

Effects of Betacyanins-Rich Extract of Beetroot (*Beta vulgaris*) on Acetaminophen-Induced Nephrotoxicity in Rats

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ABSTRACT

Acetaminophen-induced nephrotoxicity is a detrimental issue to health, current treatment options are limited and may have some adverse effects. Betacyanins may offer preferable alternative, potentially reducing the risk of side effects and improving outcomes. This research was conducted to evaluate the effects of betacyanins-rich extract against acetaminophen-induced nephrotoxicity in albino rats. The objectives of the research were to; Assess preventive and curative effects of betacyanins-rich extract on acetaminophen-induced nephrotoxicity in rats after pretreatment and co-administration with the betacyanins-rich extract of beetroot and determine some antioxidant markers *in vivo* after pretreatment and co-administration of acetaminophen with the betacyanins extract and conduct histopathological kidney tissue examination. Biochemical markers of kidney functions (Electrolytes, urea, creatinine, LDH, CRP and CAR) were determined, *in-vivo* antioxidant markers (MDA, SOD and GSH) were determined and histopathological studies of kidney tissues was conducted. Results of concentrations of biochemical and antioxidant markers revealed significant reduction in Creatinine, LDH, CRP, CAR and MDA activities with extract administration during pre-treatment phase, while SOD and GSH showed increased activities. These concentrations remain insignificant during concurrent treatment phase with increase in MDA, urea, and electrolytes. Histological examinations showed that pretreatment with betacyanins extract significantly reduced kidney damage caused by acetaminophen administration. In conclusion, pretreatment of acetaminophen-induced toxicity with betacyanins-rich extract showed reduced tissue damage and inflammation, however, its efficacy in reversing established damage in curative treatment groups was limited.

Keywords: Beetroot, Betacyanins, Kidney, Acetaminophen, Curative, Preventive, Nephrotoxicity, Antioxidants.

Introduction

Plant-derived foods and phytochemicals have been utilized to treat various disorders and diseases (Almasi *et al.*, 2022). This emphasizes the importance of incorporating functional foods, fruits, vegetables and plant-based diets into daily meals due to their effects on health, synthetic products are questioned due to allergic reactions and health risks (Sarker *et al.*, 2023).

Beetroot (*Beta vulgaris* L.) is a health-promoting food used by humans (Sivakumar *et al.*, 2009), it is rich in numerous phytochemicals (Fu *et al.*, 2020) which provide numerous nutritional and health benefits (Righi Pessoa Da Silva *et al.*, 2018). Its compounds have been studied for antiproliferative, antioxidant, antihypertensive and hypolipidemic effects.

This makes it a popular choice for dietary remediation (Salehi *et al.*, 2020). Betalains is the main beetroot plant pigment with N-heterocyclic core structure (betalamic acid) which formed the major compounds comprising of red-violet (betacyanins) and yellow-orange (betaxanthins) when condensed with imino compounds or amines (Sarker *et al.*, 2023). Therapeutic functions of beetroot is attributed to either of the betalains component with betacyanins being more pronounced (Fu *et al.*, 2020).

Kidneys are crucial for waste management and electrolyte balance, however, they can be harmed by toxicants due to the large volume of blood flowing through them (Eweka *et al.*, 2008).

This can lead to decreased excretion of waste, malfunctioned electrolyte balance, and reduced hormone synthesis (Emadi *et al.*, 2020).

Renal dysfunctions are often caused by nephritic stress from antibiotics, food colors, and other drugs (Jwad *et al.*, 2015; Baponwa *et al.*, 2022). Synthetic drugs (Lopes *et al.*, 2023), Poor dietary practices and medical complications can harm kidney tissues (Yang *et al.*, 2020), disrupting normal metabolic functions by producing reactive oxygen metabolites causing tissue injuries and mitochondrial dysfunction, (Salehi *et al.*, 2020; Ogbe *et al.*, 2022; Baothman *et al.*, 2023). Nephrotoxicity can lead to rapid deterioration in renal function and kidney failure (Abood *et al.*, 2021; Mahmood and Askar, 2022; Lopes *et al.*, 2023). Chronic kidney dysfunctions are linked to conditions like hypertension, obesity, diabetes, and primary renal disorders (Emadi *et al.*, 2020).

Acetaminophen (paracetamol) is a widely-used over-the-counter medication used to address multiple symptoms, it has analgesic and antipyretic properties (Abood *et al.*, 2021; Baponwa *et al.*, 2022) and works by inhibiting cyclooxygenase (COX) enzyme, reducing the production of prostaglandins, which promote pain and fever (Salehi *et al.*, 2020; Abood *et al.*, 2021; Lopes *et al.*, 2023), dosages vary based on individual age, weight, product, and use (Abood *et al.*, 2021). Overdosing can lead to kidney damage (Salehi *et al.*, 2020; Lopes *et al.*, 2023).

Chronic kidney disease (CKD) affects over 10% of the global population and is the fifth-fastest growing cause of death, expected to become the third-leading cause by 2040 (Priyadarshani *et al.*, 2023). Acetaminophen misuse and overdose is one of the causes of drug-induced kidney toxicity (Salehi *et al.*, 2020). It affects over 800 million individuals and occurs in 20%–25% of patients with liver disease (Mahmood and Askar, 2022), millions of people die each year (Levin *et al.*, 2023) and over 2 million currently receive treatment with dialysis or a kidney transplant to stay alive (Kovesdy, 2022). About 14% of adults are estimated to have CKD with 1 in 3 adults with severe CKD unaware of their condition (Emadi *et al.*, 2020). In Nigeria, self-medication, lack of awareness regarding proper dosage, and limited access to healthcare facilities are the contributory factors for the prevalence of acetaminophen toxicity (Levin *et al.*, 2023).

The nephrotoxic effects of acetaminophen overdose present a critical public health challenge, exacerbating the burden on the healthcare system (Omorodion *et al.*, 2021; Offor *et al.*, 2022). There is a need to explore potential preventive or curative measures to mitigate acetaminophen's adverse effects using natural substances like betacyanins, this could lead to safer and more accessible measures. The aim of this work was to study the effects of betacyanins-rich extract of beetroot (*Beta vulgaris*) on acetaminophen-induced nephrotoxicity in albino rats.

