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IN VITRO ANTIOXIDANT PROPERTIES OF *DIALIUM GUINEENSE* AND EFFECTS OF ETHANOL EXTRACTS ON ELECTROLYTES IN WISTAR RATS

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ABSTRACT

Dialium guineense (*D. guineense*), a plant known for its ethno medicinal uses in West Africa, contains a variety of photochemical that may contribute to its antioxidant potential. This study investigated the *in vitro* antioxidant properties of *D. guineense* and evaluated the effects of its ethanol extracts on electrolyte balance in Wistar rats. The *in vitro* activities were assessed using standard assays including lipoxygenase scavenging, xanthine oxisae, 2,2-diphenyl-1-picrylhydrazyl radical scavenging, hydroxyl radical and 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid. For the *in vivo* component, sixteen adult Wistar rats were randomly assigned into control and treatment groups. The treated groups received oral doses of ethanol extracts of *D. guineense* for 14 days, after which serum electrolytes-sodium (Na^+), potassium (K^+), chloride (Cl^-), and bicarbonate (HCO_3^-) were analysed. The *in vitro* antioxidant results obtained show a concentration-dependent antioxidant activity, indicating that the activity increases with increasing concentration. The serum electrolytes results showed that the extract prevent alteration of the electrolyte levels which suggest a modulatory effect. These findings indicate that *D. guineense* possesses strong antioxidant properties and may influence electrolyte homeostasis, supporting its traditional use and potential therapeutic application in oxidative stress-related and electrolyte imbalance disorders.

Keywords: *Dialium guineense,* antioxidant activity, oxidative stress, electrolytes.

Introduction

Dialium guineense (black velvet tamarind) is a widely consumed fruit tree indigenous to West Africa and is extensively used in traditional medicine for the management of ailments such as malaria, diarrhoea, infections, and inflammatory conditions (Arogba et al., 2006; Ajiboye et al., 2015; Lawal et al., 2017). Various parts of the plant, including the fruit pulp, leaves, bark, and roots, are valued for their nutritional and medicinal properties. The fruit pulp is particularly rich in carbohydrates, dietary fiber, vitamins, minerals, and bioactive compounds such as flavonoids, tannins, and phenolic acids, which have been associated with antioxidant activity (Arogba, 2000; Adepoju et al., 2016; Lawal et al., 2017).

Oxidative stress, resulting from an imbalance between free radicals and endogenous antioxidant defenses, plays a significant role in the development of several chronic diseases, including cardiovascular disorders, diabetes mellitus, renal dysfunction, and neurodegenerative conditions (Valko et al., 2007; Birben et al., 2012). Natural antioxidants derived from plants have gained increasing attention due to concerns surrounding the safety and long-term use of synthetic antioxidants (Prior et al., 2005; Pham-Huy et al., 2008). Previous studies have demonstrated that extracts of *Dialium guineense* exhibit notable free-radical-scavenging activity in in vitro assays, suggesting its potential as a natural antioxidant source (Ajiboye et al., 2015; Lawal et al., 2017).

Electrolytes such as sodium, potassium, chloride, and bicarbonate are essential for maintaining fluid balance, nerve transmission, muscle contraction, and acid–base homeostasis (Guyton & Hall, 2016). Disruption of electrolyte balance is often linked to impaired renal function and systemic metabolic disturbances (Palmer & Clegg, 2016). Although several studies have focused on the antioxidant properties of *D. guineense* (Ajiboye et al., 2015; Lawal et al., 2017), there is limited scientific evidence linking its antioxidant activity to its effects on electrolyte balance and renal function, particularly following repeated oral administration.

Furthermore, variations in phytochemical composition due to extraction methods and dosage raise concerns regarding the safety profile of the plant at different concentrations. The lack of integrated studies assessing both antioxidant potential and electrolyte-modulating effects of the ethanolic extract of *D. guineense* represents a significant gap in existing literature. Findings from this study are expected to provide scientific evidence supporting the traditional use of *Dialium guineense* and contribute to the growing body of knowledge on plant-derived antioxidants with potential therapeutic relevance in oxidative stress-related and electrolyte-imbalance disorders. This study aimed to investigate the in vitro antioxidant properties of the ethanolic extract of *Dialium guineense* and evaluate its effects on serum electrolyte levels and kidney function markers in Wistar rats.

Methodology

Fruits Collection and Identification

Dialium guineense fruits were obtained in March 2025 from Okada Market, Edo State, Nigeria. Identification was carried out by Dr. Adebayo M. A. in the Department of Pharmacognosy, Igbinedion University Okada. A voucher specimen number IUO/13/070 was assigned to the fruit.

Preparation of Fruit Extract

The *Dialium guineense* fruits were washed thoroughly with clean water to remove dirt and unwanted materials. The pulp was manually separated from the seeds and air-dried at room temperature for seven days until a constant weight was obtained. The dried pulp was grounded into fine powder using an electric blender. 500g of the powdered sample was macerated in 2L of 70% ethanol for 72 hours with occasional stirring. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40°C. The concentrated extract was further dried in a water bath at 45°C to obtain the crude ethanol extract of *Dialium guineense* (EEDG). The extract was stored in an airtight container(5ml) at 4°C until further use. Extraction of 500 g of dried *Dialium guineense* fruit pulp yielded approximately 52 g of crude ethanolic extract, corresponding to a percentage yield of 10.4%.

In Vitro Antioxidant Assays

Lipoxygenase and Xanthine Oxidase Assays

Lipoxygenase and xanthine oxidase inhibitory activities were measured spectrophotometrically using ascorbic acid and allopurinol as respective reference standards. Percent inhibition and IC_{50} values were calculated for all assays (Baylac & Racine, 2003; Owen & Johns, 1999).

