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Investigation of Renal Protective Effects of *Bryophyllum pinnatum (Lam)* Extract in Acetaminophen-Induced Toxicity in Albino Rats

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Abstract

Background: Acetaminophen is strongly linked to a notable rise in the likelihood of developing new kidney damage. Thus, it affects the kidney and its biomarkers, as it is a vital organ in the removal of toxicants from the body.

Objective: The research assessed renal biomarkers such as serum electrolytes (Na^+, K^+), chloride, bicarbonate, creatinine, and urea in acetaminophen-induced toxicity in albino rats and also observed the activities of these biomarkers in the albino rats when treated with *Bryophyllum pinnatum.*

Methods: Twenty-five (25) healthy male albino rats weighing between 150-200g were randomly grouped into five (5) groups (n-6) in a standard plastic rat cage and were pre-treated as follows: group I served as normal control: distilled water, group II served as positive control: acetaminophen (2g/kg) + distilled water. Group III served as treatment group 1: acetaminophen (2g/kg) + plant extract (200 mg/kg b.wt), group IV served as treatment group 2: acetaminophen (2g/kg) and plant extract (400 mg/kg b.wt) and group V served as standard Control: acetaminophen (2g/kg) and vitamin E (200 mg/kg b.wt) all for 14 days.

Results: When intoxicated with acetaminophen, the concentration of serum electrolytes, creatinine, urea, and chloride significantly increased, while the concentration of serum bicarbonate decreased. However, treatment with Bryophyllum pinnatum at various doses relative to body weight (200mg/kg and 400mg/kg) and Vitamin E at 200mg/kg yielded positive responses in the activities of these renal biomarkers.

Conclusion: The study showed that the administration of *Bryophyllum pinnatum* leaf extracts reduced the acetaminophen-induced changes in kidney function parameters. Thus satisfying the renal protective effects of *Bryophyllum pinnatum.*

Keywords: *Bryophyllum pinnatum*, Renal, Electrolytes, Biomarkers, Serum, Kidney, Acetaminophen, Albino rats.

INTRODUCTION

Acetaminophen, also known as paracetamol, is a non-narcotic pain reliever and fever reducer [1]. It possesses the same level of effectiveness as aspirin, particularly in the central nervous system. Acetaminophen is generally well-tolerated, with few occurrences of adverse effects and medication interactions at regular dosages. Excessive consumption and long-term usage of acetaminophen can lead to liver damage and the initiation of oxidative stress [2]. When cellular reactive oxygen species synthesis outstrips antioxidant defenses, a condition known as oxidative stress develops. This can lead to tissue damage, lipid peroxidation, changes in the plasma membrane, and the deactivation of enzymes. While overdosing on prescription acetaminophen is rare, obtaining acetaminophen over-the-counter for self-medication without a prescription might potentially harm organs [3-5].

The kidney is an essential organ for maintaining the body's internal environment. The kidney is responsible for regulating homeostatic processes and removing metabolic waste, as per Luevano and Damodaran [6]. Renal disease frequently results in metabolic acidosis. The disease is subsequently caused by the accumulation of fixed acid catabolites, such as phosphates and sulfates, and elevated levels of non-protein nitrogen in the blood, which is a result of a low glomerular filtration rate. The renal tubular system, which is responsible for the excretion of hydrogen ions and the production of ammonia, may also be malfunctioning. This defect would result in the excretion of a substantial quantity of cations in the urine. In this scenario, dehydration could result from electrolyte loss if plasma sodium levels plummeted significantly. Reduced blood flow leads to an additional impairment of kidney function, which in turn reduces the volume of extracellular fluid [6].

A member of the Crassulaceae family, *Bryophyllum pinnatum* is a plant genus that inhabits Madagascar and Africa. Gardens, parks, roadsides, railway lines, waste areas, coastal locations, watercourse banks, open woodlands, forests, and forest borders are just a few of the locations where this plant, which is frequently considered a weed, can be found. It has been implemented in tropical, subtropical, and milder temperate countries [7]. This plant is known by a variety of names, including "miracle leaf" and "never-die plant." Traditional medicine practitioners have employed this perennial herb in numerous countries. This herb contains diverse chemical components, including alkaloids, flavonoids, steroids, lipids, and organic acids, as per Ogidi et al. [8]. Furthermore, Yahya et al. [7] pointed out that it contains cardiac glycosides and bufadienolide.