Materials and Methods

Ethical Approval: Ethical clearance was granted for this study by the Research Ethics Committee, Gombe State Ministry of Agriculture, Animal Husbandry and Cooperatives, Gombe State, Nigeria (Ref: MAAH/HQ/S/GEN/423/V.1/00068 of 05/04/2024).

Collection and Processing of Beetroot Root

Fresh beetroot was obtained from Yan Kaba market, Kano State, authenticated by a taxonomist and processed as described by Tutunchi *et al.*, (2019). Betacyanins were extracted using ultrasonic-assisted extraction method as described by Righi Pessoa Da Silva *et al.*, (2018). The extract was then air-dried, wrapped in an aluminum foil, packed in a zip lock transparent bag and stored in a refrigerator until administration to the rats.

Collection and Processing of Male White Albino Rats

The study was conducted on male white albino rats weighing 100-200g, caged in the Animal House of Department of Human Physiology, College of Health Sciences, Gombe State University, Gombe. The research was carried out in two phases; preventive treatment phase and curative treatment phase. The rats were pretreated with the extract before damage to the kidney was induced for two weeks in the pretreatment group, followed by administration of acetaminophen (500mg/kg body weight) for another two weeks. In the curative treatment group, the extract and acetaminophen were administered concurrently for two weeks. The administration was done orally for both phases (Motawi *et al.*, 2020).

Animal Grouping

Each of the phases contained six groups (n = 3) the groups were: Group I: Normal Control, Group II: Positive Control: Rats in this group were given 100mg of betacyanins extract Kg⁻¹ body weight for two weeks. Group III: Negative Control: Rats in this group were given 500mg of paracetamol Kg⁻¹ body weight for two weeks. Group IV: Rats in this group were given 50mg of betacyanins extract Kg⁻¹ body weight and 500mg of paracetamol Kg⁻¹ body weight for two weeks. Group V: Rats in this group were treated with 100mg of betacyanins extract Kg⁻¹ body weight day⁻¹ and 500mg of paracetamol Kg⁻¹ body weight for two weeks. Group VI: Rats in this group were treated with 200mg of betacyanins extract Kg⁻¹ body weight and 500mg of paracetamol Kg⁻¹ body weight for two weeks. After the treatment period, the rats were sacrificed; blood samples were collected for analysis of biochemical parameters such as electrolytes (Na, K and Cl), urea, creatinine, C-Reactive Protein (CRP) C-Reactive protein-albumin Ratio (CAR), while kidney tissues were collected for histopathological examination.

Methods

CBS-500 Electrolyte analyser (Scantrik Medical Supplies, UK) was used to estimate the serum electrolyte as described by Sarker *et al.*, (2023), urea, creatinine and LDH were performed by a fully automated chemistry analyser (COBASS c 111, Roche, UK) as described by Al-Salem *et al.*, (2020). Finecare Machine (SURE SIGN CIGA HEALTHCARE, UK) was used to determine serum C-Reactive protein (CRP) (Charlton *et al.*, 2023) Malondialdehyde (MDA) concentration was determined as described by Baponwa *et al.*, (2022). Glutathione (GSH) and Superoxide dismutase (SOD) activities were determined as per protocol devised by Al-Dosari, (2011). The tissue samples of kidney from animals of each group were collected in 10% Neutral Buffered Formalin (NBF) standard procedures was followed using automated tissue processor (Scitek TP-A1; TP-A3A UK), stained by routine Hematoxylin and Eosin staining as described by Eweka *et al.* (2008). Data sets were analyzed using One-Way Analysis of Variance (ANOVA) with the Tukey Post-Hoc test with the aid of Jasp software, version 20.0. Values were expressed as mean $\pm$ standard deviation (n = 3), p<0.05 was considered statistically significant.

Results

Results are presented on the basis of administration periods as preventive treatment and curative treatment phases respectively.

Preventive Treatment phase of Kidney Function Indices

Electrolytes

The Negative Control group showed significant elevation of chloride levels and insignificant elevation of sodium and potassium levels when compared to the control groups, indicating potential kidney dysfunction. Significance was observed in Na and Cl levels respectively with 100mg/kg body weight being significant to the positive control groups. The 50mg/kg body weight group showed insignificance when compared with the negative, 100 and 200mg/kg body weight groups in Na level. Administration of betacyanins-rich extract reduced potassium levels, particularly at the 50mg/kg dosage, indicating a potential protective effect against acetaminophen-induced potassium elevation (Table 1).

Other Markers of Kidney Function

Normal control group shows the lowest uric acid, LDH, CRP and CAR levels, Negative control showed a significant increase in uric acid, CRP and CAR indicating severe inflammation or kidney damage which may be due to paracetamol. In the case of urea and LDH, insignificant reduction was observed in negative control groups when compared to normal and positive controls. Creatinine level in the negative control group showed significant reduction when compared with normal and positive controls respectively. Reduced urea and LDH levels in negative control indicate renal impairment due to paracetamol (Table 2). In the various preventive treatment groups, CRP, CAR and Uric Acid showed dose-dependent significant decrease down the groups, however, significant increase was observed with respect to administered doses of the extract in LDH levels. Significant reduction in urea was observed in the preventive treatment groups while creatinine showed dose-dependent significant increase. Dose-dependent significant reduction in uric acid, CRP and CAR levels in preventive treatment groups indicates dose-dependent anti-inflammatory effects of the extract against kidney damage induced by paracetamol.