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Assay

The free radical scavenging activity of the extract was evaluated using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described by Brand-Williams et al. (1995). Various concentrations of the extract (10, 5, 2.5, 1.25, 0.625, 0.3125 mg/mL) or standard (vitamin C) were mixed with an equal volume of 0.3 mM DPPH in methanol. The mixture was incubated in the dark at room temperature for 30 min, and absorbance was

measured at 517 nm. Radical scavenging activity was calculated as:

$$\text{Inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

The IC_{50} value, defined as the concentration of extract required to scavenge 50% of DPPH radicals, was determined from a plot of percentage inhibition versus extract concentration.

Hydroxyl Radical Scavenging Assay

Hydroxyl radical scavenging activity was determined based on the inhibition of hydroxyl radical-induced degradation, with butylated hydroxytoluene (BHT) used as the reference standard, following the method described by Halliwell et al. (1987) and Smirnoff and Cumbes (1989).

2,2'-Azinobis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) Radical Scavenging Assay

The ABTS radical scavenging activity was assessed by reacting the extract with pre-formed $ABTS^{+}$ radicals according to the method described by Re et al. (1999). Reduction in absorbance was measured at 734 nm using Trolox as the standard antioxidant. Percent inhibition and IC_{50} values were calculated as described for the DPPH assay

Experimental Animals

Sixteen female Wistar rats weighing between 100 and 120 g were obtained from a certified veterinary breeder in Ajibode, Ibadan. The rats were housed in plastic cages under standard laboratory conditions, with free access to food and water. They were allowed to acclimatize for 14 days before the commencement of the study.

Experimental Design

The rats were randomly divided into four groups, with four animals in each group:
Group 1: Control

Group 2: 50 mg/kg ethanolic extract of D. guineense
Group 3: 100 mg/kg ethanolic extract of D. guineense
Group 4: 150 mg/kg ethanolic extract of D. guineense

Each rat received its assigned dose orally once daily for 14 consecutive days. No changes were made to the treatment regimen throughout the dosing period. The doses of the ethanolic extract of *Dialium guineense* used in this study were based on previous studies that investigated the biological and antioxidant activities of the plant at comparable dose ranges (Ajiboye et al., 2015; Lawal et al., 2017). Oral administration was employed to mimic the traditional route of consumption of *Dialium guineense* in herbal medicine and dietary use, thereby enhancing the translational relevance of the findings (OECD, 2008).

Sacrifice of Animals

At the end of the extract treatment, the rats were fasted overnight and humanely sacrificed using cervical dislocation in accordance with standard laboratory animal care and ethical guidelines (AVMA, 2020). Blood samples were collected via cardiac puncture using a 2 mL syringe and dispensed into universal and lithium heparin tubes. The samples were centrifuged at 3000 rpm for approximately 10 minutes to obtain serum, which was used immediately for biochemical analyses. The kidneys and liver were also harvested for histological examination.

Kidney Function Tests

Serum urea and creatinine levels were determined using commercially available diagnostic kits following the manufacturer's instructions, as commonly applied in experimental renal function assessment (Tietz, 1995; Burtis & Bruns, 2014).

Electrolyte Analysis

Serum concentrations of sodium (Na^{+}), potassium (K^{+}), chloride (Cl^{-}), and bicarbonate (HCO_3^{-}) were measured using standard colorimetric and ion-selective electrode methods to assess electrolyte balance (Tietz, 1995; Palmer & Clegg, 2016).

Histological Examination

The excised kidneys were rinsed in saline, and fixed in 10% formalin. Tissues were processed, embedded in paraffin, sectioned (5 µm), and stained with hematoxylin and eosin (H&E). Slides were examined under a light microscope at ×100 and ×200 magnification to assess tissue architecture and potential extract-induced morphological alterations.

Statistical Analysis

Data collected were analyzed using Graph pad prism 8.0.1. The test and control group were compared using One-way ANOVA. Tukey's multiple comparison test was used for the significance difference within the group. The results were presented as mean SEM (Standard error of mean) in graphs.

Results

In Vitro Antioxidants Results

Lipoxygenase Scavenging Activity of Ethanol Extract *Dialium guineense*

The results of the lipoxygenase free radical scavenging capacity showed in table 1 revealed an increase in percentage inhibition of the free radical formation. The lipoxygenase IC_{50} value for the ethanol extract *dialium guineense* is significantly higher ($p<0.05$) than the ascorbic acid used as the positive control. The lower the IC_{50}, the more effective the DPPH activity.

Table 1: Lipoxygenase scavenging activity of ethanol extracts *Dialium guineense*

Concentration (mg/mL)	% inhibition Dialium guineense	Concentration (µg mL^{-1})	% inhibition Standard (ascorbic acid)
1	51.3 ± 0.19	0.1	75.6 ± 0.41
0.5	38.9 ± 1.54	0.05	67.0 ± 0.38
0.25	29.0 ± 2.07	0.025	50.3 ± 2.42
0.125	22.1 ± 0.19	0.0125	34.6 ± 0.38
0.0625	16.7 ± 0.19	0.00625	18.3 ± 5.08
0.03125	10.6 ± 1.86	0.003125	10.1 ± 1.13
r^2	0.9074	-	0.7542
IC_{50} (mg/mL)	0.89 ± 0.01028		0.03 ± 0.00024

Data presented as the mean±SEM of three determinations

Xanthine Oxidase Radical Scavenging Activity of Ethanol Extract *Dialium guineense*

The results of the hydroxyl free radical scavenging capacity showed in table 2 revealed an increase in percentage inhibition of the free radical formation. The xanthione oxidase IC_{50} value for the ethanol extract *dialium guineense* is significantly higher ($p<0.05$) than the allopurinol used as the positive control. The lower the IC_{50}, the more effective the DPPH activity.

