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Evaluation of the Antioxidant and Protective Effects of *Dioscorea villosa* Extracts on Gentamicin-Induced Kidney Damage in Albino Wistar Rats

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Abstract

Antioxidants can be effective in the prevention of oxidative stress. The study aimed to evaluate the antioxidant and protective effects of *Dioscorea villosa* extracts on gentamicin-induced kidney damage in albino wistar rats. A total of 114 albino rats were divided into 19 groups of 6 rats each, categorized into four subgroups (A-D, E-H, I-L, M-P) and control groups (Q, R, S). Kidney damage was induced in all groups except the normal control (Q) by administering 100 mg/kg of gentamicin intraperitoneally. Groups A-D received 200, 400, 600, and 800 mg/kg of deionized water leaf extract (DWL), while groups E-H received the same doses of deionized water root extract (DWR). Groups I-L were given diethyl ether leaf extract (DER) in similar doses, and groups M-P received diethyl ether root extract (DEL). Group Q received normal saline, group R (negative control) also received normal saline, and group S (positive control) was given 25 mg/kg of silymarin. All treatments were administered orally for 14 days. Administration of the extract significantly increased the superoxide dismutase (SOD) levels at all doses compared to the untreated group. In terms of catalase activity, there was no significant difference ($p<0.05$) between the groups treated with 800 mg/kg and 600 mg/kg of DWL and DWR extracts. Assessment of glutathione reductase (GR) and Malondialdehyde (MDA) activity after treatment showed a dose-dependent increase in the groups treated with DWL, DWR, DEL, and DER extracts. The extracts helped in mitigation of oxidative damage, and may function as alternative remedies to conventional therapeutic approaches.

Keyword: Antioxidants, oxidative stress, gentamicin induced, wistar rats, treatment, *D. villosa* extracts.

Introduction

The kidney is the primary organ essential for the human body to perform various crucial functions (Al-Naimi et al., 2019). Many medications that are used to treat and manage a variety of illnesses, such as diabetes, hypertension, infections, and other pathologies, are nephrotoxic. Millions of individuals worldwide suffer from kidney disease, making it a major public health issue with various implications and identified risk factors (Alomar, 2020). Nephrotoxicity refers to a significant decline in kidney performance due to the damaging impacts of drugs and chemicals and this condition can manifest in various forms, with some medications potentially affecting renal function in multiple ways (Al-Naimi et al., 2019). Gentamicin, a widely used aminoglycoside antibiotic, is known for its nephrotoxic effects (Althunibat et al., 2022a), which can lead to acute

kidney injury (AKI). It has been shown that gentamicin (GM) damages tubules by altering how important components of cells that facilitate the transport of solutes and water function (Lopez-Novoa et al., 2011; Randjelović et al., 2017).

Various Dioscorea species have been adopted as food sources across different ethnic populations and geographical regions due to their high nutritional content and potential therapeutic advantages in treating and curing various health issues (Elkhadragy et al., 2018). *D. villosa*, commonly known as wild yam, has been traditionally used for its medicinal properties because it contains significant levels of bioactive compounds (Obidiegwu et al., 2020). These compounds positively affect metabolic reactions by inhibiting receptor activities, modulating enzyme actions, providing antioxidant, antihypertensive, anti-inflammatory, and antidiabetic benefits, and regulating gene expression through both activation and suppression (Correia et al., 2012). Previous studies have highlighted the potential of various plant extracts in mitigating drug-induced organ damage through their antioxidant properties (Elkhadragy et al., 2018; El-Said et al., 2023; Ke et al., 2019). However, the specific protective effects of *D. villosa* on gentamicin-induced kidney damage have not been extensively studied.

The present study aims to evaluate the antioxidant and protective effects of *D. villosa* extracts on gentamicin-induced kidney damage in albino Wistar rats. An antioxidant prevent oxidation, acting at various levels in the oxidative sequence with multiple mechanisms of action (Diplock, 1994). A diet rich in antioxidants prevents many diseases by scavenging free radicals and lessening oxidative harm to cells. Natural antioxidants are found in a wide variety of fruits and vegetables, and both the general public and scientists are very interested in them (Aluyor & Oboh, 2014).
This study demonstrates the therapeutic potential of *D. villosa* and its effectiveness in preventing or mitigating drug-induced nephrotoxicity through the assessment of the impact on oxidative stress markers.