Table 1: Electrolytes Concentrations across Various Betacyanins-Rich Extract Preventive Treatment Groups

Treatment	Grouping of Experimental Male Abino Rats					
	Control			Betacyanins-rich Extract		
	I- Normal	II - Positive	III - Negative	IV- 50mg/kg	V- 100mg/kg	VI- 200mg/kg
Na (mmol/L)	137.67±1.53 ^a	138.00±1.00 ^{a,c}	146.33±2.08 ^{b,d,f,h}	140.33±1.53 ^{a,c,e}	138.00±1.00 ^{a,c,e,g}	141.00±1.00 ^{a,c,e}
K (mmol/L)	4.17±0.15 ^a	4.87±0.21 ^{a,c}	5.70±0.36 ^{b,c,e,f,g}	6.03±0.12 ^{b,d,e}	5.67±0.15 ^{b,c,e,f}	5.80±0.10 ^{b,c,e,f,g}
Cl (mmol/L)	100.33±1.53 ^a	101.67±1.53 ^{a,b}	106.00±2.00 ^{a,b,c,d,e}	103.00±1.00 ^{a,b,c}	100.67±1.16 ^{a,b,c,d}	102.00±2.00 ^{a,b,c,d,e}

Note: Values are expressed as Mean ± S.D, (n=3), values with different superscripts in the same column are statistically significant at p<0.05; Preventive treatment= pretreated with extract followed by 500mg/kg body weight of paracetamol administration.

Table 2: Kidney Function Indices across Various Betacyanins-Rich Extract Preventive Treatment Groups

Treatment	Grouping of Experimental Male Abino Rats					
	Control			Betacyanins-rich Extract		
	I - Normal	II - Positive	III - Negative	IV - 50mg/kg	V - 100mg/kg	VI - 200mg/kg
Urea (mmol/L)	8.13±1.23 ^a	4.50±0.10 ^{b, c}	5.60±0.89 ^{b,c,e,f,g}	6.77±0.47 ^{a,d,e}	5.97±0.29 ^{b,c,e,f}	5.70±0.10 ^{b,c,e,f,g}
Creatinine (μmol/L)	45.67±11.02 ^a	43.67±1.53 ^{a, b}	40.67±5.51 ^{a,b,d,f,g}	51.33±1.53 ^{a,b,d}	58.00±2.65 ^{a,c,d,e}	57.33±2.08 ^{a,c,d,e,g}
CRP (mg/L)	6.33±0.58 ^a	7.67±1.53 ^{a, b}	11.00±1.73 ^{a,b,c,d,e}	8.33±0.58 ^{a,b,c}	7.33±1.53 ^{a,b,c,d}	7.67±0.58 ^{a,b,c,d,e}
CAR (g/L)	1.71±0.26 ^a	1.99±0.45 ^{a,b}	3.01±0.93 ^{a,b,c,d,e}	2.28±0.25 ^{a,b,c}	1.98±0.47 ^{a,b,c,d}	1.98±0.08 ^{a,b,c,d,e}
Uric Acid (μmol/L)	196.33±5.69 ^a	177.67±1.53 ^{a, c}	279.00±1.53 ^{b,d,f,h,j}	188.00±4.00 ^{a,c,e}	179.67±1.53 ^{a,c,e,g}	177.67±1.53 ^{a,c,e,g,i}
LDH (U/L)	362.33±34.70 ^a	321.33±22.14 ^{a,c}	87.33±34.43 ^{b,d,f,g,h}	323.00±106.86 ^{a,c,e}	422.67±21.50 ^{a,c,e,g}	5.60±0.89 ^{b,c,e,f,g}

Note: Values are expressed as Mean ± S.D, (n=3), values with different superscripts in the same column are statistically significant at p<0.05.

Curative Treatment Phase of Kidney Function Indices

Electrolytes

The Negative Control group exhibits insignificant elevation in sodium and potassium levels, and significant elevation in chloride level when compared to the other control groups, this may indicate impaired renal function induced by acetaminophen. Among the treatment groups, K and Cl levels showed significant increases. Na levels showed significant decrease with respect to doses from 100mg/kg to 200mg/kg body weight groups. Negative control and 50mg/kg body weight groups showed significant reductions in Na, K and Cl levels respectively (Table 3).

Other Markers of Kidney Function

The lowest uric acid, LDH, CRP and CAR levels observed in normal and positive controls, together with a significant increase in uric acid, CRP and highest CAR, in the negative control group is indicating inflammation. Treatment groups show a dose-dependent increase in CRP level particularly at 50mg/kg which show very high CRP and CAR levels, indicating severe inflammation, (Table 4), moreover, significant dose-dependent increase in urea and LDH was observed. In the case of creatinine, significant decrease was observed (Table 4).

Table 3: Electrolytes Concentration Across Various Betacyanins-Rich Extract Curative Treatment Groups

Treatment	Grouping of Experimental Male Abino Rats					
	Control			Betacyanins-rich Extract		
	I - Normal	II - Positive	III - Negative	IV - 50mg/kg	V - 100mg/kg	VI - 200mg/kg
Na (mmol/L)	137.67±1.53 ^a	138.00±1.00 ^{a,c}	146.33±2.08 ^{b,d,e}	141.67±1.53 ^{b,c,e,g}	146.67±1.53 ^{b,d,f,g,h}	144.33±0.58 ^{b,d,f,g,h}
K (mmol/L)	4.17±0.15 ^a	4.87±0.21 ^{a,c}	5.70±0.36 ^{b,c,d}	5.23±0.25 ^{b,c,d,e}	5.20±0.46 ^{b,c,d,e,f}	5.23±0.15 ^{b,c,d,e,f}
Cl (mmol/L)	100.33±1.53 ^a	101.67±1.53 ^{a,b}	106.00±2.00 ^{a,b,c}	102.33±2.08 ^{a,b,c,d}	104.33±3.79 ^{a,b,c,d,e}	104.67±3.06 ^{a,b,c,d,e}

Note: Values are expressed as Mean ± S.D, (n=3), values with different superscripts in the same column are statistically significant at p<0.05.

