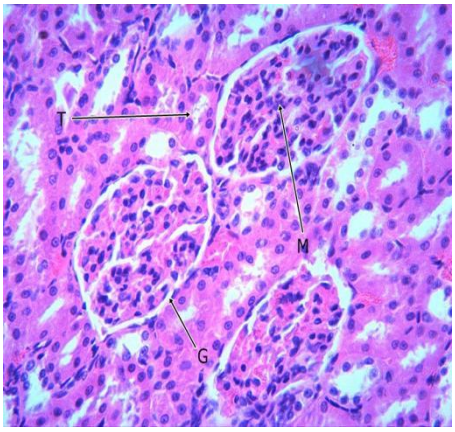

Plate 1: photomicrograph of kidney from control group (H&E; x 400)

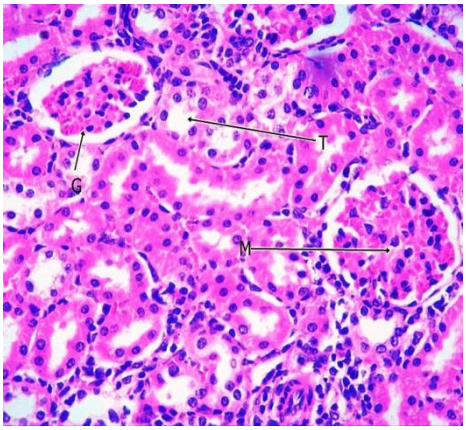

Plate 3: photomicrograph of kidney from extract (150 mg/kg b.w.t) treated group (H&E; x400)

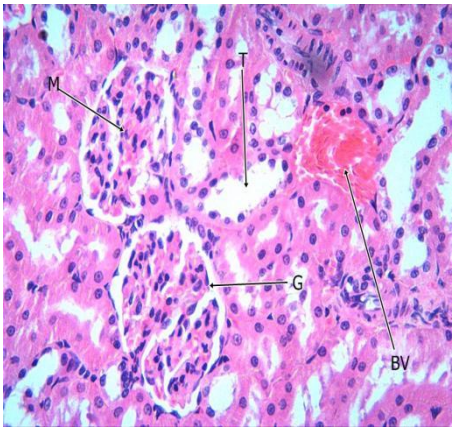

Plate 2: photomicrograph of kidney from cadmium (2 mg/kg b.w.t) treated group (H&E; x 400)

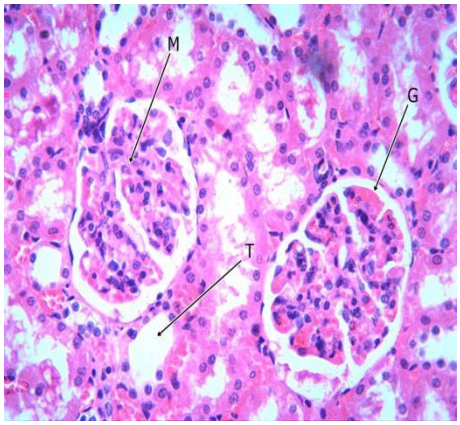

Plate 4: photomicrograph of kidney from cadmium (2 mg/kg b.w.t) and extract (150 mg/kg b.w.t) treated group (H&E; x400)

Histopathological examination of the kidney reveals distinctive electron microscopy findings for each group as follows:

Plate 1 shows the photomicrograph of the kidney removed from rats in the control group. The kidney exhibits a normative architecture characterized by well-arranged glomeruli, tubules, and blood vessels. The mesangium remains structurally intact, displaying no anomalies. Notably, there is an absence of any indications of haemorrhage or significant irregularities.

Plate 2 shows the photomicrograph of the kidney obtained from rats in the cadmium-exposed group. Evident kidney damage is noticeable through visible alterations in the glomerular structure. The mesangium exhibits conspicuous signs of expansion or impairment. Glomeruli display evident degenerative changes or disruptions. Additionally, localized haemorrhaging is observable

within specific kidney regions. Concurrently, the tubules exhibit manifestations of injury or necrosis.

Plate 3 presents the photomicrograph of the kidney obtained from rats administered with *Loranthus micranthus* extract. The kidney shows minor alterations in glomerular structure, as well as tubules and blood vessels. There were no significant changes or damage to the mesangium.