Materials and Methods

Materials

Collection of roots and leaves of D. villosa

Roots and leaves of *D. villosa* were harvested from Uffioboto Amike Ezzangbo in the Ohaukwu Local Government Area of Ebonyi State, Nigeria. The roots and leaves of *D. villosa* were identified by Mr. O.E. Nwankwo, a taxonomist, at, Ebonyi State University, and were assigned the voucher number EBSU-H-782.

Animals

Male albino Wistar rats used for the study were procured from the Department of Pharmacology, University of Nigeria Nsukka (UNN), Enugu State, Nigeria. Upon arrival, the rats were given a seven-day acclimatization period. They were housed in cages, provided with commercial poultry feed (growers mesh), and had unrestricted access to clean water.

Methods

Preparation and extraction of plant samples

The roots and leaves were air-dried for 7-8 days at room temperature. Once dried, the materials were ground into powder using an electric grinder and stored at 4°C in airtight containers.

For extraction, 1000 g of powdered leaves were separately soaked in 1500 mL of deionized water and diethyl ether for 48 h, with occasional stirring. After filtering the suspensions through muslin cloth, the filtrates were evaporated using a rotary evaporator set at 30°C. The resulting extracts, named deionized water-leaf extract (DWL extract) and diethyl ether-leaf extract (DEL extract), were stored at 4°C in airtight containers.

Similarly, 750 g of powdered roots were each soaked in 1000 mL of deionized water and diethyl ether for 48 h, with occasional shaking. After filtering through muslin cloth, the filtrates were evaporated with a rotary evaporator. The final products, deionized water-root extract (DWR extract) and diethyl ether-root extract (DER extract) were also stored in airtight containers at 4°C for future use.

In vivo administration of extracts

A total of 114 adult male albino rats weighing 150-300 g were randomly assigned to 19 groups, each with 6 rats. The groups were divided into four subgroups (A-D, E-H, I-L, and M-P), with three additional groups, Q, R, and S, serving as

normal, negative, and positive controls, respectively. Kidney damage was induced in all groups (except the normal control group) by injecting 100 mg/kg gentamicin intraperitoneally.

Groups A-D received 200, 400, 600, and 800 mg/kg of deionized water leaf extract (DWL extract) respectively. Groups E-H received the same doses of deionized water root extract (DWR extract) as above. Groups I-L received 200, 400, 600, and 800 mg/kg diethyl ether leaf extract (DER extract) respectively. Groups M-P received 200, 400, 600, and 800 mg/kg diethyl ether root-extract (DEL-extract) respectively. All treatments were administered via oral intubation for 14 days.

Group Q, the normal control, was not induced with kidney damage and received normal saline. Group R, the negative control, was induced with kidney damage and received normal saline. Group S, the positive control, was induced with kidney damage and treated with 25 mg/kg silymarin.

Preparation of tissue homogenate and assessment of antioxidant activities

Portions of the kidneys and hearts were removed, washed in normal saline, and homogenized in potassium phosphate buffer at a pH of 7.4. The homogenates were centrifuged (Allegra X-15R, Beckman Coulter) at 10,000 rpm for 15 minutes, and the antioxidant parameters were determined using the supernatants. Superoxide dismutase (SOD) activity was assayed by the method of Kakkar *et al*. (Kakkar et al., 1984). The method of Aebi (Aebi, 1983)was used to assay catalase activity in the heart and kidney tissue homogenates, while glutathione reductase (GR) activity was measured following Rumley & Paterson (Rumley & Paterson, 1998). The quantification of malondialdehyde (MDA) in the heart and kidney tissue homogenates was conducted using the method of Buege and Aust (Buege & Aust, 1978).