Table 4: Kidney Function Indices across Various Betacyanins-Rich Extract Curative Treatment Groups

Treatment	Grouping of Experimental Male Abino Rats					
	Control			Betacyanins-rich Extract		
	I - Normal	II - Positive	III - Negative	IV - 50mg/kg	V - 100mg/kg	VI - 200mg/kg
Urea (mmol/L)	8.13±1.23 ^a	4.50±0.10 ^{b,c}	5.60±0.89 ^{b,c,d}	5.73±0.25 ^{b,c,d,e}	5.70±0.27 ^{b,c,d,e,f}	5.83±0.15 ^{b,c,d,e,f}
Creatinine (μmol/L)	45.67±11.02 ^a	43.67±1.53 ^{a,b}	40.67±5.51 ^{a,b,c}	42.33±2.08 ^{a,b,c,d}	44.67±2.52 ^{a,b,c,d,e}	38.67±1.53 ^{a,b,c,d,e}
CRP (mg/L)	6.33±0.58 ^a	7.67±1.53 ^{a,c}	11.00±1.73 ^{a,c,e}	22.00±2.00 ^{b,d,f,g}	15.00±3.61 ^{b,d,e,h,i}	16.00±1.00 ^{b,d,f,h,i}
CAR (g/L)	1.71±0.26 ^a	1.99±0.45 ^{a,c}	3.01±0.93 ^{a,c,e}	6.40±0.31 ^{b,d,f,g}	4.25±1.03 ^{b,d,e,h,i}	5.22±0.35 ^{b,d,f,g,i}
Uric Acid (μmol/L)	196.33±5.69 ^a	177.67±1.53 ^{a,c}	279.00±1.53 ^{b,d,e}	284.00±4.58 ^{b,d,e,g}	303.67±6.43 ^{b,d,f,g,h}	295.00±5.29 ^{b,d,e,g,h}
LDH (U/L)	362.33±34.70 ^a	321.33±22.14 ^{a,c}	487.33±34.43 ^{b,d,e}	424.00±22.27 ^{a,c,e,f}	457.33±11.59 ^{a,d,e,f,g}	508.67±11.72 ^{b,d,e,f,g}

Values are expressed as Mean ± S.D, (n=3), values with different superscripts in the same column are statistically significant at p < 0.05.

Preventive Treatment Phase of Oxidative Stress Markers

Normal and positive control groups showed significant SOD concentrations; however, normal and positive control groups are statistically insignificant in GSH and MDA concentrations. A remarkable significant increase was observed in MDA concentration from normal to positive control, negative control group showed insignificant increase in MDA levels when compared with other control groups indicating high lipid peroxidation and oxidative damage (Table 5).

Treatment groups showed significant increases in SOD and GSH levels compared to control groups. Dose dependent effect of the extract on SOD and GSH concentration showed that the extract has potential effects on SOD and GSH concentrations elevation with 200mg/kg body weight group being more effective (Table 5). Treatment groups show a significant reduction in MDA levels compared to the negative control, indicating that the extract helps reduce lipid peroxidation and oxidative stress (Table 5).

Table 5: Antioxidant Markers Levels across Various Betacyanins-Rich Extract Preventive Treatment Groups

Treatment	Grouping of Experimental Male Abino Rats					
	Control			Betacyanins-rich Extract		
	I - Normal	II - Positive	III - Negative	IV - 50mg/kg	V - 100mg/kg	VI - 200mg/kg
SOD (µg)	92.90±1.40 ^a	92.40±3.58 ^{a,c}	25.97±1.25 ^{b,d,f,h,j}	62.97±6.35 ^{b,d,e}	66.13±6.64 ^{b,d,e,g}	64.77±4.77 ^{b,d,e,g,i}
GSH (ng/mL)	0.15±0.06 ^a	0.55±0.13 ^{b,c}	1.65±0.17 ^{b,d,f,h,j}	0.74±0.28 ^{b,c,e}	0.81±0.09 ^{b,c,e,g}	1.91±0.07 ^{b,c,e,g,i}
MDA (nmol/g)	101.67±0.58 ^a	160.67±1.16 ^{b,c}	247.00±12.12 ^{b,d,f,h,j}	180.67±3.06 ^{b,c,e}	180.00±2.00 ^{b,d,f,g}	180.67±3.06 ^{b,d,f,g,i}

Note: Values are expressed as Mean ± S.D, (n=3), values with different superscripts in the same column are statistically significant at p<0.05.

Curative Treatment Phase of Oxidative Stress Markers

Significant decrease in SOD activities was observed in the negative control group compared with the normal and positive control groups. Significant increase in SOD and GSH activities were observed with increase in the dose of the extract after the concurrent administration of the extract when compared with the negative control group (Table 6).

The negative control group showed significant increase in MDA levels when compared with positive control group indicating high lipid peroxidation and oxidative damage. Significant decrease with respect to MDA concentrations from negative control group down to the various treatment group was observed, this showed that there is a dose dependent effect with respect to extract treatment in MDA concentrations down the group with 200mg/kg body weight group been more effective (Table 6).

Table 6: Antioxidant Markers Levels across Various Betacyanins-Rich Extract Curative Treatment Groups

Treatment	Grouping of Experimental Male Abino Rats					
	Control			Betacyanins-rich Extract		
	I - Normal	II - Positive	III - Negative	IV - 50mg/kg	V - 100mg/kg	VI - 200mg/kg
SOD (µg)	92.90±1.40 ^a	92.40±3.58 ^{a,c}	25.97±1.25 ^{b,d,e}	30.20±4.47 ^{b,d,e,g}	68.87±6.68 ^{b,d,f,h,i}	58.47±1.35 ^{b,d,f,h,i}
GSH (ng/mL)	0.15±0.06 ^a	0.55±0.13 ^{b,c}	1.65±0.17 ^{b,d,e}	1.81±0.06 ^{b,d,e,f}	1.69±0.02 ^{b,d,e,f,g}	1.91±0.07 ^{b,d,e,f,g}
MDA (nmol/g)	101.67±0.58 ^a	160.67±1.16 ^{b,c}	247.0±12.12 ^{b,d,e}	246.3±12.47 ^{b,d,e,g}	236.0±3.00 ^{b,d,e,g,i}	154.67±1.16 ^{b,c,f,h,j}

Note: Values are expressed as Mean ± S.D, (n=3), values with different superscripts in the same column are statistically significant at p<0.05.