Table 2: Xanthine oxidase scavenging activity of ethanol extract *Dialium guineense*

Concentration (mg/mL)	% inhibition Dialium guineense	Concentration (µg mL^{-1})	% inhibition Standard (Allopurinol)
1	59.5 ± 0.35	0.1	55.4 ± 0.70
0.5	55.9 ± 1.04	0.05	53.9 ± 0.12
0.25	31.1 ± 0.70	0.025	51.8 ± 1.39
0.125	17.4 ± 0.93	0.0125	31.1 ± 2.09
0.0625	10.8 ± 0.19	0.00625	23.4 ± 1.04
0.03125	3.3 ± 0.70	0.003125	6.2 ± 2.09
r^2	0.9873	-	0.9307
IC_{50} (mg/mL)	0.440 ± 0.0065*		0.023 ± 0.00038

Data presented as the mean ± SEM of three determinations

DPPH Scavenging Activity of Ethanol Extract *Dialium guineense*

The results of the DPPH free radical scavenging capacity showed in table 3 revealed an increase in percentage inhibition of the free radical formation. The DPPH IC_{50} value for the ethanol extract *dialium guineense* is significantly higher ($p<0.05$) than the ascorbic acid used as the positive control. The lower the IC_{50}, the more effective the DPPH activity.

Table 3: DPPH scavenging activity of ethanol extract *Dialium guineense*

Concentration (mg/mL)	% inhibition Dialium guineense	Concentration (µg mL^{-1})	% inhibition Standard (Ascorbic acid)
1	48.1 ± 1.55	0.1	72.6 ± 0.40
0.5	40.4 ± 0.48	0.05	62.1 ± 0.32
0.25	33.0 ± 0.28	0.025	49.9 ± 0.04
0.125	27.8 ± 0.36	0.0125	37.3 ± 1.27
0.0625	24.0 ± 0.12	0.00625	29.6 ± 0.91
0.03125	19.1 ± 0.12	0.003125	20.5 ± 0.12
r^2	0.9039	-	0.9186
IC_{50} (mg/mL)	0.98 ± 0.05071*		0.03 ± 0.000258

Data presented as the mean±SEM of three determinations

Hydroxyl Radical Scavenging Activity of Ethanol Extract *Dialium guineense*

The results of the hydroxyl free radical scavenging capacity showed in table 4 revealed an increase in percentage inhibition of the free radical formation. The hydroxyl IC_{50} value for the ethanol extract *dialium guineense* is significantly higher ($p<0.05$) than the BHT used as the positive control. The lower the IC_{50}, the more effective the DPPH activity.

Table 4: Hydroxyl radical scavenging activity of ethanol extract *Dialium guineense*

Concentration (mg/mL)	% inhibition Dialium guineense	Concentration (µg mL^{-1})	% inhibition Standard (BHT)
1	66.7 ± 0.30	0.1	63.7 ± 1.55
0.5	53.6 ± 0.30	0.05	44.0 ± 0.48
0.25	40.4 ± 0.51	0.025	38.9 ± 0.28
0.125	25.9 ± 0.51	0.0125	25.0 ± 0.36
0.0625	18.9 ± 1.21	0.00625	5.9 ± 0.12
0.03125	11.6 ± 0.91	0.003125	4.4 ± 0.12
r^2	0.8858	-	0.8440
IC_{50} (mg/mL)	0.58 ± 0.001179*		0.07 ± 0.000382

Data presented as the mean±SEM of three determinations

ABTS Radical Scavenging Activity of Ethanol Extract *Dialium guineense*

The results of the ABTS free radical scavenging capacity showed in table 5 revealed an increase in percentage inhibition of the free radical formation. The ABTS IC_{50} value for the ethanol extract *dialium guineense* is significantly higher ($p<0.05$) than the Trolox used as the positive control. The lower the IC_{50}, the more effective the DPPH activity.

Table 5: ABTS radical scavenging activity of ethanol extract *Dialium guineense*

Concentration (mg/mL)	% inhibition Dialium guineense	Concentration (µg mL^{-1})	% inhibition Standard (Trolox)
1	70.8 ± 0.42	0.1	83.9 ± 1.26
0.5	67.3 ± 1.26	0.05	64.3 ± 1.68
0.25	57.1 ± 1.68	0.025	58.3 ± 0.84
0.125	45.2 ± 0.84	0.0125	41.1 ± 0.42
0.0625	32.0 ± 0.76	0.00625	27.4 ± 0.84
0.03125	28.0 ± 1.26	0.003125	18.5 ± 0.42
r^2	0.8889	-	0.8218
IC_{50} (mg/mL)	0.24 ± 0.005306*		0.02 ± 0.000709

Data presented as the mean±SEM of three determinations

Kidney function

Urea

The effect of ethanol extract *Dialium guineense* on serum urea level in Wistar rats is shown in Fig 1. The serum urea level is 50 mg/kg and 100 mg/kg ethanol extract *Dialium guineense* is similar to that of control level, while significant increase was observed in 150 mg/kg ethanol extract *Dialium guineense* when compared with the control