Results

The study investigated the effects of various extracts (DWL, DWR, DEL, and DER) on SOD, catalase, GR, and MDA levels. The results are summarized as follows:

The group treated with 800 mg/kg of extract exhibited significantly higher ($p<0.05$) SOD levels compared to all other groups within the DWL and DWR extract categories. In contrast, there was no significant difference ($p>0.05$) in SOD levels between the 200 mg/kg and 400 mg/kg groups, whereas the 600 mg/kg group demonstrated substantially higher ($p<0.05$) SOD levels than both the 200 mg/kg and 400 mg/kg groups. SOD levels increased in a dose-dependent manner in the DEL extract study. For the DER extract, SOD levels did not differ significantly ($p>0.05$) between the groups receiving 600 mg/kg and 800 mg/kg; however, both dosages had significantly higher ($p<0.05$) SOD levels than the 200 mg/kg and 400 mg/kg groups. Additionally, the SOD levels in the group treated with 800 mg/kg were similar to those in the uninduced normal control group. The untreated group had significantly lower ($p<0.05$) SOD levels than all other groups.

In terms of catalase activity, there was no significant difference ($p<0.05$) between the groups treated with 800 mg/kg and 600 mg/kg of DWL and DWR extracts. However, the groups treated with 600 mg/kg and 800 mg/kg had significant lower ($p<0.05$) catalase activity compared to the groups treated with 200 mg/kg and 400 mg/kg. The DEL extract showed a dose-dependent decrease in catalase activity. For the DER extract, the 200 mg/kg group had higher ($p<0.05$) catalase activity compared to the 600 mg/kg and 800 mg/kg groups, but there was no significant difference ($p>0.05$) in catalase activity between the 200 mg/kg, 400 mg/kg, and 600 mg/kg groups. The uninduced control group had catalase activity comparable to the group treated with 800 mg/kg of extract.

Assessment of glutathione reductase (GR) activity after treatment showed a dose-dependent increase in the groups treated with DWL, DWR, DEL, and DER extracts. GR levels were significantly lower ($p<0.05$) in the untreated group compared to the treated groups. There was no difference ($p>0.05$) in GR activity between the standard drug group and the uninduced control group. Both the uninduced control group and the standard drug-treated

group had significantly higher ($p<0.05$) GR levels compared to all extract-treated groups.

Similarly, in the oxidative stress indices, the gentamicin-induced rats showed a significant ($P<0.05$) increase in Malondialdehyde (MDA) levels compared to the other groups. The groups of rats treated with 200, 400, 600, and 800 mg/kg of deionized water and diethyl ether extracts of the leaves and roots of *D. villosa* had significantly ($P<0.05$) reduced MDA levels, comparable to the standard control group treated with silymarin. The levels of MDA decreased with increasing concentration of the extracts in the groups treated with DWL, DWR, DEL, and DER extracts.

