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The Impact of Dioscorea *villosa* Extracts on Haematological Parameters in Gentamicin-Induced Nephrotoxicity in Albino Wistar Rats

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Abstract

Nephrotoxicity, or kidney damage, is associated with the use of various therapeutic agents, including gentamicin. This study aimed to assess the impact of *Dioscorea villosa* leaf and root extracts on hematological parameters in gentamicin-induced nephrotoxicity of albino rats. A total of 114 albino rats were randomly assigned to 19 groups of 6 rats each, divided 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 injecting 100 mg/kg of gentamicin intraperitoneally. Groups A-D received 200, 400, 600, and 800 mg/kg of deionized water leaf extract (DWL). Groups E-H received the same deionized water root extract (DWR) doses. Groups I-L received diethyl ether leaf extract (DER), and groups M-P received diethyl ether root extract (DEL). Group Q received normal saline, group R (negative control) received normal saline, and group S (positive control) received 25 mg/kg of silymarin. All treatments were administered orally for 14 days, and hematological assessments were carried out using standard methods. Results showed that Hb, PCV, and red blood cell (RBC) counts were significantly lower ($p<0.05$) in the untreated gentamicin-induced group compared to all other groups. However, administering different doses of *D. villosa* extracts significantly increased ($p<0.05$) the Hb, PCV, and RBC levels, similar to the effects of silymarin. The total WBC count was significantly higher ($p<0.05$) in the untreated group compared to all other groups. These findings suggest that *D. villosa* could be a valuable natural remedy for nephrotoxicity.

Keywords:*Dioscorea villosa,* Gentamicin-induced, Nephrotoxicity, traditional medicine, hematological parameters

Introduction

Acute kidney injury (AKI) affects over 13.3% of the world's population each year, making it a significant global health concern (Kwiatkowska et al., 2021). While the etiology of AKI is complex, the primary causes include ischemia, nephrotoxicity, and sepsis (Eluu et al., 2024; Kwiatkowska et al., 2021; Zuk & Bonventre, 2016). Nephrotoxicity, or kidney damage, is associated with the use of various therapeutic agents, including gentamicin, an aminoglycoside

antibiotic commonly used to treat severe bacterial infections (Walker et al., 1999). Gentamicin-induced nephrotoxicity is characterized by oxidative stress, inflammation, renal tubular cell apoptosis, leading to compromised kidney function (Althunibat et al., 2022; Randjelović et al., 2017). This condition necessitates exploring effective therapeutic strategies to mitigate its harmful effects. One promising approach is the use of medicinal plants with potential nephroprotective properties.

D. villosa, commonly known as wild yam, has a long history of use in traditional medicine for its health benefits (Oladebeye et al., 2023; Ziyan et al., 2024). Since prehistoric times, Indigenous peoples have utilized the roots, tubers, and rhizomes of yams both as a food source and for their medicinal properties, with the Dioscorea genus, comprising over 600 species, found in diverse regions including Africa, Asia, Latin America, the Caribbean, and Oceania (Kassie et al., 1996; Obidiegwu et al., 2020). It contains several bioactive compounds, including diosgenin, alkaloids, and flavonoids, which are believed to confer various therapeutic effects, such as anti-inflammatory, antioxidant, and hepatoprotective activities (Wang et al., 2023). These compounds, present in foods and spices, are gradually becoming more popular than synthetic medications because they act on several different molecular targets simultaneously, which effectively works to prevent or treat chronic disorders (Raju & Rao, 2012).

This therapeutic potential, rooted in the bioactive compounds of *D. villosa*, aligns with the need to explore its effects on systemic health issues, particularly those associated with nephrotoxicity. Hematological parameters, which serve as critical indicators of overall health, are often disrupted in conditions like gentamicin-induced nephrotoxicity, necessitating investigations into potential natural remedies such as *D. villosa*. Hematological parameters, are critical indicators of overall health and can be significantly impacted by nephrotoxicity (Kelada et al., 2012). Gentamicin-induced kidney damage often results in anemia and leukocytosis, reflecting the broader systemic impact of renal impairment. This study investigates the impact of *D. villosa* leaf and root extracts on hematological parameters in albino rats with gentamicin-induced nephrotoxicity. Our findings may help in understanding the therapeutic potential of *D. villosa* in nephrotoxicity management.