Histopathological Examination of Kidney Tissues

In the normal control group, sections of the kidney showed normal glomeruli, tubules, interstitium, afferent and efferent arterioles (Plate I). Sections of the positive control group rat kidney showed relatively normal glomeruli, tubules and interstitium (Plate II). Negative control group sections of the kidney showed acute tubular necrosis (Plate III). In the 50mg/kg body weight treatment group, sections of the kidney showed patchy necrosis of the tubular lining in keeping with acute tubular necrosis. The glomeruli are congested;

tubules show focal loss of tubular epithelial lining, there is focal infiltration of the interstitium by lymphocytes (neutrophils) and plasma cells (Plate IV). While in the other treatment groups (100 and 200 mg/kg), the sections of the kidney showed acute tubular necrosis, (Plate V) for group four and (Plate VI) for group 5 respectively.

However, in the curative treatment phase, the sections of the kidney in the treatment groups showed acute tubular necrosis in all groups. These are presented in Plates VII, VIII and IX respectively.

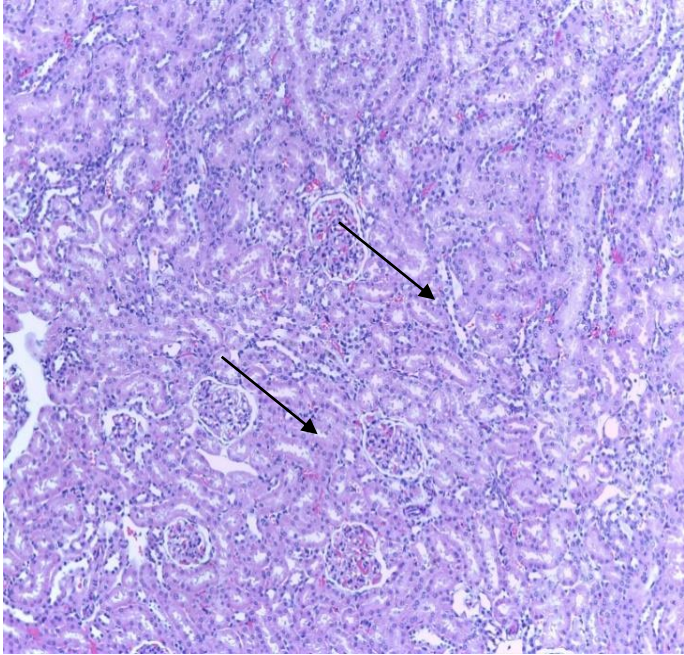


Plate I: Photomicrograph of the section of normal rat kidney with normal glomeruli, tubules and remarkable interstitium. Mg $\times$ 10

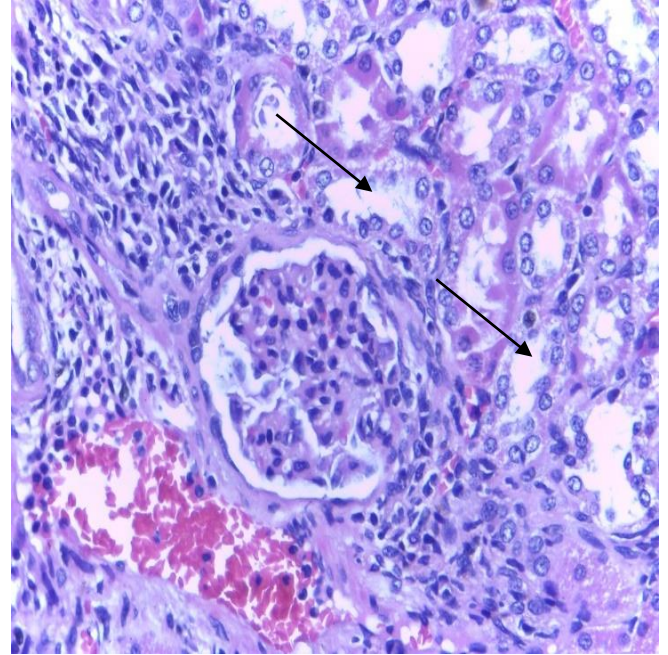


Plate III: Photomicrograph of the section of negative control (500mg/kg Paracetamol) group rat kidney showing acute tubular necrosis. Mg $\times$ 10

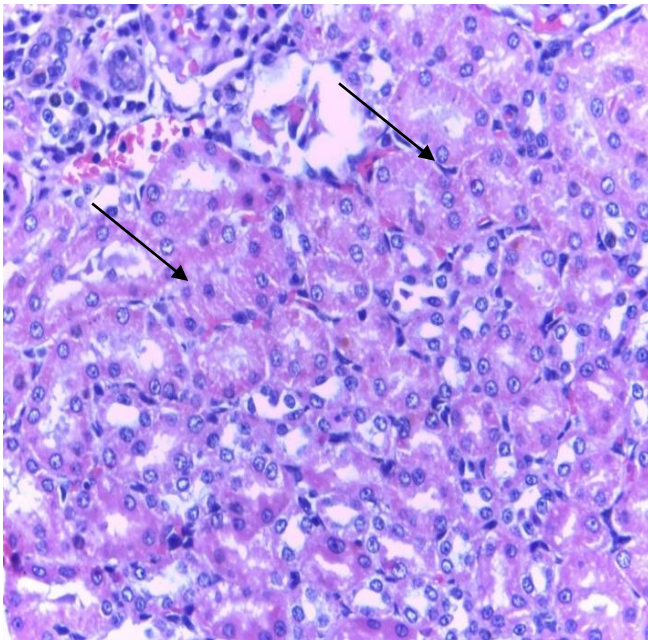


Plate II: Photomicrograph of the section of positive control (100mg/kg Betacyanins-rich Extract) group rat kidney with relatively normal glomeruli, tubules and interstitium.