The leaf extracts of the *Bryophyllum pinnatum* plant possess analgesic, antibacterial, anti-inflammatory, and sedative properties [9]. The efficacy of *B. pinnatum* in preventing and treating ethylene glycol-induced urolithiasis has been documented by Yahya et al. [7] in

relation to glycosides. A study conducted by Yahya et al. [7] discovered that when *B. pinnatum* leaf glycosides were administered as an aqueous extract, there was a marked reduction in both urine oxalate levels and calcium-oxalate deposition in the kidneys. According to Arroyo et al. [10], the plant is traditionally used in Nigerian medicine to treat a wide range of illnesses, including respiratory issues, skin conditions, gastrointestinal issues, neurological disorders, high blood pressure, and kidney problems. The herb is also known for its calming, wound-healing, anti-inflammatory, diuretic, and cough-suppressing effects. Boils, skin ulcers, intestinal parasites, bronchitis, and pneumonia glycosides are all conditions that the plant's leaf juice is used to cure [7].

Electrolyte, creatinine, and urea levels are frequently measured to evaluate renal function. According to Chin and Mei [11], serum creatinine levels do not experience a substantial increase until renal function is substantially reduced by renal disease. The objective of this investigation is to evaluate the nephroprotective properties of *Bryophyllum pinnatum* ethanol leaf extract in albino rats that have been induced to become poisoned by acetaminophen.

MATERIALS AND METHODS

Experimental Equipment

Spectrophotometer (UV/VIS. Model 752)-long/Tech. China, Conical Flask, Whatmann filter Paper, Digital electronic Weighing Balance, Spactular, Rotary evaporator, Electric blender

Reagents/Chemicals and drugs

Ethanol (96%), Distilled water, Di–Ethyl, Calcium Chloride, Urea Kit and Creatinine Kit. The Acetaminophen drug used in this study was purchased from Cynflac Pharmacy, Imgbi Road, Yenagoa, Bayelsa State, Nigeria

Collection of Never die (*Bryophyllum pinnatum*) leaves samples

The fresh *Bryophyllum pinnatum* leaves were obtained at Opolo, Yenagoa Local Government Area, Bayelsa State. The leaves were identified by Prof. Inetiminebi Arrow Ogidi from the Department of Plant Science, Faculty of Agricultural Technology, Niger Deltra University, Wilberforce Island, Bayelsa State, Nigeria. The leaves were carefully sifted to remove any deceased material and other undesirable particles. The samples were left to dry in the air for a period of two weeks, after which they were crushed into a fine powder using an electric dry mill.

Methods

Bryophyllum pinnatum leaves Extraction

200 grams of the powdered plant material was immersed in 500 milliliters of ethanol for a duration of 48 hours at the ambient temperature. The liquid was strained into a 500 ml conical flask using a Whatman filter paper. The liquid passed through a filter was dehydrated using a rotary evaporator.

Experimental Animals

A total of twenty-five (25) male albino

rats of the Wistar strain, with a weight range of 150-200g, were acquired from the animal house of the Department of Pharmacology, Faculty of Basic Clinical Sciences, College of Health Sciences, University of Port Harcourt, Rivers State. The rats were housed in conventional rat cages located in the animal facility of the Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State. Prior to the start of this study, the animals were given a 14-day period to adjust to the typical laboratory conditions. During this time, they had unrestricted access to commercial grower's mash (Delta Feeds) , water and were exposed to a 12-hour light and darkness cycle. They also had access to fresh air.

Experimental Design

The animals were randomly grouped into five (5) groups of six rats each in a standard plastic rat cage and were pre-treated as follows:

Group I: Normal control: distilled water (for 14 days).

Group II: Positive control: Acetaminophen (2g/kg) + distilled water (for 14 days)

Group III: Treatment group 1: Acetaminophen (2g/kg) + Extract (200 mg/kg b.wt for 14 days)

Group IV: Treatment group 2: Acetaminophen (2g/kg) Extract (400 mg/kg b.wt for 14 days)

Group V: Standard Control: Acetaminophen (2g/kg) Vitamin E (200 mg/kg b.wt for 14 days)

After acclimatisation, animals in groups 2, 3, 4 and 5 were administered with 2 g/kg body weight of acetaminophen orally to induce toxicity. After 24 hours, they were treated as shown in the experimental design above. At the end of 14th day, all the animals were anaesthetized with chloroform and sacrificed.