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 Ohaukwu Local Government Area of Ebonyi State, Nigeria. These samples were then identified by a taxonomist from the Department of Applied Biology at Ebonyi State University, Abakaliki.

Animals

Male albino Wistar rats used in the study was procured from the Department of Pharmacology at the 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

Acute toxicity study of the extracts

The lethal dose (LD_{50}) of the extracts was determined following the Lorke's method (Lorke, 1983).

A total of 25 rats were used for the acute toxicity test. The animals were acclimatized for seven days before being randomly divided into five groups of five rats each. Each group was orally administered varying doses of the deionized water and diethyl ether extracts (leaf and root) at 400, 600, 900, 1200, and 1500 mg/kg body weight. The rats were monitored individually for 72 hours to observe signs of toxicity, including dullness, weakness, convulsions, sedation, diarrhea, and mortality.

Subsequently, the procedure was repeated with higher doses of 2500, 3000, 3500, 4000, and 5000 mg/kg body weight.

Formula for calculating the LD_{50}, using their relationship

$$LD_{50} = \sqrt{D_0 X D_{100}}$$

Where D_0 = Highest dose that would give no mortality

D_{100} = Lowest dose that would produce 100 % mortality

Preparation and extraction of plant samples

The roots and leaves were air-dried at room temperature for 7-8 days. Once dried, the materials were ground into powder 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 to dryness 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 to dryness 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 acclimated for seven days. They were then randomly assigned to 19 groups, each with six 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). Groups E-H received the same doses of deionized water root extract (DWR extract). Groups I-L received 200, 400, 600, and 800 mg/kg of diethyl ether leaf extract (DER extract). Groups M-P received 200, 400, 600, and 800 mg/kg of diethyl ether root-extract (DEL-extract). 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.

Collection of Blood Samples

Blood samples were collected via cardiac puncture. Samples for hematological analysis were stored in EDTA containers to be used later in the research.

Determination of Hematological parameters

Hemoglobin (Hb) Concentration, Packed cell volume, and Total leukocyte count were determined as described by Cole (Coles, 1986). Red blood was determined as described by Baker and Silverton (Baker & Silverton, 1976).

Results

Effect of the extracts on hematological function

The effect of the extracts on hematological function after treatment is shown in Figures 1-4. Hemoglobin (Hb), packed cell volume (PCV), and red blood cell (RBC) count were significantly lower ($p<0.05$) in the untreated gentamicin-induced group compared to all other groups. However, administering different doses (200, 400, 600, and 800 mg/kg) of deionized water and diethyl ether leaf and root extracts of *D. villosa* significantly increased ($p<0.05$) the levels of Hb, PCV, and RBC counts, similar to the effects of the standard drug silymarin (Fig. 1-3).

Deionized water leaf extract resulted in significantly higher Hb, RBC, and PCV than deionized water root, diethyl ether leaf, and diethyl ether root extracts. Diethyl ether root extract had higher Hb levels than deionized water root and diethyl ether leaf extracts. These levels increased with higher doses of extract administration.

There was no significant difference in Hb concentration between the uninduced control

group and the group administered 800 mg/kg of deionized water leaf extract. Similarly, there was no significant difference in RBC counts between the normal control, the control group given the standard drug, and the groups administered 600 and 800 mg/kg of various extracts. Additionally, there were no significant differences in PCV between the 800 mg/kg deionized water leaf and diethyl ether root extract groups, the standard drug group, and the normal control group.

The total white blood cell (WBC) count was significantly higher ($p<0.05$) in the untreated gentamicin-induced group compared to all other groups (Fig.4). There was no significant difference in WBC count among the different extracts, even at different doses.