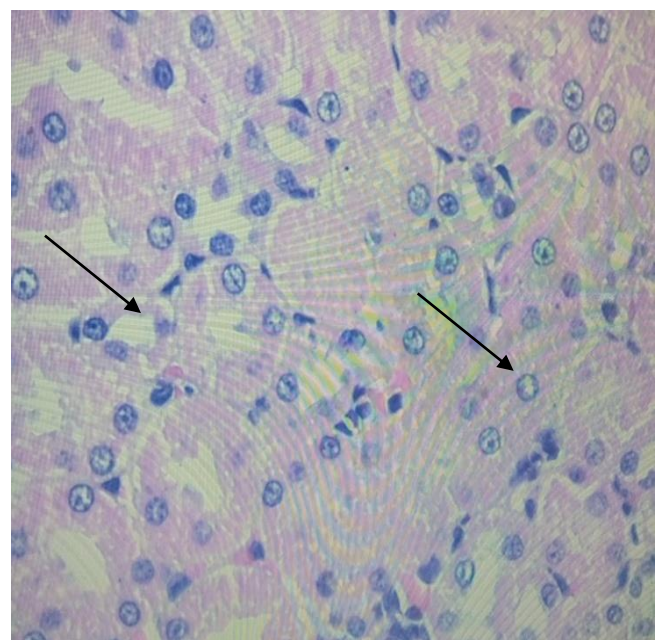


Plate IV: Photomicrograph of the section of 50mg/kg body weight preventive treatment group rat kidney showing patchy necrosis of the tubular lining and congested glomeruli. Mg $\times$ 10

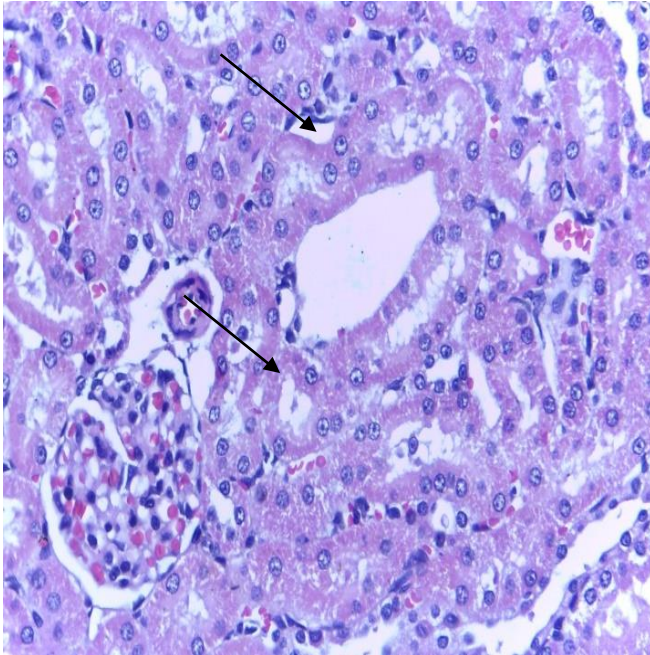


Plate V: Photomicrograph of the section of 100mg/kg body weight preventive treatment group rat kidney showing acute tubular necrosis. Mg $\times$ 10

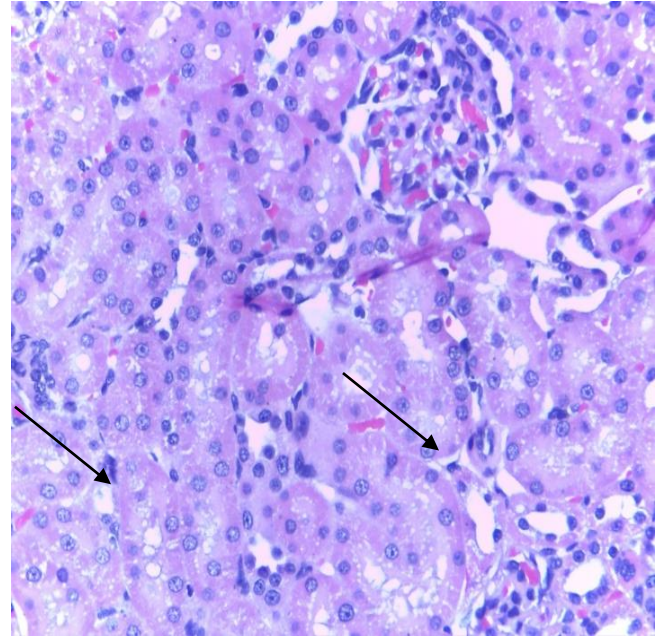


Plate VII: Photomicrograph of the section of 50mg/kg body weight curative treatment group rat kidney showing acute tubular necrosis. Mg $\times$ 10

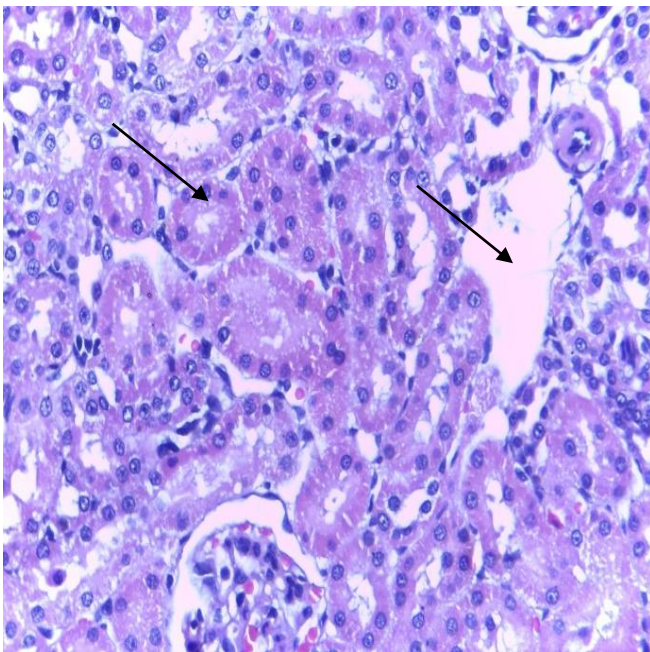


Plate VI: Photomicrograph of the section of 200mg/kg body weight preventive treatment group rat kidney showing acute tubular necrosis. Mg $\times$ 10