Blood Collection

Blood was collected via cardiac puncture into plain bottles and allowed to stand for 30 minutes for coagulation to take place. Afterward, the blood samples were centrifuged at 2000 RPM for ten minutes, and the supernatant (serum) was collected for biochemical analysis while a portion of the livers, kidneys, and hearts were collected to prepare homogenate for antioxidant assays.

Renal Function Tests

The levels of Creatinine and Urea in the serum were measured using an automated analyzer called the Biosystem A25 Random Access Analyzer. The auto-analyzer (EasyLyte Plus Analyzer) was used to determine the serum concentrations of Sodium, Potassium, Chloride, and bicarbonate. The manufacturer's instructions for the complete biochemical test were followed rigorously [12].

RESULTS AND DISCUSSION

Effects of *Bryophyllum Pinnatum* (Lam) Extract on kidney function parameters of Acetaminophen Induced Albino Rats

Table 1 and Figures 1-6 shows that

acetaminophen administration caused a significant increase ($p<0.05$) in serum urea (118.93±4.77), creatinine (2.05±0.10), sodium (95.55±2.15), potassium (3.04±0.07), chloride (57.96±1.07) and decrease in bicarbonate (21.91±0.96) activities (positive control), when compared to the normal control rats. However, treatment with *Bryophylum pinnatum* at doses of 200mg/kg body weight significantly ($p<0.05$) decreased in serum urea (91.63±2.71), sodium (86.17±3.83), potassium (2.27±0.16), chloride (43.93±1.43), creatinine (1.47±0.06) activities (positive control), while slight decrease in bicarbonate (30.44±1.40) when compared to the normal control rats.

High dose (400mh/kg body weight) treatment Group with *Bryophylum pinnatum* has significant ($p<0.05$) increase in serum urea (76.15±1.84), sodium (73.20±1.20), creatinine (1.20±0.18), potassium (1.90±0.04), chloride (34.84±1.12) activities (positive control), slight decrease in bicarbonate (37.57±0.85) when compared to the normal control rats. Treatment with standard (Vitamin E at 200mg/kg b.wt) significantly ($p<0.05$) increased in serum urea (78.41±2.78), sodium (71.54±2.02), while bicarbonate (38.15±0.71), creatinine (1.00±0.30), potassium (1.87±0.02), chloride (24.86±4.52) showed slight increase in when compared to the control rats.

Table 1: Effects of *Bryophyllum Pinnatum* (Lam) Extract on kidney function parameters of Acetaminophen Induced Albino Rats

PARAMETERS	GROUPS				
	Normal Control	Positive Control	Group 3 (Para +200 mg/kg Ext)	Group 4 (Para + 400 mg/kg Ext)	Group 5 (Para + Vit E)
Urea (mmol/L)	$57.90±1.43^{b}$	$118.93±4.77^{a}$	$91.63±2.71^{ab}$	$76.15±1.84^{ab}$	$78.41±2.78^{ab}$
Creatinine (mmol/L)	$0.70±0.02^{b}$	$2.05±0.10^{a}$	$1.47±0.06^{a}$	$1.20±0.18^{b}$	$1.00±0.30^{b}$
Sodium (mmol/L)	$59.93±1.52^{b}$	$95.55±2.15^{a}$	$86.17±3.83^{ab}$	$73.20±1.20^{ab}$	$71.54±2.02^{ab}$
Potassium (mmol/L)	$1.47±1.37^{b}$	$3.04±0.07^{a}$	$2.27±0.16^{ab}$	$1.90±0.04^{ab}$	$1.87±0.02^{ab}$

Chloride (mmol/L)	24.80±0.71[b]	57.96±1.07[a]	43.93±1.43[ab]	34.84±1.12[ab]	24.86±4.52[b]
Bicarbonate (mmol/L)	37.71±1.05[b]	21.91±0.96[a]	30.44±1.40[ab]	37.57±0.85[b]	38.15±0.71[b]

Values are given as Mean ± SEM for each group where SEM is the Standard Error of Mean. Superscript 'a' and 'b' indicate significant difference ($p<0.05$) compared to Normal Control (NC) and Positive Control (PC) respectively. P: statistical level of significance was determined by one-way Analysis of Variance (ANOVA) followed by Tukey post-hoc test. Para: Paracetamol. Ext: Extract. Vit E: Vitamin E.