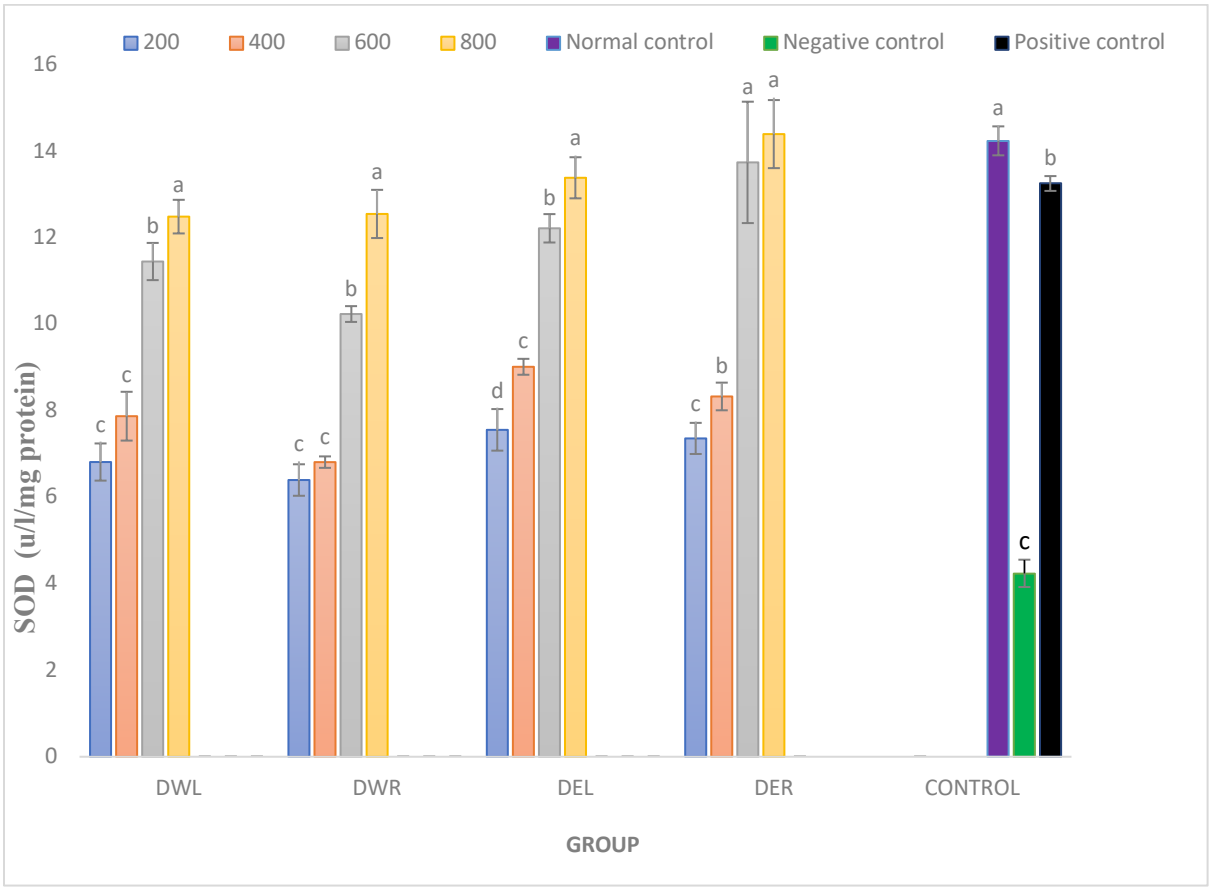

Fig 1: Superoxide dismutase activity in rats administered *D. villosa* leaf and root extracts following kidney injury. Variables with distinct alphabets exhibit a significant difference at ($P<0.05$). DWL represents deionized water leaf extract, DWR represents deionized water root extract, DER represents diethyl ether leaf extract, and DEL represents diethyl ether root extract.