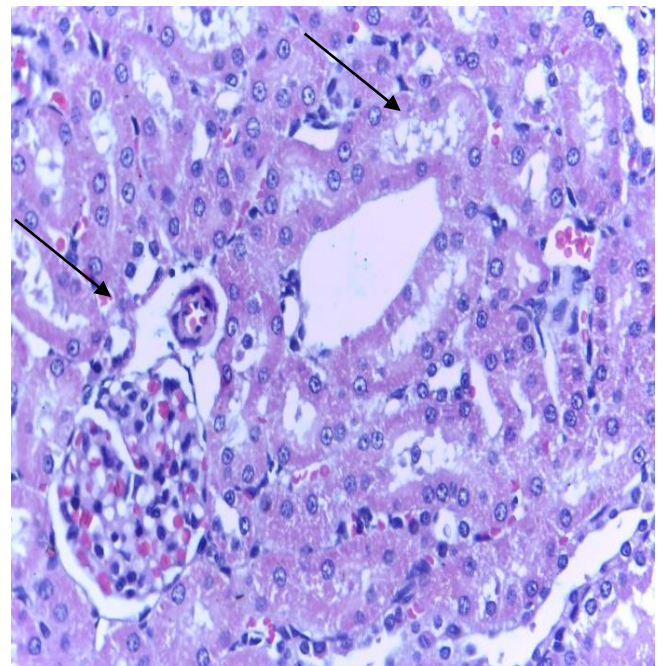


Plate VIII: Photomicrograph of the section of 100mg/kg body weight curative treatment group rat kidney showing acute tubular necrosis. Mg $\times$ 10

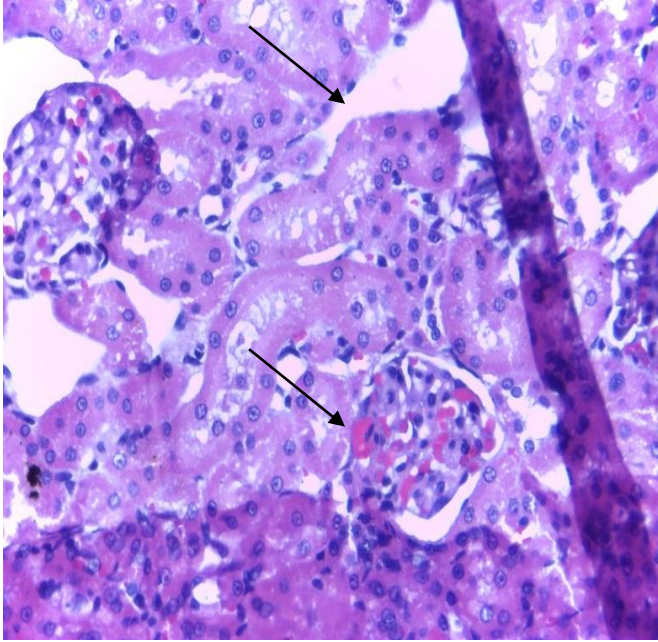


Plate IX: Photomicrograph of the section of 200mg/kg body weight curative treatment group rat kidney showing acute tubular necrosis. Mg×10

Discussion

Acetaminophen-induced kidney injury can lead to sodium and/or potassium accumulation due to impaired renal excretion (Çoban *et al.*, 2023), this is consistent with the findings of this research where elevation in Na and K levels in the Negative Control group was observed, indicating that acetaminophen-induced nephrotoxicity disrupts electrolyte balance leading to Na and K retention. The reduction in potassium levels, particularly at the 50mg/kg dosage of betacyanins-rich extract, suggests a potential nephroprotective effect of the extract in restoring potassium homeostasis. Elevation in chloride levels in the Neg. Control group suggests impaired chloride homeostasis, a common feature of kidney injury (Betzler *et al.*, 2022). The observed trend towards normalization of sodium, potassium and chloride levels with betacyanins-rich extract administration aligns with studies demonstrating the potential nephroprotective effects of beetroot-derived compounds on electrolyte balance (Zarbock *et al.*, 2024) and supports the hypothesis that the extract may protect against acetaminophen-induced kidney dysfunction.

Significant reduction from normal control in positive control suggests that urea might be affected by the diet of the animals; dose dependent slight increase in urea level among pre-treatment groups indicates partial protection of the extract against acetaminophen-induced urea elevation. Creatinine is less affected in all the control groups, however, treatment groups show increment with respect to dose, this indicates less or no protective effect. In the concurrent administration phase, the extract has limited effect on urea level but can significantly affect creatinine dose-dependently. Betacyanins-rich beetroot extract shows significant protective effects by maintaining normal levels of urea and creatinine (Betzler *et al.*, 2022). Plant extracts with high antioxidant content could prevent nephrotoxicity induced by drugs like acetaminophen by maintaining normal urea and creatinine (Khan, 2016). Antioxidants have curative potential that could partially restore kidney function markers but were less effective compared to pre-treatments (Bellomo *et al.*, 2023). Lactate dehydrogenase (LDH) release is a highly reliable way of assessing degrees of cell death, however, it is released from multiple extrarenal organs in response to diverse forms of injury, increase in circulating levels could potentially indicate kidney and other organs injury (Liu *et al.*, 2020; Çoban *et al.*, 2023). The trend towards lower LDH levels in the betacyanins-rich extract groups indicates a potential dose-dependent effect. Elevated uric acid levels in the betacyanins-rich extract groups may suggest a potential nephrotoxic effect at higher dosages.

The pre-treatment appears to be more effective in maintaining normal kidney function markers compared to the concurrent treatment.

Serum C-reactive protein to albumin ratio (CAR) was recently identified as a an independent prognostic marker and a composite indicator of inflammation and nutritional status among kidney injury patients (Liu *et al.*, 2020; Zarbock *et al.*, 2024). CRP levels reflect the severity of inflammation, albumin may be used as a nutritional marker in critically ill patients (Wald *et al.*, 2023; Zarbock *et al.*, 2024). Betacyanins-rich beetroot extract shows significant protective effects by maintaining normal levels of CRP and CAR, this suggests its potential in preventing oxidative stress and inflammation-induced kidney damage. The concurrent treatment effects of the extract are less pronounced, with only partial restoration of kidney function markers.