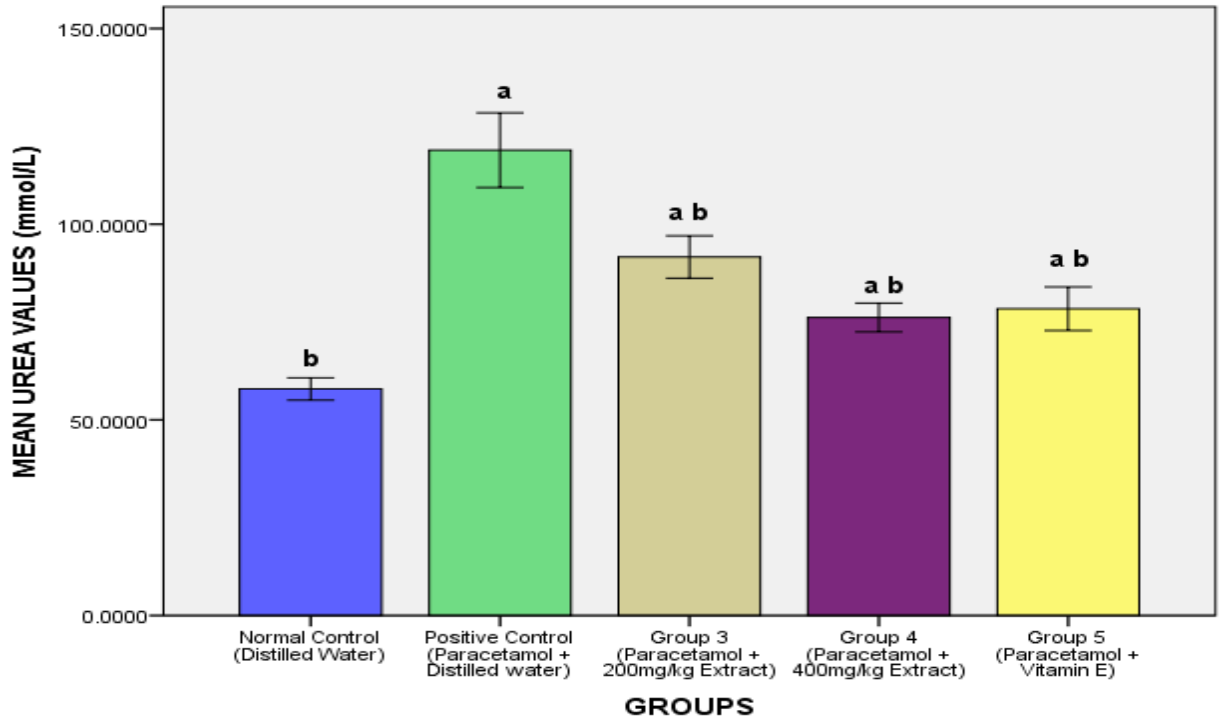

Figure 4.1: Showing the comparative value of urea across the groups

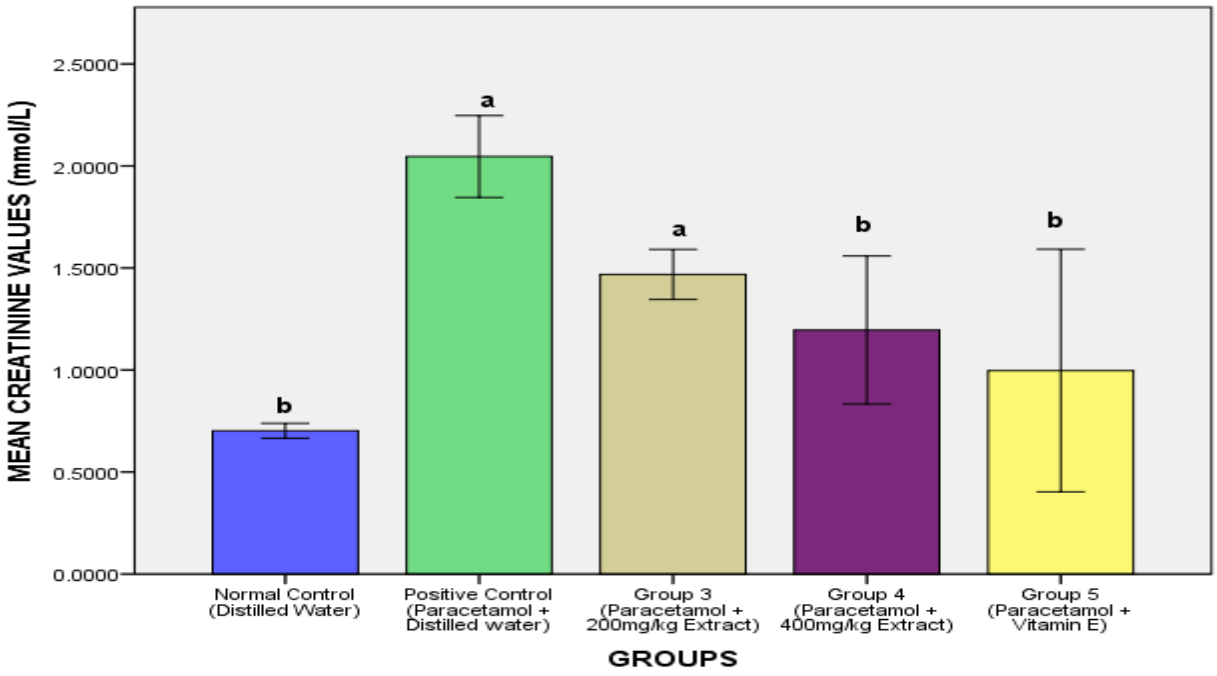

Figure 2: Showing the comparative value of creatinine across the groups

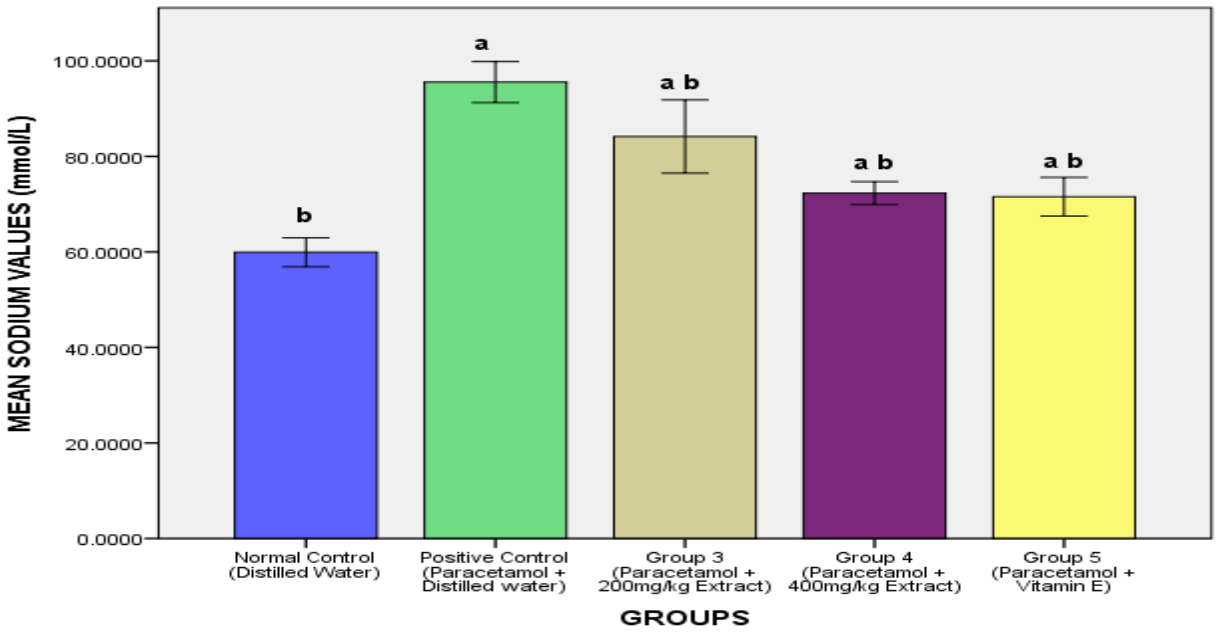

Figure 3: Showing the comparative value of sodium across the groups

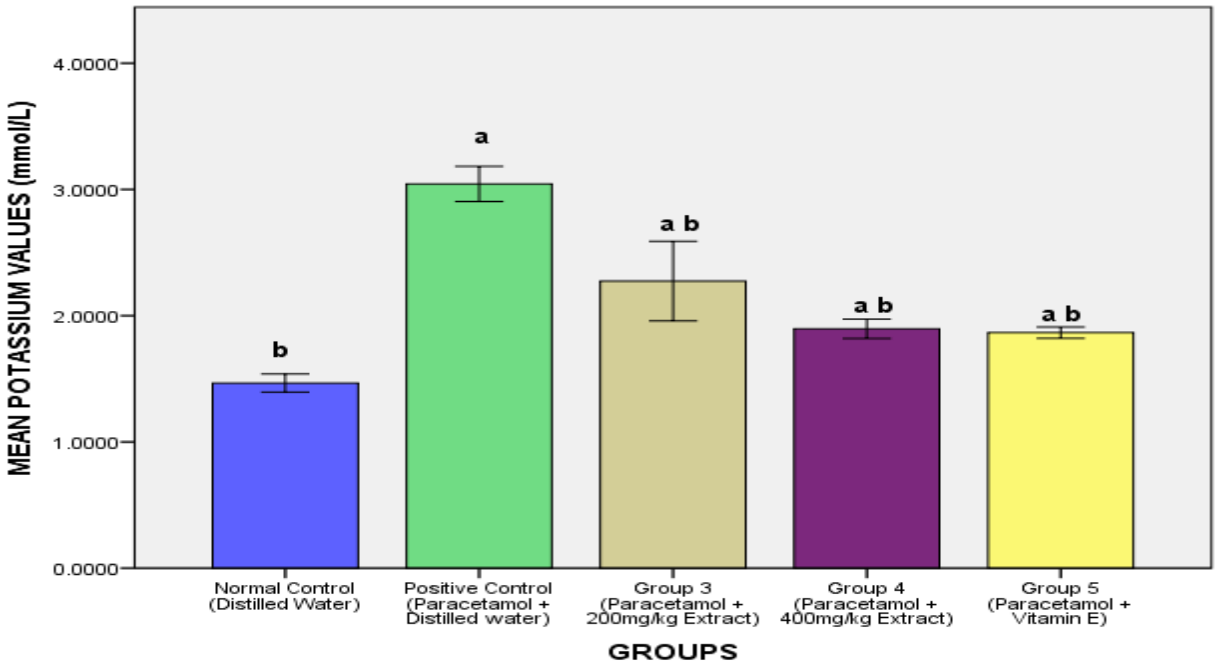

Figure 4: Showing the comparative value of potassium across the groups