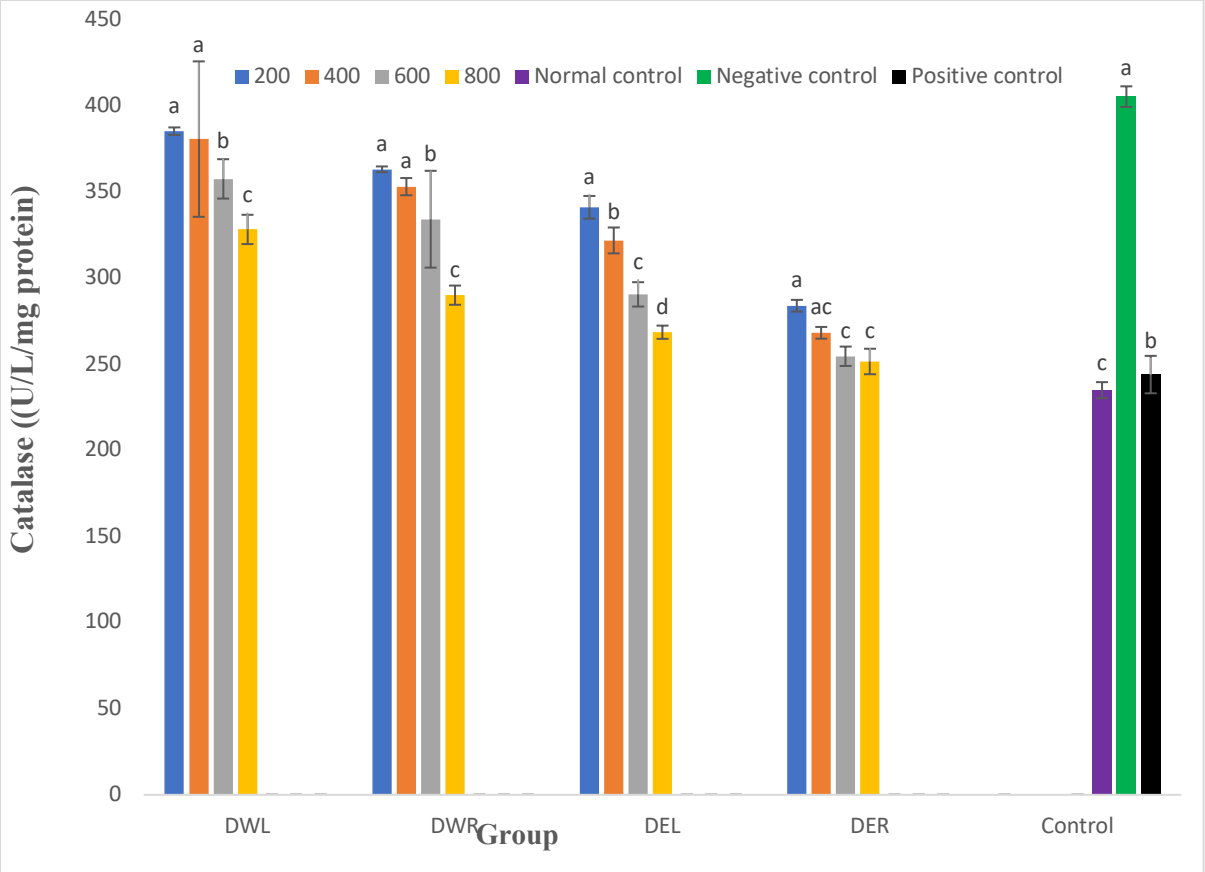

Fig 2: Catalase activity in rats administered *D. villosa* leaf and root extracts following kidney injury. Variables with distinct alphabets exhibit a significant difference at ($P<0.05$). DWL represents deionized water leaf extract, DWR represents deionized water root extract, DER represents diethyl ether leaf extract, and DEL represents diethyl ether root extract.