Plate 4 shows the photomicrograph of the kidney from rats exposed to both cadmium and *Loranthus micranthus* extract treatment. The kidney reveals glomeruli that show less degeneration or disruption compared to the cadmium-exposed group. The mesangium also shows diminished expansion or damage, while haemorrhage appears to be notably attenuated or less severe. Moreover, the tubules exhibit fewer indications of injury or necrosis when juxtaposed with the cadmium-exposed group.

Discussion

In toxicology, the LD_{50} is a measure of the lethality or toxicity of a substance, indicating the dose required to cause death in 50% of the tested population. In this study, the LD_{50} of the aqueous extract is greater than 5000 mg/kg, which suggests that the extract is relatively safe and has a low level of acute toxicity, as a higher dose is required to cause lethal effects in a significant portion of the population.

Phytochemical screening helps to identify and assess potential bioactive compounds present in the plant extract. These compounds may contribute to the plant's medicinal properties and can guide further research for potential therapeutic uses or pharmacological activities. Phytochemicals are naturally occurring compounds found in plants that have potential health benefits and often possess therapeutic properties. In this study, phytochemical analysis of the extract demonstrated the existence of alkaloids, flavonoids, saponins, tannins, and glycosides. These findings align with earlier research as reported by Ebhohon et al. (2022).

Alkaloids are nitrogenous compounds known for their diverse pharmacological activities, including antioxidant properties. They scavenge free radicals and inhibit oxidative stress, contributing to the plant extract's antioxidant potential. Alkaloids can neutralize harmful reactive oxygen species (ROS), protecting cells from oxidative damage (Macáková et al., 2019). Flavonoids are potent antioxidants that help neutralize free radicals and prevent oxidative damage. They also have anti-inflammatory, anti-cancer, and anti-ageing properties. Flavonoids are known to contribute significantly to the antioxidant potential of plant extracts, making them valuable for human health (Ullah et al., 2020). Saponins possess antioxidant properties and are known to scavenge free radicals, reduce oxidative stress, and inhibit lipid peroxidation. They can help protect cells from oxidative damage and improve overall antioxidant defences (Khan et al., 2022). Tannins have antioxidant properties due to their ability to scavenge free radicals and metal ions. They help in reducing oxidative stress and preventing cell damage by neutralizing harmful compounds. Tannins are often associated with anti-inflammatory and anti-cancer effects as well (Andrade et al., 2005). Glycosides are compounds that can have antioxidant potential, helping to protect cells and tissues from oxidative stress (Khan et al., 2019). They may contribute to the overall antioxidant activity of the plant extract.

The combined presence of these phytochemicals in this extract indicates its potential to act as a powerful antioxidant. Antioxidants help counteract the damaging effects of free radicals and oxidative stress, which are linked to various chronic diseases such as cardiovascular disease, cancer, and neurodegenerative disorders (Ayoka et al., 2022). Therefore, this plant extract with alkaloids, flavonoids, saponins, tannins, and glycosides can be explored for its antioxidant potential and potential health benefits in preventing or mitigating oxidative damage in the human body.

Cadmium, a harmful heavy metal not essential for human health, occurs naturally in the Earth's crust and is discharged into the environment due to industrial operations, mining, and agricultural practices. Persistent exposure to cadmium mainly happens through polluted foods, air, and water (Saikat et al., 2022). The primary focus of cadmium's detrimental effects is on the kidneys, causing significant renal harm over prolonged exposure (Satarug, 2018). The findings in this study indicate that *Loranthus micranthus* extract may have both beneficial and potentially adverse effects on kidney function, depending on the context of administration. When administered alongside cadmium exposure, the extract exhibited protective effects and mitigated the negative impact of cadmium on kidney function. This discovery closely resembles a study documented by Ebokaiwe et al. (2019). On the other hand, when given alone, it altered certain kidney parameters, suggesting that it might have direct effects on renal physiology unrelated to cadmium toxicity. Further investigation is needed to understand the mechanisms of action of *Loranthus micranthus* extract on kidney function and its potential interactions with cadmium. Additionally, determining the optimal dosage and administration regimen of the extract would be crucial to maximizing its protective effects while minimizing any unintended adverse outcomes.