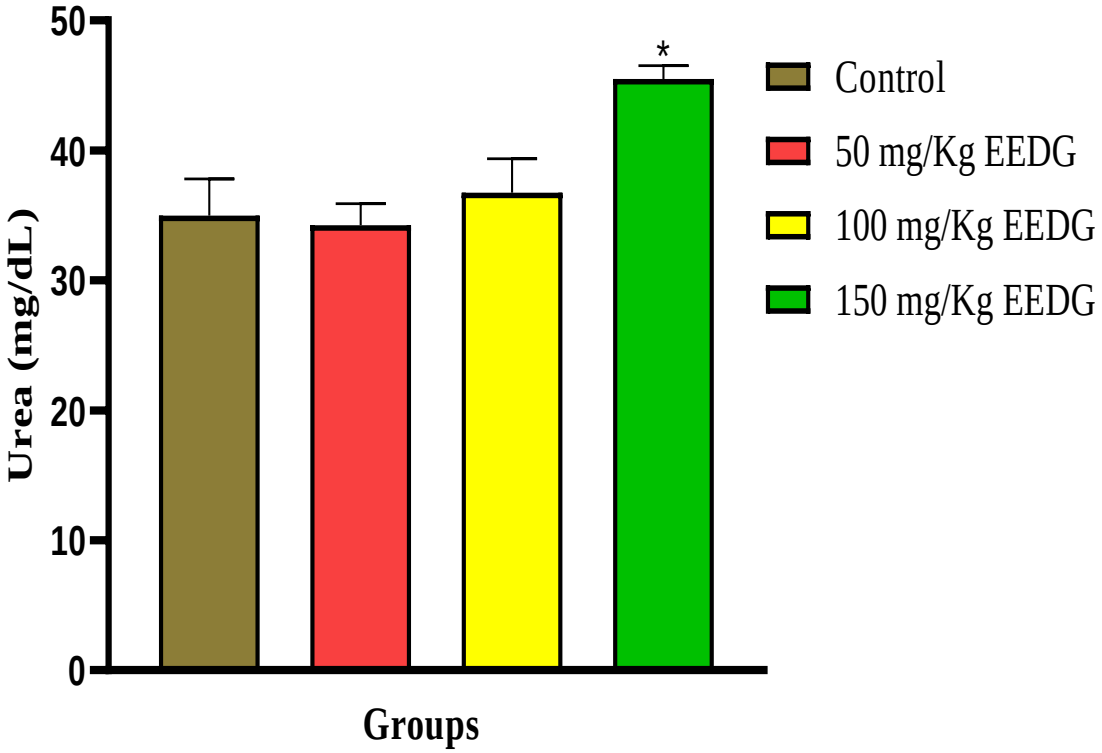

Figure 1: Effect of ethanol extract *Dialium guineense* on serum urea level of Wistar rats.
EEDG = Ethanol extract *Dialium guineense*
*p< 0.05 is significant compared to the control

Sodium ion

Figure 2 shows no significant difference in serum sodium ion level of 50 mg/kg, 100 mg/kg and 150 mg/kg ethanol extract *Dialium guineense* groups when compared with the control.

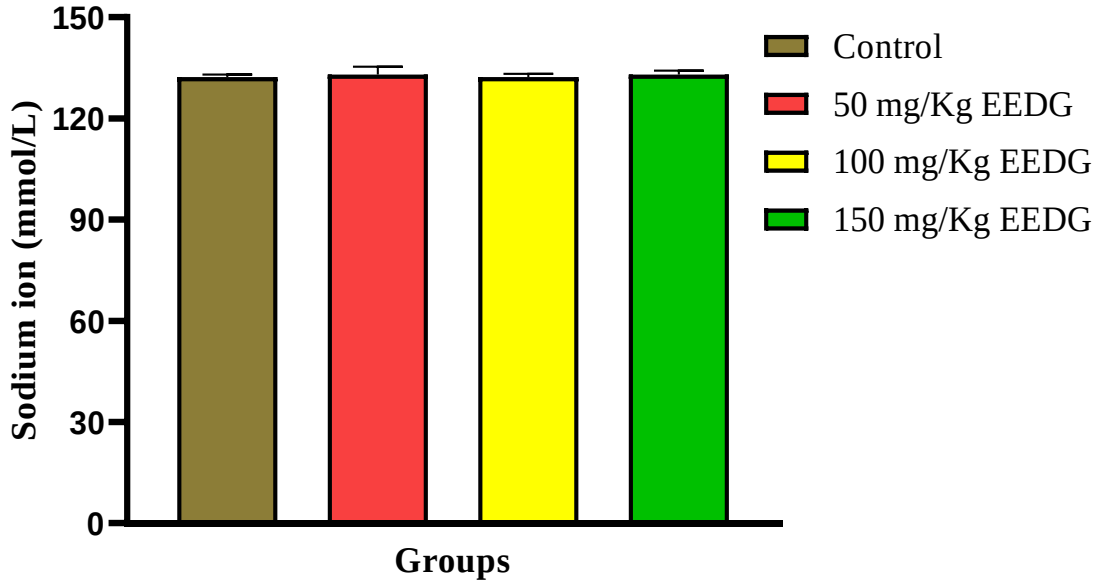

Figure 2: Effect of ethanol extract *Dialium guineense* on serum sodium ion level of Wistar rats.
EEDG = Ethanol extract *Dialium guineense*

Potassium ion

Figure 3 displays no significant difference in serum potassium ion level of 50 mg/kg, 100 mg/kg and 150 mg/kg ethanol extract *Dialium guineense* groups when compared with the control.