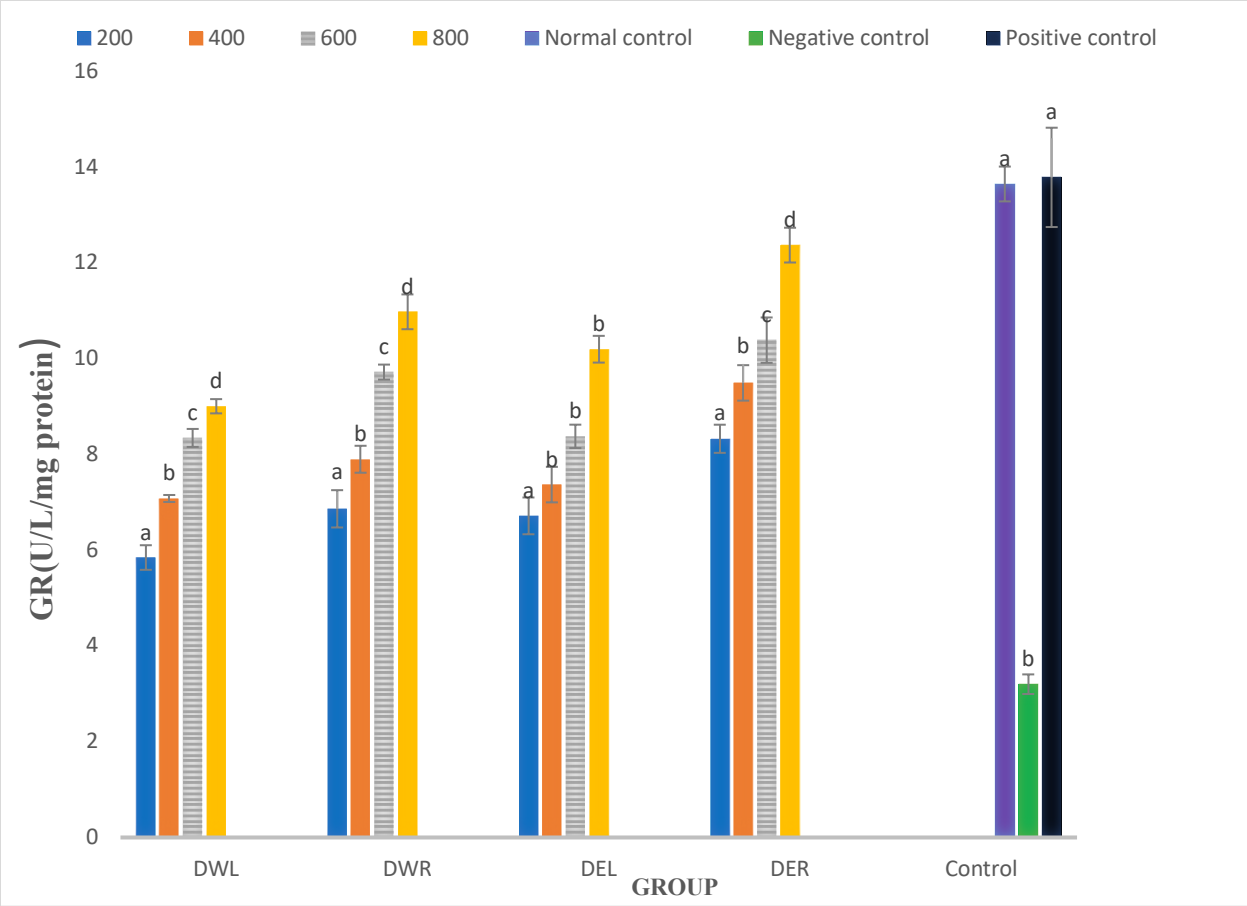

Fig 3: Glutathion reductase activity in rats administered *D. villosa* leaf and root extracts following kidney injury. Variables with distinct alphabets exhibit a significant difference at ($P<0.05$). DWL represents deionized water leaf extract, DWR represents deionized water root extract, DER represents diethyl ether leaf extract, and DEL represents diethyl ether root extract.