The significant increases in the activities of SOD and CAT indicate enhanced ROS scavenging capacity and protection against oxidative stress (Ighodaro and Akinloye, 2018). The elevated GSH levels suggest improved antioxidant status, facilitating cellular protection against oxidative damage. The significant decrease in MDA levels indicates a reduction in lipid peroxidation (Cordiano et al., 2023), implying that *Loranthus micranthus* extract helped mitigate the

oxidative damage induced by cadmium exposure. Administration of the extract alone also showed antioxidant effects on the rats' serum parameters. There were significant increases ($p \leq 0.05$) in SOD and CAT activities, indicating enhanced ROS scavenging and antioxidative capacity. The elevated GSH levels suggest improved antioxidant defence even in the absence of cadmium-induced oxidative stress. The significant decrease ($p \leq 0.05$) in MDA levels indicates reduced lipid peroxidation, demonstrating that *Loranthus micranthus* extract alone can protect against oxidative damage. The results suggest that *Loranthus micranthus* extract had a more substantial impact on increasing antioxidant defences (SOD and CAT activities, GSH levels) and reducing oxidative damage (MDA levels) when administered alongside cadmium exposure. When the extract was administered alone, its effect on antioxidant markers and oxidative stress was still significant compared to both the control group and the cadmium + extract group, indicating potential intrinsic antioxidative properties of the extract.

Overall, the findings highlight the protective effects of *Loranthus micranthus* extract against cadmium-induced oxidative stress in the kidney. The extract's ability to enhance antioxidant defences and reduce oxidative damage suggests its potential as a therapeutic agent to combat oxidative stress-related conditions, including those induced by heavy metal toxicity. This discovery mirrors the results of studies conducted by Dkhil et al. (2020) and Imade et al. (2014). However, further research is needed to elucidate the underlying molecular mechanisms and to determine the most effective dosage and administration regimens for therapeutic purposes.

In this study, significant renal impairments after exposure to cadmium (Cd) were observed, as evidenced by histological analysis. These observations are consistent with prior studies conducted by Wan et al. (2022) and Wongmekiat et al. (2018). The changes in glomerular structure induced by cadmium, along with mesangial impairment and tubular injury or necrosis are also in agreement with the findings reported by Adi et al. (2016) and Rafati et al. (2015). Gobe and Crane (2010) linked the relationship between cadmium intoxication and renal cell damage to the vulnerability of the proximal tubular epithelium to oxidative stress. Administering the extract to rats intoxicated with cadmium led to an improvement in the compromised renal structure caused by cadmium treatment. However, further investigations are necessary to comprehensively comprehend the precise mechanisms accountable for this protective effect.

CONCLUSION

Overall, the results present promising evidence of the protective potential of *Loranthus micranthus* extract against cadmium-induced kidney toxicity, warranting further research to explore its potential as a therapeutic agent for kidney-related conditions

REFERENCES

Adi, P.J., Burra, S.P., Vataparti, A.R. & Matcha, B. (2016) *Calcium, zinc and vitamin E ameliorate cadmium-induced renal oxidative damage in albino Wistar rats. Toxicology Report, 3, 591-597.*

Andrade, R. G., Jr, Dalvi, L. T., Silva, J. M., Jr, Lopes, G. K., Alonso, A. & Hermes-Lima, M. (2005) *The antioxidant effect of tannic acid on the in vitro copper-mediated formation of free radicals. Archives of Biochemistry and Biophysics, 437(1), 1–9.*

Arao, T. (2019) *Cadmium Toxicity, Mitigation strategies for cadmium and arsenic in rice*, Springer, pp. 125–138.

Arunachalam, K. & Sasidharan, S.P. (2021) *General considerations and collection of animals' blood. In: bioassays in experimental and preclinical pharmacology*, New York: Humana, New York, NY Springer Protocols Handbooks, pp. 51-55.

Atawodi, S. (2011) *Evaluation of the hypoglycaemic, hypolipidemic and antioxidant effects of methanolic extract of "Ata-Ofa" polyherbal tea (A- polyherbal) in alloxan- induced diabetic rats. Drug Invention Today, 3, 270-276.*

Ayoka, T.O., Ezema, B.O., Eze, C.N. & Nnadi, C.O. (2022) *Antioxidants for the Prevention and Treatment of Non-communicable Diseases. Journal of Exploratory Research in Pharmacology, 7(3),178-188.*

Baloch, S., Kazi, T. G., Baig, J. A., Afridi, H. I. & Arain, M. B. (2020) *Occupational exposure of lead and cadmium on adolescent and adult workers of battery recycling and welding workshops: Adverse impact on health. The Science of the Total Environment, 720, 137549*.