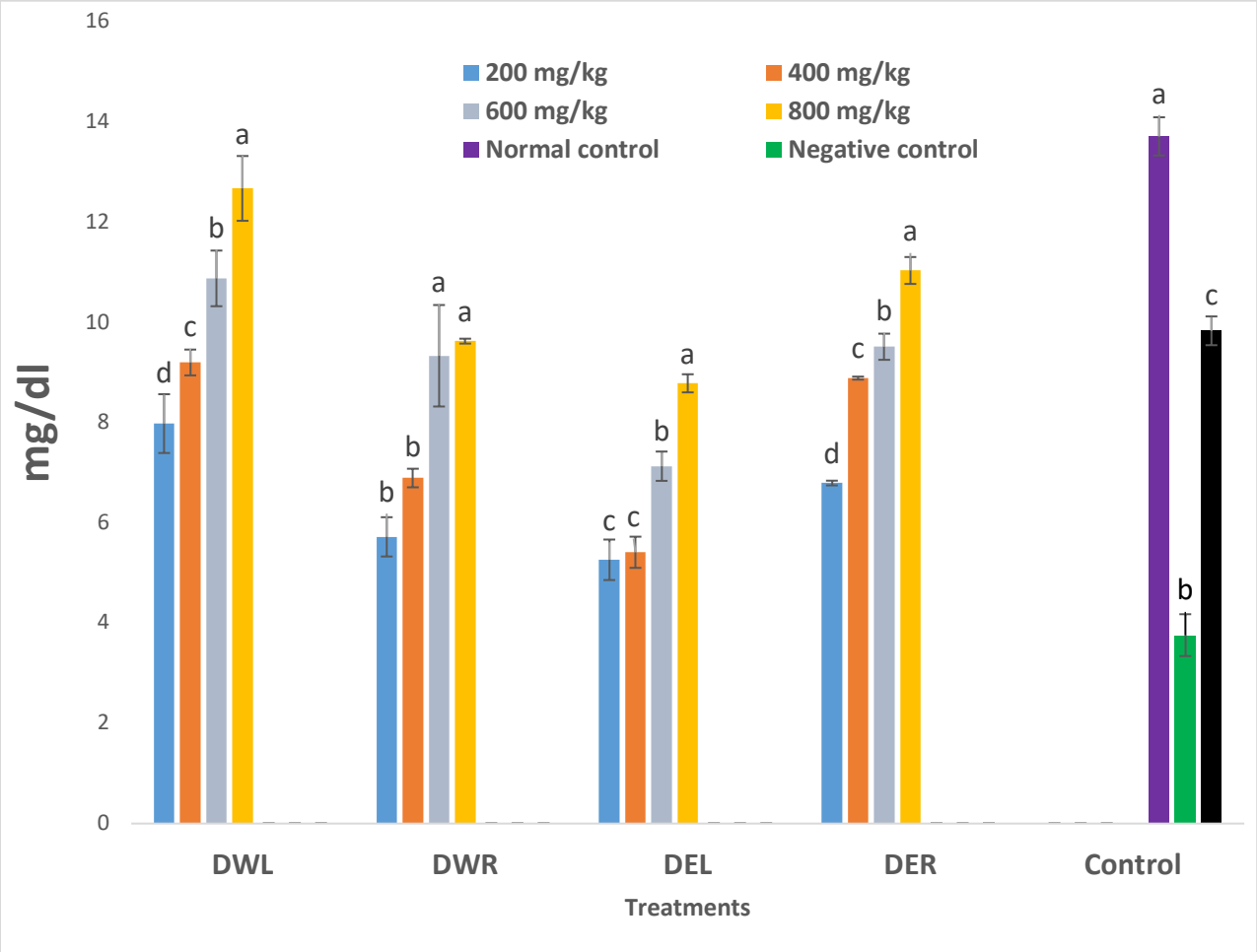

Fig 1: Haemoglobin level in in Rats Administered *D. villosa* Leaf and Root Extracts Following Kidney Injury. Variables with distinct alphabets exhibit a significant difference at ($P<0.05$).