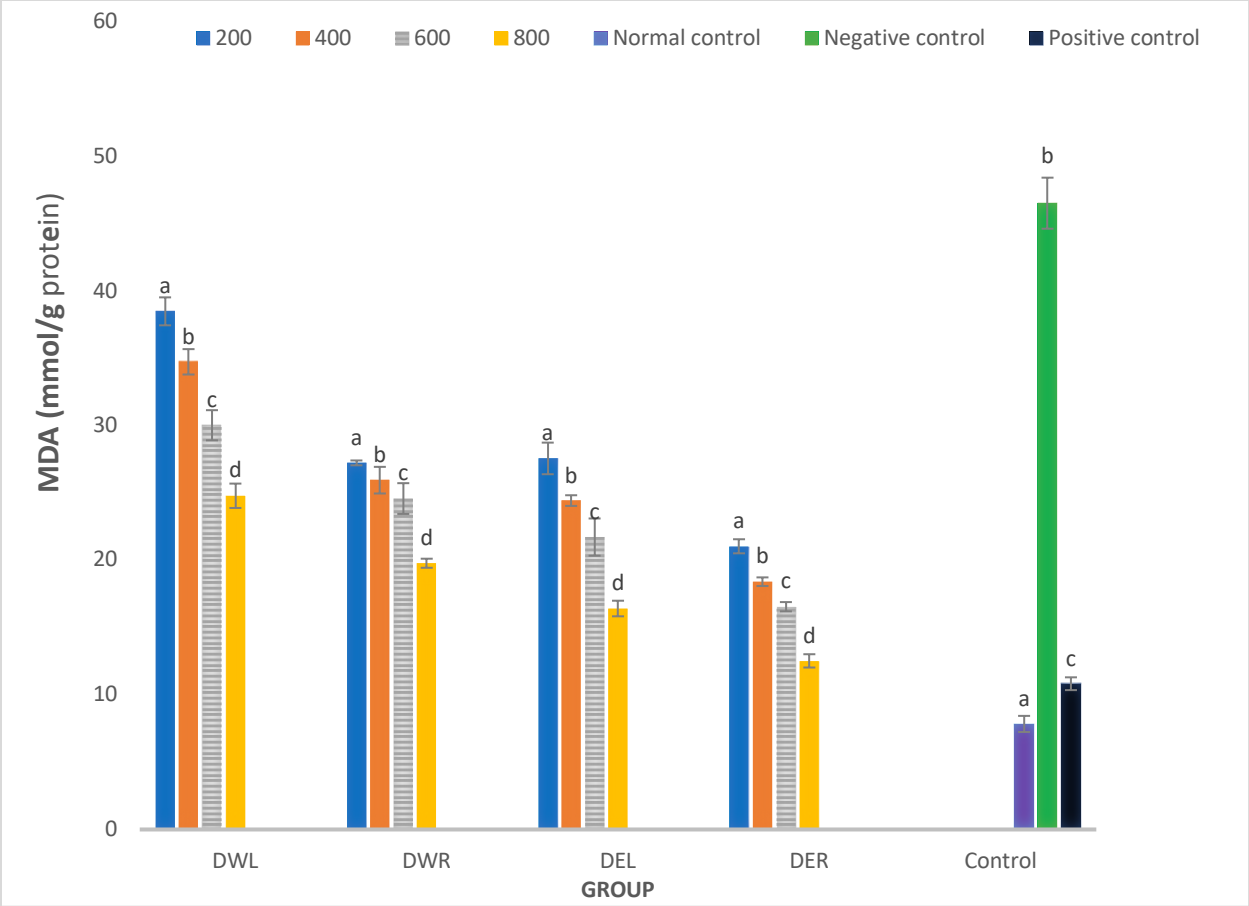

Fig 4: Malonedialdehyde level in rats administered *D. villosa* leaf and root extracts following kidney injury. Variables with distinct alphabets exhibit a significant difference at (P<0.05). DWL represents deionized water leaf extract, DWR represents deionized water root extract, DER represents diethyl ether leaf extract, and DEL represents diethyl ether root extract.

Discussion

Antioxidant are useful in the prevention of oxidative stress (Atawodi et al., 2014) and function through a variety of ways, such as lowering power, improving the ability to scavenge free radicals, and inhibiting the production of free radicals (Gan et al., 2017). The study investigated the effects of various extracts (DWL, DWR, DEL, and DER) from *D. villosa* on superoxide dismutase SOD, catalase, GR, and MDA levels in gentamicin-induced kidney damage in rats. The results demonstrated significant protective effects ($p<0.05$) of the extracts on oxidative stress parameters, highlighting their potential as therapeutic agents in oxidative stress-related conditions. Several species of Dioscorea are commonly used for various medicinal purposes, with their therapeutic effects attributed to the presence of phytochemicals that exhibit antioxidant properties. These properties are primarily linked to their radical-scavenging ability in chemical assays and their beneficial effects on the endogenous antioxidant system (Adomėnienė & Venskutonis, 2022).

Metalloenzymes known as superoxide dismutases (SODs), present in every life form, serve as the first line of protection against damage caused by reactive oxygen species (ROS) (Younus, 2018). The results revealed that the group treated with 800 mg/kg of DWL and DWR extracts had significantly higher ($p<0.05$) SOD levels compared to all other groups. Similarly, the 600 mg/kg group showed significantly higher ($p<0.05$) SOD levels compared to the 200 mg/kg

and 400 mg/kg groups, indicating a dose-dependent increase. Notably, the DEL extract also exhibited a dose-dependent increase in SOD levels. For the DER extract, both 600 mg/kg and 800 mg/kg doses resulted in significantly higher ($p<0.05$) SOD levels compared to the 200 mg/kg and 400 mg/kg groups, with the 800 mg/kg group comparable to the uninduced normal control. This suggests that the higher doses of the extracts are more effective in enhancing SOD activity. SOD is a crucial enzyme in the antioxidant defense system that mitigates oxidative damage by dismutating superoxide radicals into hydrogen peroxide and oxygen (Saxena et al., 2023; Subi M et al., 2022). Enhanced SOD activity indicates improved capacity to counteract oxidative stress, which is essential in protecting kidney tissue from gentamicin-induced damage (Althunibat et al., 2022b; Eluu, et al., 2024).