Branca, J.J.V., Fiorillo, C., Carrino, D., Paternostro, F., Taddei, N., Gulisano, M., Pacini, A. & Becatti, M. (2020) *Cadmium-Induced Oxidative Stress: Focus on the Central Nervous System. Antioxidants, 9(6), 492.*

Brookes, E.M. & Power, D.A. (2022) *Elevated serum urea-to-creatinine ratio is associated with adverse inpatient clinical outcomes in non-end stage chronic kidney disease. Scientific Reports, 12, 20827.*

Cordiano, R., Di Gioacchino, M., Mangifesta, R., Panzera, C., Gangemi, S. & Minciullo, P.L. (2023) *Malondialdehyde as a Potential Oxidative Stress Marker for Allergy-Oriented Diseases: An Update. Molecules, 28(16),5979.*

Cravedi, P. & Remuzzi, G. (2013) *Pathophysiology of proteinuria and its value as an outcome measure in chronic kidney disease*. *British Journal of Clinical Pharmacology, 76(4), 516–523.*

Cui, Z. G., Ahmed, K., Zaidi, S. F. & Muhammad, J. S. (2021) *Ins and outs of cadmium-induced carcinogenesis: Mechanism and prevention. Cancer Treatment and Research Communications, 27, 100372.*

Dkhil, M.A., Diab, M.S.M., Lokman, M.S., El-Sayed, H., Bauomy, A.A., Al-Shaebi, E.M. & Al-Quraishy, S. (2020) *Nephroprotective effect of Pleurotus ostreatus extract against cadmium chloride toxicity in rats. Anais da Academia Brasileira de Ciências | Annals of the Brazilian Academy of Sciences, 2(1), e20191121.*

Draper, H. & Hadley, M. (1990) *Malondialdehyde determination as index of lipid peroxidation. Methods in Enzymology, 186, 421- 431.*

Ebhohon, S.O**.**, Ojowu, J.O., Osibemhe, M. & Onwudo, J.I. (2022). *Loranthus micranthus Linn Aqueous Leaf Extract Exhibited Protection Against Tissue Damage by Paracetamol Toxicity in Male Wistar Rats. SAU Science-Tech Journal, 7(1), 13-25.*

Ebokaiwe, A., Ijomone, O., Edeh, O., Oteh, I. & Ebuka, D. (2018) *Influence of Loranthus micranthus on hepatic and renal antioxidant status and impaired glycolytic flux in streptozotocin-induced diabetic rats. Journal of Basic and Clinical Physiology and Pharmacology, 29(5), 447-461.*

Ebokaiwe, A.P., Ijomone, O.M., Griffin, S., Ehiri, R.C., Obeten, K.E., Nwankwo, J.O., Ejike, C.E.C.C. & Keck. C.M. (2019) *Nanosized selenium and Loranthus micranthus leaves ameliorate streptozotocin-induced hepato-renal dysfunction in rats via enhancement of antioxidant system, regulation of caspase 3 and Nrf2 protein expression. Pharma Nutrition 9, 100150.*

Gaweł, S., Wardas, M., Niedworok, E. & Wardas, P. (2004) *Dialdehyd malonowy (MDA) jako wskaźnik procesów peroksydacji lipidów w organizmie [Malondialdehyde (MDA) as a lipid peroxidation marker]. Wiadomosci Lekarskie (Warsaw, Poland: 1960), 57(9-10), 453–455.*

Genchi, G., Sinicropi, M. S., Lauria, G., Carocci, A. & Catalano, A. (2020) *The Effects of Cadmium Toxicity. International Journal of Environmental Research and Public Health 17(11): 3782.*

Gobe, G. & Crane, D. (2010) *Mitochondria, reactive oxygen species and cadmium toxicity in the kidney. Toxicology Letters, 198(1), 49-55.*

Haider, M.Z. & Aslam, A. (2022) *Proteinuria.* In: StatPearls Treasure Island (FL): StatPearls Publishing.

Harborne, J.B. (1984) *Phytochemical methods: a guide to modern techniques of plant analysis,* London., (2nd ed.) Chapman & Hall.