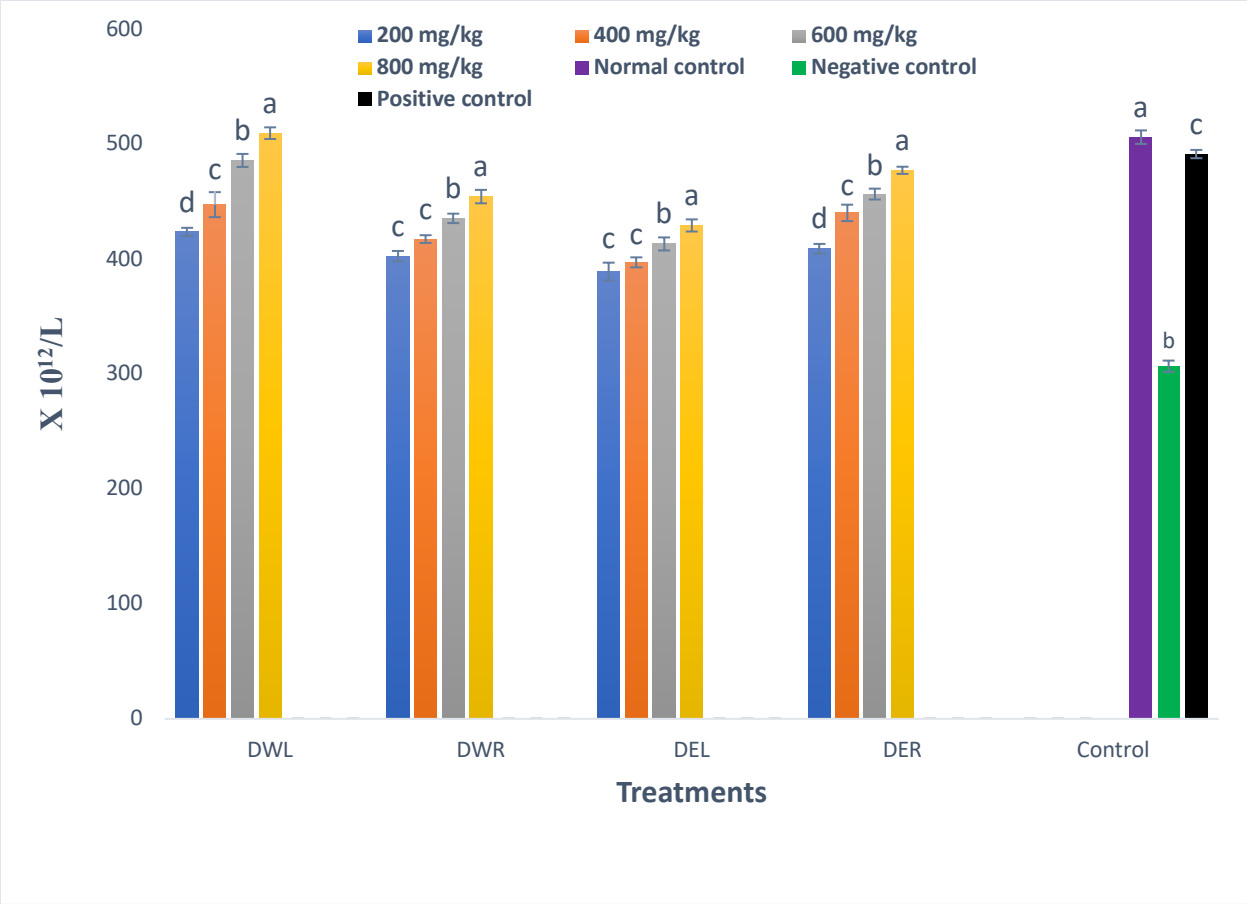

Fig 2: Red Blood Cell Count in in Rats Administered *D. villosa* Leaf and Root Extracts Following Kidney Injury. Variables with distinct alphabets exhibit a significant difference at ($P<0.05$).