However, higher doses show some improvement in CRP and CAR, indicating partial anti-inflammatory and renal protective effects. Effectiveness of betacyanins depends on the dosage and timing of administration (Motawi *et al.*, 2020).

Synthetic and natural antioxidants could reduce oxidative stress markers and increase antioxidant enzyme activities (Georgieva and Mihaylova, 2015). Increased MDA levels in negative control group indicate high lipid peroxidation. Pre-treatment with betacyanins-rich extract significantly improved antioxidant defenses, as evidenced by increased SOD and GSH levels and decreased MDA levels. This suggests that betacyanins can preemptively counteract oxidative stress induced by acetaminophen, thereby preventing cellular damage. Elevated GSH levels in the treatment groups compared to controls indicate a protective effect of the extract. Paracetamol-induced toxicity leads to depletion of GSH but can later cause a compensatory increase, treatments with antioxidant-rich extracts have been shown to restore GSH levels, mitigating the damage caused by oxidative stress (Kaur *et al.*, 2019; Afful *et al.*, 2022; Chidiac *et al.*, 2023).

The concurrent treatment study demonstrated that betacyanins-rich beetroot extract could restore antioxidant enzyme activities and reduce lipid peroxidation even when administered alongside acetaminophen. The significant reduction in MDA levels and increase in SOD and GSH levels in the higher dosage group indicates a strong protective effect. Betacyanins effectively restored antioxidant enzyme activities and reduced oxidative damage markers in a rat model of liver injury when administered concurrently (Mahmood and Askar, 2022). This corroborates with the 200 mg/kg concurrent group, where there was a significant reduction in MDA levels and increase in GSH and SOD level. The slight increase in GSH levels in the concurrent groups compared to the negative control suggests a protective role of the extract. The mechanisms of paracetamol-induced oxidative stress and the depletion of antioxidant defenses supports the observations in the negative control group (Baponwa *et al.*, 2022; Chiew and Isbister, 2023).

The kidney, particularly the proximal tubules, is susceptible to APAP-induced injury due to its role in drug excretion and its high metabolic activity (Eweka *et al.*, 2008; Toth *et al.*, 2019; Dallak *et al.*, 2022).

Although most cases of intrinsic acute kidney injury are associated with prerenal triggers and typically thought to be due to acute tubular necrosis, in some cases, the illness is secondary to inflammatory parenchymal disease such as vasculitis, glomerulonephritis, and interstitial nephritis (Tavares *et al.*, 2012; Alchin *et al.*, 2022). Normal glomeruli, tubules, interstitium, and arterioles observed in normal control group suggests the group to serve as a baseline showing normal kidney structure and function, the positive control treatment does not cause significant harm to kidney tissues, it could be a control to show the effects of acetaminophen toxicity. Acute tubular necrosis observed in plate III confirms that acetaminophen administration induces significant kidney damage, as expected in a model of acetaminophen-induced nephrotoxicity.

Acetaminophen is known to cause oxidative stress and depletion of glutathione in renal tissues, leading to tubular necrosis and kidney injury (Tsapenko *et al.*, 2012). Renal histologic changes are notoriously patchy in their presentation, with areas of severe necrosis adjacent to areas which demonstrate near normal histology (Tavares *et al.*, 2012). Patchy necrosis of tubular lining, congested glomeruli, focal loss of tubular epithelial lining, focal lymphocyte and plasma cell infiltration observed in low-dose pre-treatment group indicates partial protective effect, but significant damage is still present. The low dose of betacyanins might not be sufficient to prevent acetaminophen-induced toxicity. Acute tubular necrosis similar to the negative control observed in the medial and high dose pre-treatment groups indicate that even higher doses of betacyanins do not prevent acetaminophen-induced nephrotoxicity in the pre-treatment phase. Betacyanins, found in beetroot and other sources, are known for their antioxidant and anti-inflammatory properties, their effectiveness depend on dosage, bioavailability, and the extent of oxidative damage (McGill and Curry, 2023; Wald *et al.*, 2023). The damage induced by acetaminophen might be too severe for the protective effects of betacyanins to counteract. The dosages used and the timing of administration in the study might have been insufficient to confer protection in the context of high oxidative stress and tissue damage induced by acetaminophen. Betacyanins could reduce the severity of organ damage when administered prior to toxin exposure and less effective when given after the onset of damage (Ninfali *et al.*, 2017).

Concurrent treatment groups show acute tubular necrosis otherwise unremarkable; this suggests that the betacyanins do not exhibit a protective effect when administered concurrently with acetaminophen, the extent of tubular damage remains similar across all groups, this might be because, renal damage has commenced when the antioxidant and anti-inflammatory properties of betacyanins are insufficient to reverse the damage.

This aligns with literature indicating that antioxidants are often more effective in a preventive capacity rather than as treatments for established damage (Liu and Kehrer, 1996; Ninfali *et al.*, 2017; Toth *et al.*, 2019). Jain and Janmeda (2023), reported that antioxidants could reduce markers of oxidative stress and tissue damage in pre-treated groups but were less effective post-exposure, this corroborates with the concurrent treatment phase findings.

Conclusion

The betacyanins-rich extract of beetroot demonstrates potential as a preventive agent against acetaminophen-induced nephrotoxicity particularly when administered as a pre-treatment, its antioxidant and anti-inflammatory properties play a key role in counteracting the oxidative stress and inflammation induced by paracetamol in kidney tissues. However, its protective effects are limited as their efficacy in reversing established damage during concurrent treatment is limited emphasizing the importance of early intervention to ensure efficacy, safety and overall risk-benefit profile of betacyanins-rich extract in kidney protection. The dose-dependent response observed in the betacyanins-rich extract groups suggests that higher dosages may not necessarily confer greater protection and could even lead to adverse effects.

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Author Contributions

All of the authors contributed in design and conducting the experiments, drafting the manuscript, reviewing the relevant literature and revising the manuscript format.

Conflict of Interests

The authors declare no conflict of interest in this research.

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