Hernández-Cruz, E. Y., Amador-Martínez, I., Aranda-Rivera, A. K., Cruz-Gregorio, A. & Pedraza-Chaverri, J. (2022) *Renal damage induced by cadmium and its possible therapy by mitochondrial transplantation. Chemico-Biological Interactions, 361, 109961.*

Hlophe, S. & Bassey K. (2023) *Phytochemical Profiling, and Antioxidant Potentials of South African and Nigerian Loranthus micranthus Linn.: The African Mistletoe Exposé. Plants, 12(10), 2016.*

Ighodaro, O.M & Akinloye, O.A (2018) *First-line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine, 54 (4), 287-293.*

Imade, F., Imade, M. & Oyewole, B. (2014) *Effect of aqueous extract of Loranthus micranthus (mistletoe) on total protein, catalase and NOS activity in the kidney of albino rats induced with Pb acetate. Journal of Medicinal Plants Studies, 2(3), 72-79.*

Khan, H., Saeedi, M., Nabavi, M. S., Mubarak S. M. & Bishayee, A. (2019) *Glycosides from Medicinal Plants as Potential Anticancer Agents: Emerging Trends Towards Future Drugs. Current Medicinal Chemistry, 26(13), 2389-2406.*

Khan, M. I., Karima, G., Khan, M. Z., Shin, J. H. & Kim, J. D. (2022) *Therapeutic Effects of Saponins for the Prevention and Treatment of Cancer by Ameliorating Inflammation and Angiogenesis and Inducing Antioxidant and Apoptotic Effects in Human Cells. International Journal of Molecular Sciences, 23(18), 10665.*

Lorke, D. (1983) *A new approach to practical acute toxicity testing. Archives of Toxicology 54: 275-87. 1983.*

Macáková, K., Afonso, R., Saso, L. & Mladěnka, P. (2019) *The influence of alkaloids on oxidative stress and related signalling pathways. Free Radical Biology and Medicine. 134, 429-444.*

Mitra, S., Chakraborty, A.J., Tareq, A.M., Emran, T.B., Nainu, F., Khusro, A., Idris, A.M., Khandaker, M.U., Osman, H., Alhumaydhi, F.A. & Simal-Gandara, J. (2022) *Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. Journal of King Saud University – Science, 34(3), 101865.*

Moghadamtousi, S.Z., Hajrezaei, M., Abdul Kadir, H. & Zandi, K. (2013) *Loranthus micranthus Linn.: Biological Activities and Phytochemistry. Evidence-based complementary and alternative medicine: eCAM, 2013, 273712.*

National Institutes of Health Office of Laboratory Animal Welfare Public Health Service policy on the humane care and use of laboratory animals. Bethesda, MD: NIH, 2002.

Nicoletti, M. (2023) *The Antioxidant Activity of Mistletoes (Viscum album and Other Species). Plants (Basel, Switzerland), 12(14), 2707.*

Norris, K.C., Smoyer, K.E., Rolland, C. *et al.* (2018) *Albuminuria, serum creatinine, and estimated glomerular filtration rate as predictors of cardio-renal outcomes in patients with type 2 diabetes mellitus and kidney disease: a systematic literature review. BMC Nephrology, 19, 36.*

Orr, S.E. & Bridges, C.C. (2017). *Chronic kidney disease and exposure to nephrotoxic metals. International Journal of Molecular Science, 18,1039.*

Prozialeck, W. C. & Edwards, J. R. (2010) Early biomarkers of cadmium exposure and nephrotoxicity. *Biometals: An International Journal on the Role of Metal Ions in Biology, Biochemistry, and Medicine, 23(5), 793–809.*

Prozialeck, W. C., VanDreel, A., Ackerman, C. D., Stock, I., Papaeliou, A., Yasmine, C., Wilson, K., Lamar, P. C., Sears, V. L., Gasiorowski, J. Z., DiNovo, K. M., Vaidya, V. S. & Edwards, J. R. (2016) *Evaluation of cystatin C as an early biomarker of cadmium nephrotoxicity in the rat. Biometals: An International Journal on the Role of Metal Ions in Biology, Biochemistry, and Medicine, 29(1), 131–146.*

Rafati, A., Hoseini, L., Babai, A., Noorafshan, A., Haghbin, H. & Karbalay-Doust, S. (2015) *Mitigating the effect of resveratrol on the structural changes of mice liver and kidney induced by cadmium; a stereological study. Preventive Nutrition and Food Science, 20(4), 266-275.*

Rennke, H.G. & Denker, B.M. (2020) *Renal Pathology: The Essentials*. 5th ed. Wolters Kluwer; New York, NY, USA.