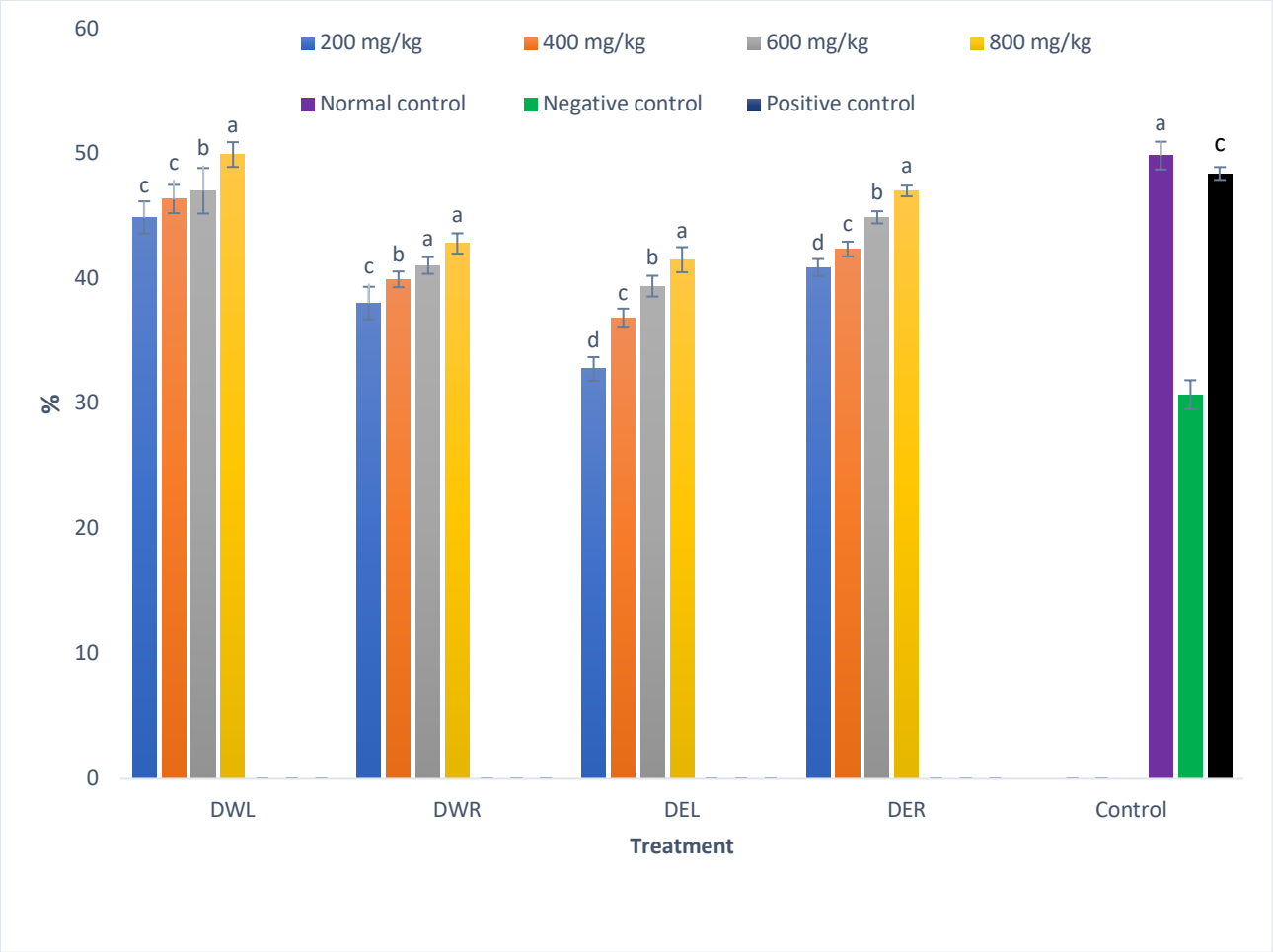

Fig 3: Packed Cell Volume Level in in Rats Administered *D. villosa* Leaf and Root Extracts Following Kidney Injury. Variables with distinct alphabets exhibit a significant difference at (P<0.05).