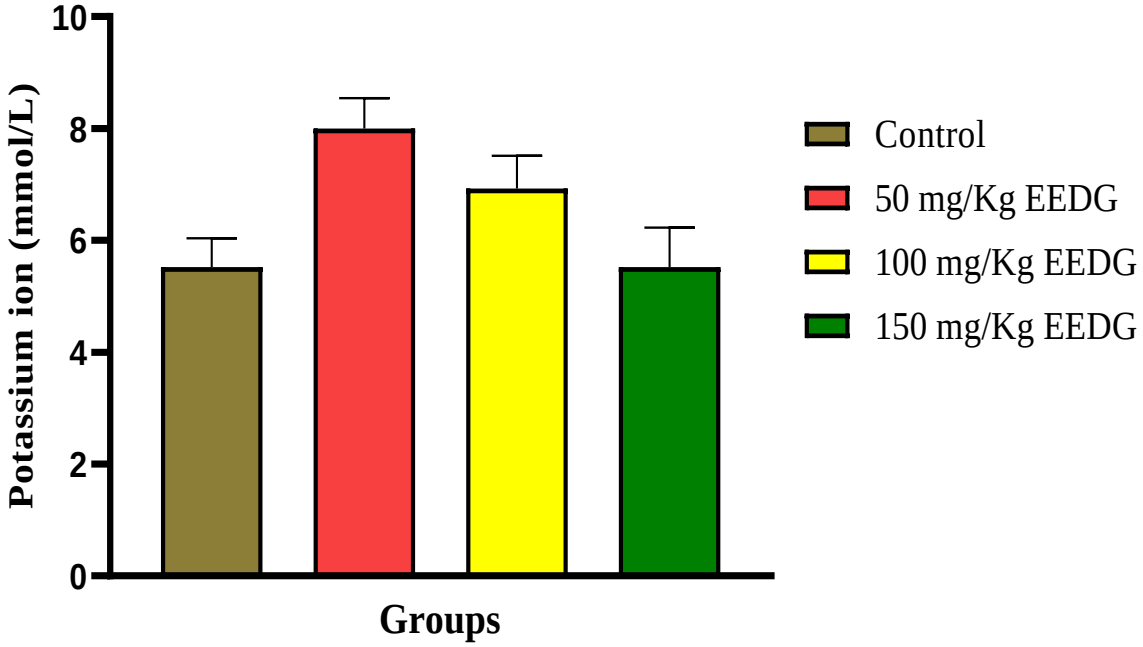

Figure 3: Effect of ethanol extract *Dialium guineense* on serum potassium ion level of Wistar rats.
EEDG = Ethanol extract *Dialium guineense*

Bicarbonate ion

The level of serum bicarbonate ion is similar in all the groups (Fig 4).

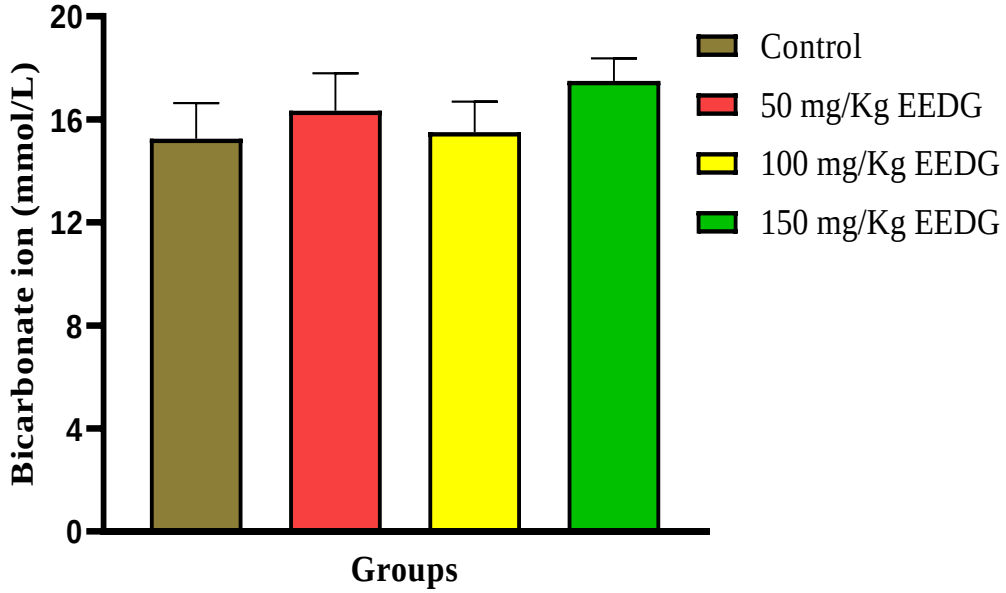

Figure 4: Effect of ethanol extract *Dialium guineense* on serum bicarbonate ion level of Wistar rats.
EEDG = Ethanol extract *Dialium guineense*

Chloride ion

Figure 5 shows no significant difference in serum chloride ion level of 50 mg/kg, 100 mg/kg and 150 mg/kg ethanol extract *Dialium guineense* groups when compared with the control.