Catalase activity assessment indicated no significant difference ($p>0.05$) between the groups treated with 800 mg/kg and 600 mg/kg of DWL and DWR extracts. However, both these groups had significantly lower ($p<0.05$) catalase activity compared to the 200 mg/kg and 400 mg/kg groups. The DEL extract showed a dose-dependent decrease in catalase activity, while the DER extract had higher catalase activity at 200 mg/kg compared to the 600 mg/kg and 800 mg/kg groups. The uninduced control group's catalase activity was comparable to that of the 800 mg/kg extract-treated group. Of all the antioxidant enzymes, catalase is one of the most significant. Catalase is a therapeutic agent used to treat a variety of oxidative stress-related disorders because it breaks down hydrogen peroxide into harmless molecules like oxygen and water(Nandi et al., 2019). The lower catalase activity at higher doses might indicate a complex regulatory mechanism or a compensatory response due to enhanced SOD activity. This interplay between SOD and catalase suggests that while SOD neutralizes superoxide radicals, the role of catalase in breaking down hydrogen peroxide may be regulated differently in response to varying oxidative stress levels (Weydert & Cullen, 2010). GR activity exhibited a dose-dependent increase in the groups treated with DWL, DWR, DEL, and DER extracts. The untreated group had significantly lower ($p<0.05$) GR activity compared to the treated groups. Both the uninduced control group and the standard drug-treated group (silymarin) had significantly higher ($p<0.05$) GR levels compared to all extract-treated groups. Glutathione plays a role in endogenous and xenobiotic chemical detoxification by directly neutralizing many oxidative compounds, aiding excretion from the body and cells, and facilitating toxin transport across plasma membranes through at least four distinct processes, the most significant being the generation of glutathione S-conjugates (Eluu, Oko, et al., 2024; Pizzorno, 2014). The increase in GR activity with extract treatment suggests an enhanced capacity for detoxifying reactive oxygen species (ROS), further supporting the protective effects of these extracts against oxidative stress.

In the MDA result, gentamicin-induced rats showed a significant ($P<0.05$) increase in MDA levels, indicating elevated lipid peroxidation and oxidative stress. Treatment with varying doses of DWL, DWR, DEL, and DER extracts significantly ($P<0.05$) reduced MDA levels, comparable to the standard control group treated with silymarin. The dose-dependent decrease in MDA levels with extract treatment highlights the potential of these extracts in mitigating oxidative damage. MDA is a marker of lipid peroxidation, and its reduction signifies a decrease in cellular membrane damage and overall oxidative stress. The ability of these extracts to lower MDA levels suggests their efficacy in protecting renal tissues from gentamicin-induced oxidative damage, potentially offering a therapeutic advantage in managing nephrotoxicity.

Several phytochemicals have been identified to be present in *Dioscorea* (Ezeabara & Anona, 2018). Numerous phytochemicals especially alkaloids in the leaves of the experimental plants have been shown to exhibit antioxidant activity (Ferreira et al., 2015; Gan et al., 2017; Pu et al., 2013), which explains the antioxidant effects we observed in our study.

Conclusion

The results have significant implications for the possible therapeutic application of *D. villosa* extracts in disorders associated with oxidative stress. Through enhancement of significant antioxidant enzyme functions and mitigation of

oxidative damage indicators, these extracts may function as alternative remedies to conventional therapeutic approaches. The molecular mechanisms behind these effects and the long-term safety and efficacy of these extracts in clinical settings should be investigated in details.

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