Satarug, S. (2018) *Dietary Cadmium Intake and Its Effects on Kidneys. Toxics, 6(1), 15.*

Satarug, S., Boonprasert, K., Gobe, G. C., Ruenweerayut, R., Johnson, D. W., Na-Bangchang, K. & Vesey, D. A. (2018) *Chronic exposure to cadmium is associated with a marked reduction in glomerular filtration rate. Clinical Kidney Journal, 12(4), 468–475.*

Simmons, R. W., Pongsakul, P., Saiyasitpanich, D. & Klinphoklap, S. (2005) *Elevated levels of cadmium and zinc in paddy soils and elevated levels of cadmium in rice grain downstream of a zinc mineralized area in Thailand: implications for public health. Environmental Geochemistry and Health, 27(5-6), 501–511.*

Sofowora, A. (1993) *Medicinal plants and Traditional medicine in Africa*, Ibadan., Spectrum Books Ltd.

Sripada, K. & Lager, A. M. (2022) *Interventions to reduce cadmium exposure in low- and middle-income countries during pregnancy and childhood: A systematic review. Journal of Global Health, 12, 04089.*

Sun, Y., Oberley, W. & Li, Y. (1988) *A simple method for clinical assay of superoxide dismutase. Clinical Chemistry, 34, 497- 500.*

Szurpnicka, A., Kowalczuk, A. & Szterk, A. (2020) *Biological activity of mistletoe: in vitro and in vivo studies and mechanisms of action. Archives of Pharmacal Research, 43, 593–629.*

Tietze, F. (1969) *Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Analytical Biochemistry, 27, 502 – 522.*

T
rease, G.E. & Evans, W.C. (2002) *Pharmacognosy*, (15th ed.) London., Saunders Publishers.

Ullah, A., Munir, S., Badshah, S. L., Khan, N., Ghani, L., Poulson, B. G., Emwas, A. H. & Jaremko, M. (2020) *Important Flavonoids and Their Role as a Therapeutic Agent. Molecules (Basel, Switzerland), 25(22), 243.*

Wan, X., Xing, Z., Ouyang, J., Liu, H., Cheng, C., Luo, T., Yu, S., Meihua, L. & Huang, S. (2022) *Histomorphological and ultrastructural cadmium-induced kidney injuries and Precancerous lesions in rats and screening for biomarkers. Bioscience Reports, 42(6), BSR20212516.*

Wang, C., Nie, G., Zhuang, Y., Hu, R., Wu, H., Xing, C., Li, G., Hu, G., Yang, F. & Zhang, C. (2020) *Inhibition of autophagy enhances cadmium-induced apoptosis in duck renal tubular epithelial cells. Ecotoxicology and Environmental Safety, 205, 111188.*

Wang, P., Chen, H., Kopittke, P. M. & Zhao, F. J. (2019) *Cadmium contamination in agricultural soils of China and the impact on food safety. Environmental Pollution (Barking, Essex: 1987), 249, 1038–1048.*

Wongmekiat, O., Peerapanyasut, W. & Kobroob, A. (2018) *Catechin supplementation prevents kidney damage in rats repeatedly exposed to cadmium through mitochondrial protection. Naunyn-Schmiedeberg's Archives of Pharmacology 391(4), 385-394.*

Yan, L. J. & Allen, D. C. (2021) *Cadmium-Induced Kidney Injury: Oxidative Damage as a Unifying Mechanism. Biomolecules, 11(11), 1575.*

Yimthiang, S., Vesey, D. A., Pouyfung, P., Khamphaya, T., Gobe, G. C. & Satarug, S. (2023) *Chronic Kidney Disease Induced by Cadmium and Diabetes: A Quantitative Case-Control Study. International Journal of Molecular Sciences, 24(10): 9050.*

Moghadamtousi, S.Z., Hajrezaei, M., Abdul Kadir, H. & Zandi, K. (2013) *Loranthus micranthus Linn.: Biological Activities and Phytochemistry. Evidence-based complementary and alternative medicine: eCAM, 2013: 273712.*