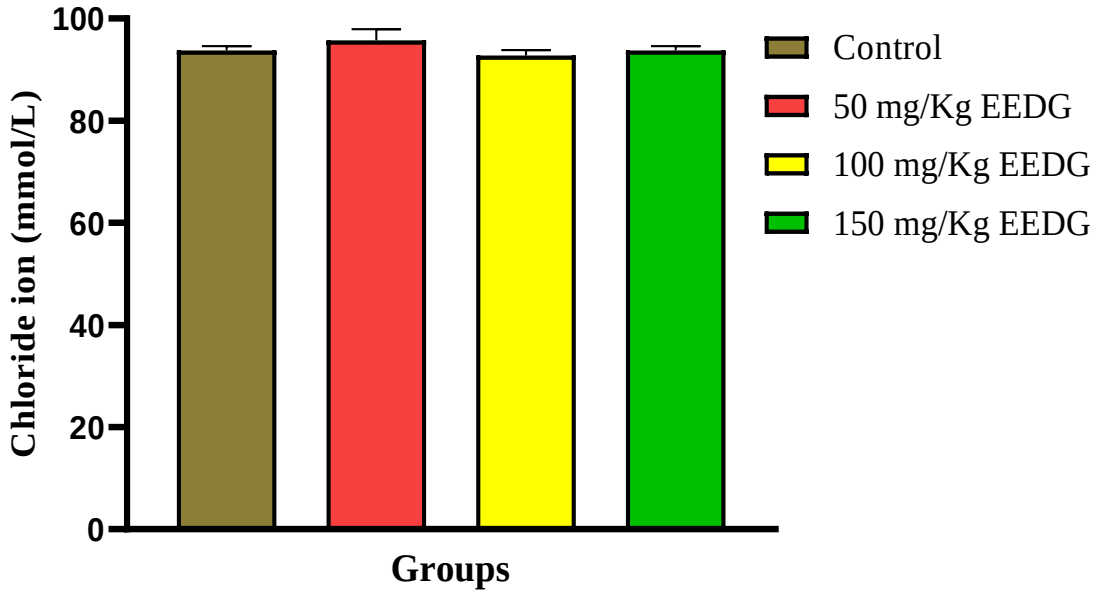

Figure 5: Effect of ethanol extract *Dialium guineense* on serum chloride ion level of Wistar rats.
EEDG = Ethanol extract *Dialium guineense*

Creatinine level

There is no significant difference in serum creatinine level of 50 mg/kg, 100 mg/kg and 150 mg/kg ethanol extract *Dialium guineense* groups when compared with the control (Fig 6).

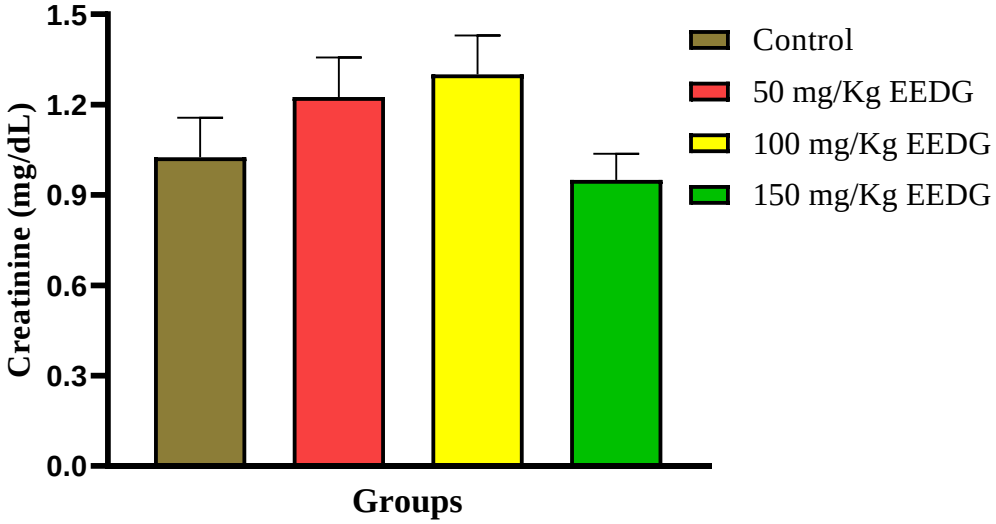

Figure 6: Effect of ethanol extract *Dialium guineense* on serum creatinine level of Wistar rats.
EEDG = Ethanol extract *Dialium guineense*

Kidney Histology

Histological examination of kidney sections from the control group showed normal renal architecture with intact glomeruli and well-defined renal tubules. Sections from rats treated with 50 mg/kg and 100 mg/kg of the ethanolic extract revealed no observable pathological alterations, indicating preserved renal integrity. However, mild tubular congestion was observed in the 150 mg/kg group (Fig 7).