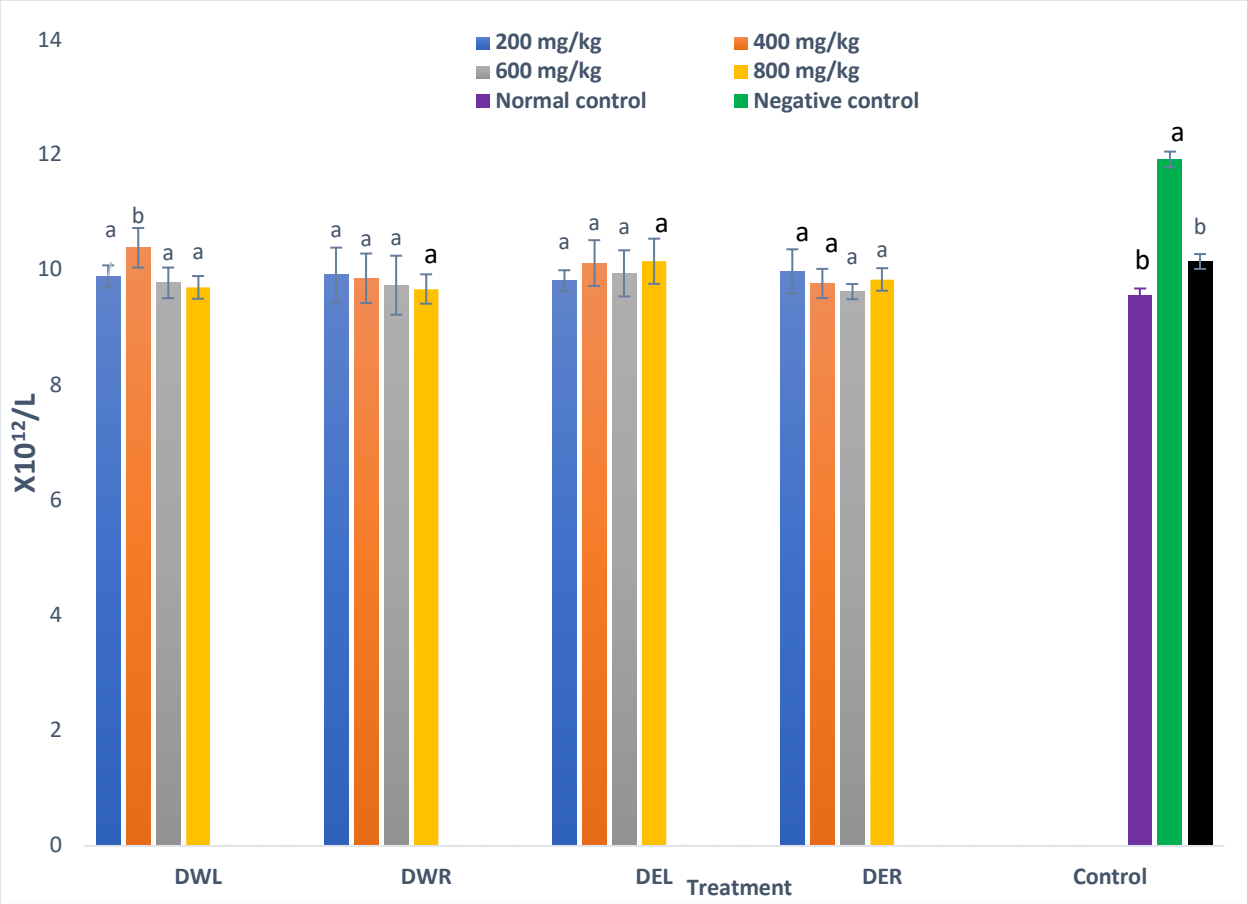

Fig 4: White Blood Cell Count in in Rats Administered *D. villosa* Leaf and Root Extracts Following Kidney Injury. Variables with distinct alphabets exhibit a significant difference at (P<0.05).