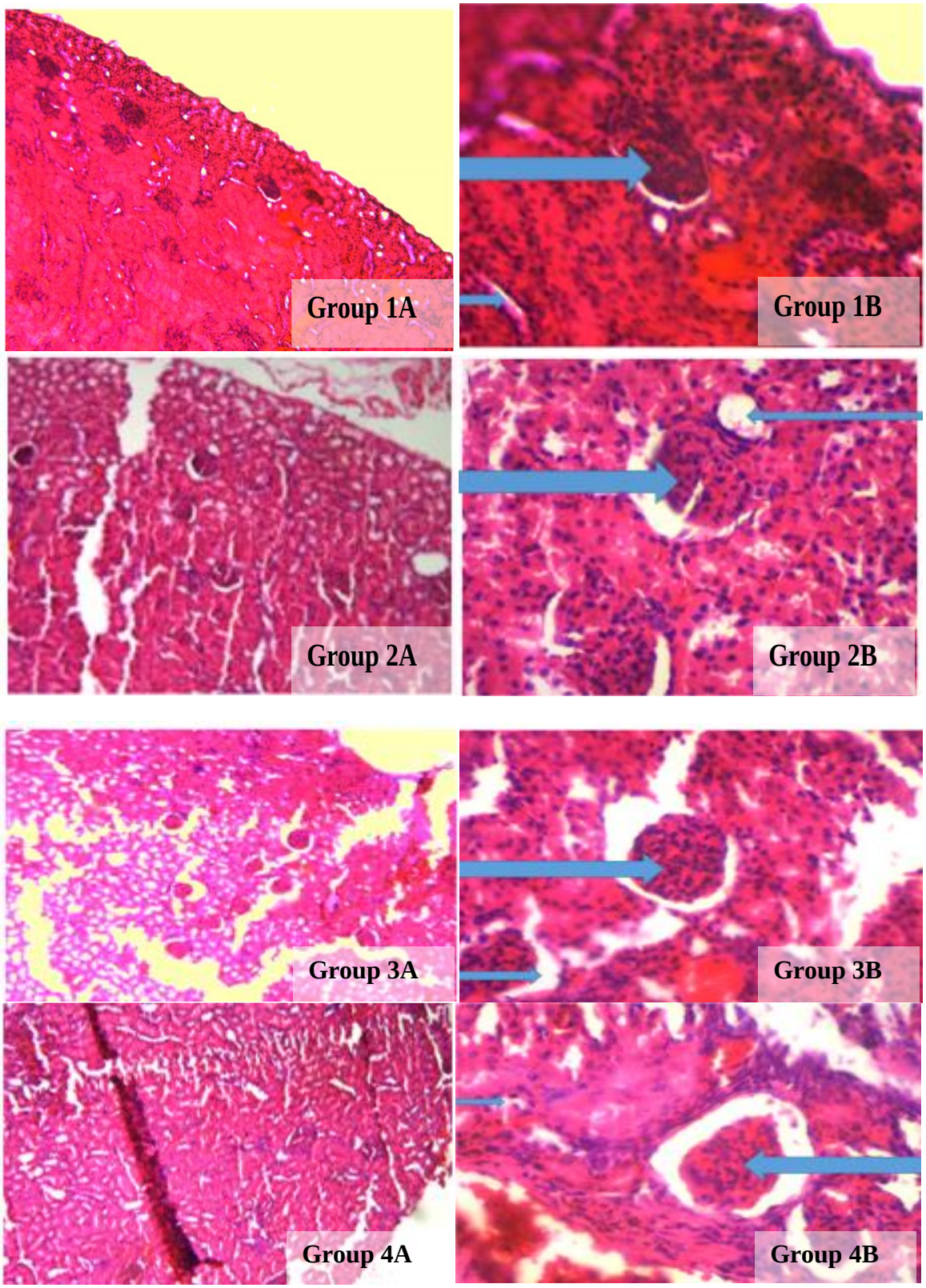

Figure 7: Effect of ethanol extract *dialium guineense* on kidney histology of Wistar rats (H&E stained). The magnification of A and B is × 100 and × 200

Liver Histology

Liver sections show in Fig 8 that the control rats displayed normal hepatic architecture with intact hepatocytes, central veins, and sinusoids. Similarly, rats treated with 50 mg/kg and 100 mg/kg of the extract

showed preserved liver morphology with no evidence of necrosis or inflammation. In contrast, mild hepatocellular distortion was observed in the 150 mg/kg group.