Discussion

Fruits, vegetables, and plants have been vital to humankind as sustainable, natural supplies of critical nutrients and health-promoting compounds (Adomėnienė & Venskutonis, 2022). As an alternative to clinical therapy, herbal medicines have drawn more attention, and demand for these therapies has recently risen (Igbinaduwa et al., 2012). The results indicate that gentamicin-induced kidney damage significantly lowered hemoglobin (Hb), packed cell volume (PCV), and red blood cell (RBC) counts in untreated rats. However, treatment with different doses (200, 400, 600, and 800 mg/kg) of deionized water and diethyl ether leaf and root extracts of *D. villosa* significantly improved ($p<0.05$) these hematological parameters, producing effects comparable to those of the standard drug silymarin (Fig.1-4). The reversal effects of the extracts on the parameters that were assessed may be due to the components of the plants. *Dioscorea* are rich in bioactive components including polysaccharides, diosgenin, polyphenols, allantoin, phenols, tannins, and flavonoids (Satija et al., 2018; Wang et al., 2023). These components have been shown to have antioxidant properties and to be potent superoxide anions scavengers (Adomėnienė & Venskutonis, 2022). The antioxidant property of the extract may have contributed to its hepatoprotective action. Remarkably, allantoin, phenols, tannins, and flavonoids —are said to improve the body's inherent resilience and healing abilities (Tungmunnithum et al., 2018; Eluu et al., 2024b).

The administration of the deionized water leaf extract resulted in significantly higher Hb, RBC, and PCV levels compared to rats treated with the deionized water root extract, diethyl ether leaf extract, or diethyl ether root extract. Additionally, the diethyl ether root extract increased Hb levels more effectively than the deionized water root extract and diethyl ether leaf extract. These findings suggest that leaf extracts, especially in deionized water, might have a more potent effect on hematological recovery than root extracts. The efficacy of these treatments increased with higher doses, indicating a dose-dependent response.

Interestingly, there was no significant difference in Hb concentration between the uninduced control group and the group administered 800 mg/kg of deionized water leaf extract. Additionally, RBC counts did not significantly differ among the normal control, the standard drug group and the groups administered 600 and 800 mg/kg of the extracts. Similarly, PCV levels did not differ significantly between the 800 mg/kg deionized water leaf and diethyl ether root extract groups, the standard drug group, and the normal control group. These results suggest that higher doses of these extracts can restore hematological parameters to near-normal levels, comparable to the standard drug.

The total white blood cell (WBC) count was significantly higher ($p<0.05$) in the untreated gentamicin-induced group compared to all other groups, indicating an inflammatory response to kidney damage. White blood cell counts, or the quantity of WBCs in a volume of blood, are commonly measured in clinical settings to assess patient health, evaluate drug toxicity, and pinpoint the origins of disease (Mosley et al., 2023).The increase in WBC may be due to the production of white blood cells caused by the induced kidney damage. However, administration of the extracts significantly reduced the WBC. There was no significant difference in WBC count among the different extract treatments, even at varying doses. These findings align with existing literature highlighting the hematoprotective effects of *D. villosa*. For instance, studies have shown that certain plant extracts can ameliorate drug-induced hematological toxicity by enhancing erythropoiesis and reducing oxidative stress (Abdallah et al., 2019; Semwal et al., 2022)(Abdallah et al., 2019; Semwal et al., 2022).

Conclusion

These findings suggest that *D. villosa* could serve as a natural therapeutic agent for managing hematological imbalances associated with nephrotoxicity, offering a promising alternative or complement to conventional treatments like silymarin.

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