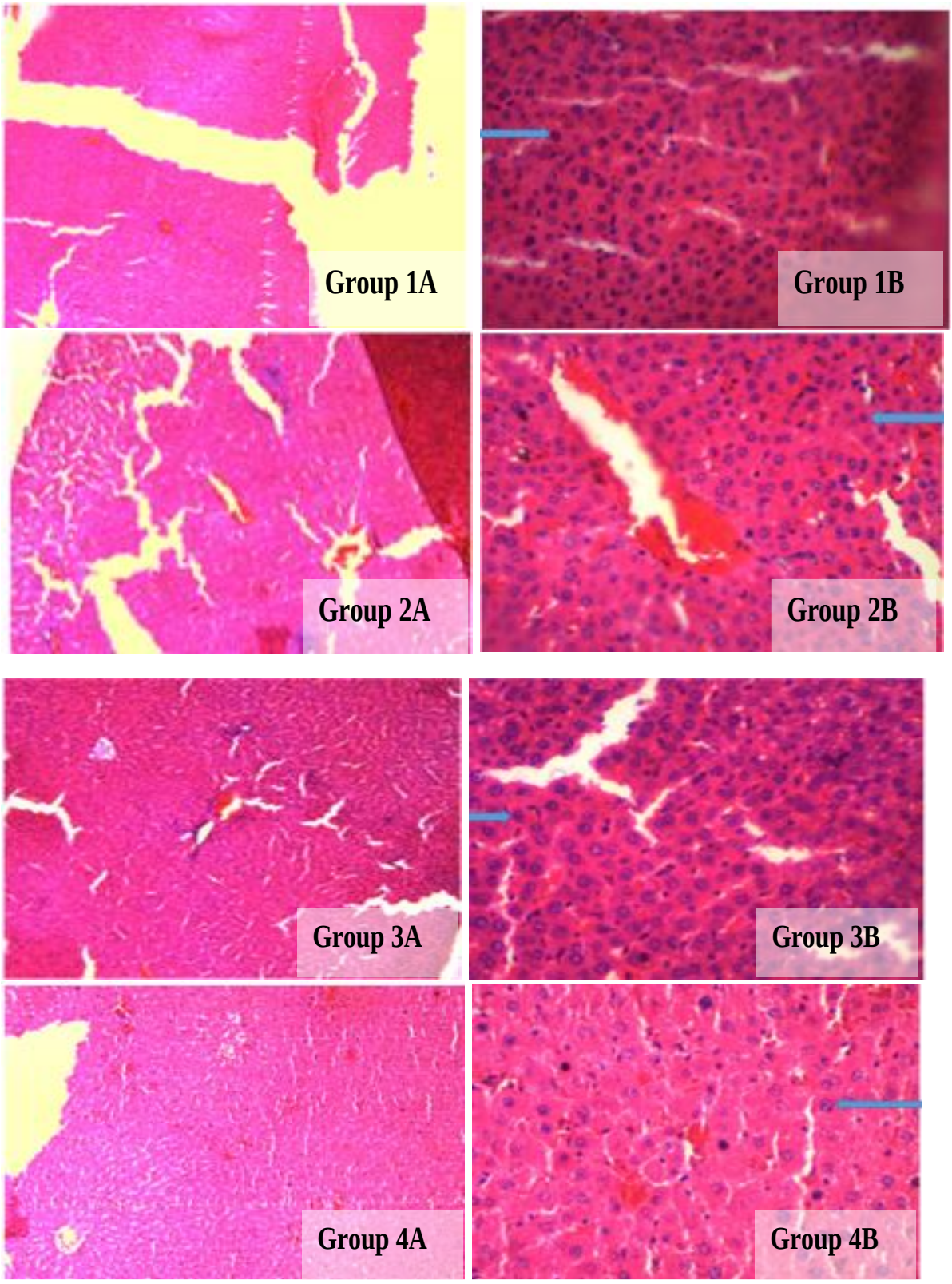

Figure 8: Effect of ethanol extract *dialium guineense* on liver histology of Wistar rats (H&E stained). The magnification of A and B is × 100 and × 200

Discussion

This study evaluated the in vitro antioxidant properties of the ethanolic extract of *Dialium guineense* and examined its effects on kidney function, electrolyte balance, and tissue histology in Wistar rats following repeated oral administration. The findings provide experimental support for the antioxidant potential of *D. guineense* while also offering insight into its physiological safety profile.

The in vitro antioxidant assays demonstrated that the ethanolic extract of *D. guineense* exhibited significant free radical scavenging activity across multiple test systems, including DPPH, ABTS, hydroxyl radical, lipoxygenase, and xanthine oxidase assays. The concentration-dependent increase in percentage inhibition observed across these assays indicates that the antioxidant activity of the extract is dose-responsive. Although the extract showed lower potency compared to standard antioxidants such as ascorbic acid, Trolox, and allopurinol, its consistent activity across different radical systems suggests the presence of multiple bioactive phytochemicals capable of neutralizing reactive oxygen species (Ajiboye et al., 2015; Lawal et al., 2017). This observation aligns with previous reports attributing the antioxidant capacity of *D. guineense* to its high content of phenolic compounds, flavonoids, and tannins (Arogba, 2000; Lawal et al., 2017).

Oxidative stress is a known contributor to renal dysfunction and electrolyte imbalance through damage to renal tubular cells and disruption of ion transport mechanisms (Valko et al., 2007; Birben et al., 2012). Therefore, the strong antioxidant activity observed in this study provides a plausible basis for the evaluation of renal and electrolyte parameters following extract administration. In the present study, serum electrolyte concentrations—including sodium, potassium, chloride, and bicarbonate remained largely unchanged across treatment groups when compared with the control. The absence of significant electrolyte disturbances suggests that the extract did not adversely affect renal electrolyte handling at the administered doses over the 14-day treatment period (Palmer & Clegg, 2016).

Kidney function markers further support this observation. Serum creatinine levels remained unchanged across all treated groups, indicating preserved glomerular filtration. However, a significant increase in serum urea was observed in rats administered 150 mg/kg of the extract. Elevated urea in the presence of normal creatinine may reflect altered tubular handling of nitrogenous waste or increased protein metabolism rather than overt renal damage (Burtis & Bruns, 2014; Tietz, 1995). This interpretation is supported by histological findings, which revealed preserved renal architecture at lower doses (50 and 100 mg/kg) but mild tubular congestion at the highest dose. Together, these results suggest a dose-dependent physiological response, with higher extract concentrations exerting mild renal stress without causing severe structural or functional impairment.

Histopathological evaluation of kidney and liver tissues provided further insight into the safety profile of the extract. Kidney sections from rats treated with lower doses showed normal glomerular and tubular structures, corroborating the biochemical findings of stable creatinine and electrolyte levels. Mild renal alterations observed at 150 mg/kg may explain the corresponding increase in serum urea. Similarly, liver histology revealed preserved hepatic architecture at lower doses, while mild hepatocellular distortion at the highest dose suggests possible dose-related hepatic stress. Importantly, these changes were subtle and not indicative of severe toxicity, implying that the extract is relatively safe at low to moderate doses.

Although this study did not directly investigate molecular pathways, the combined in vitro antioxidant activity, stable electrolyte profile, and largely preserved tissue architecture suggest a possible antioxidant-mediated modulation of renal and hepatic function. The ability of plant polyphenols to protect cellular membranes and enzymes from oxidative damage may contribute to the maintenance of electrolyte homeostasis observed in this study (Prior et al., 2005; Pham-Huy et al., 2008). However, definitive mechanistic conclusions cannot be drawn without direct assessment of oxidative stress biomarkers and renal ion transport proteins.

A limitation of the present study is the absence of in vivo oxidative stress markers and transporter-level analyses, which would be required to establish a definitive mechanistic link between antioxidant activity and electrolyte regulation. Nonetheless, the findings provide valuable preliminary data supporting the traditional use of *D. guineense* and highlight the importance of dose optimization.

Conclusion

This study demonstrated that *Dialium guineense* contains abundant bioactive phytochemicals and exhibits significant *in vitro* antioxidant activity across multiple free radical scavenging assays. The ethanolic extract produced minimal alterations in serum electrolyte levels and kidney function markers at lower doses, indicating relative safety. However, dose-dependent changes in serum urea and mild histological alterations at higher doses

suggest the need for caution and further toxicological evaluation. Overall, the findings support the traditional use of *D. guineense* while emphasizing the importance of controlled dosing and extended studies to fully establish its therapeutic potential.

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