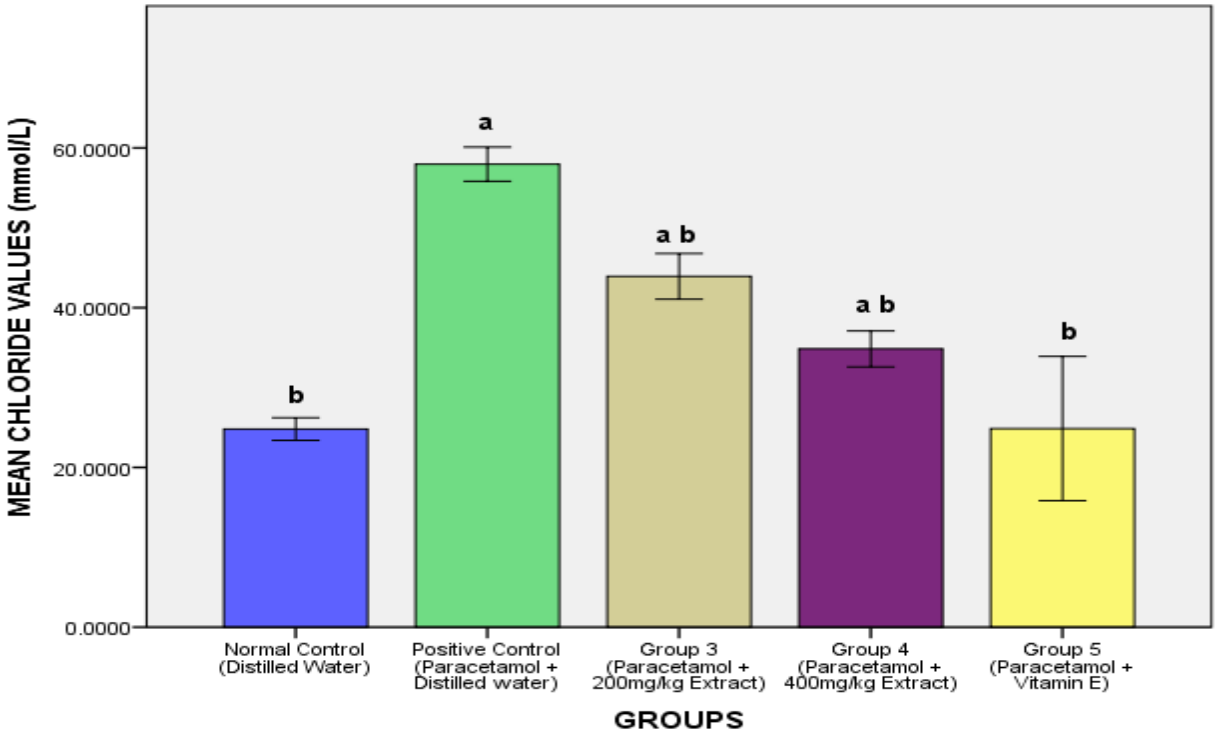

Figure 5: Showing the comparative value of chloride across the groups

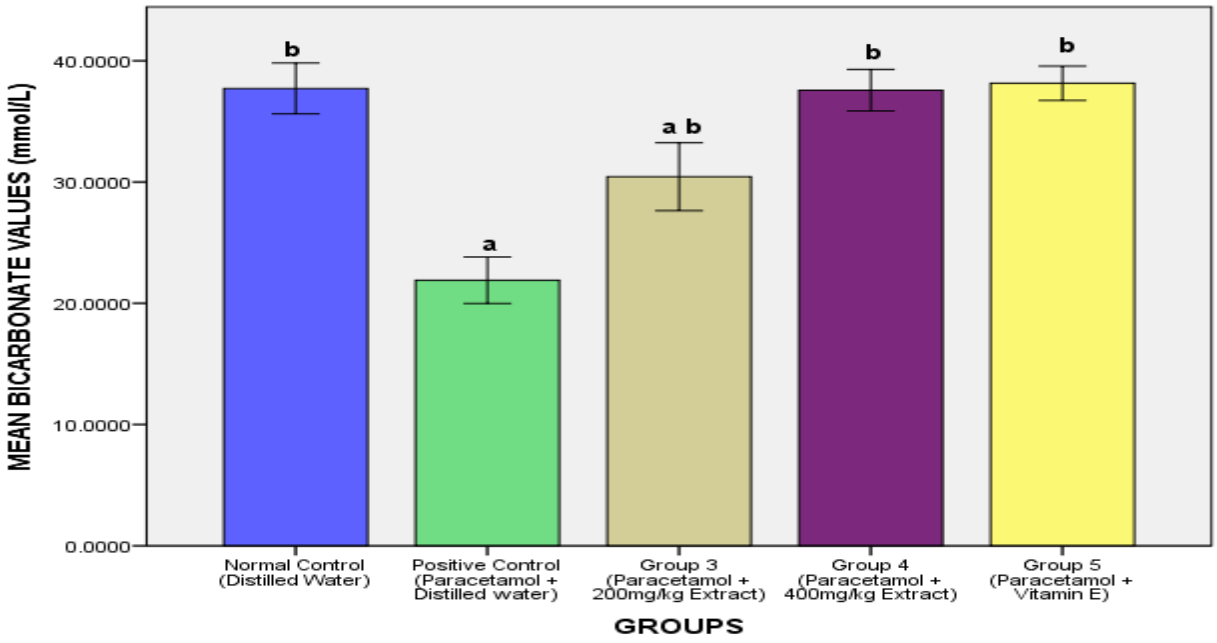

Figure 6: Showing the comparative value of bicarbonate across the groups

Acetaminophen is a widely used pain reliever and fever reducer, and it is also one of the primary causes of sudden liver toxicity on a global scale [12]. Urea is the primary final product of the protein breakdown. The process of amino acid deamination occurs in the liver, which is also the location of the urea cycle. The urea cycle transforms ammonia into urea, which urine then eliminates. It accounts for 90% of the overall removal of nitrogen from urine. The body's level of urea directly correlates with the amount of protein consumed, and its excretion pace is inversely proportional [13]. Renal disorders that reduce glomerular filtration cause urea accumulation. Paracetamol administration (positive control group) causes nephrotoxicity, as evidenced by a significant (P 0.05) increase in urea serum level (Table 1). This study clearly demonstrates the relationship between an increase in urea levels and the impairment of the structural integrity of the nephrons. The ethanolic leaf extract of *Bryophyllum pinnatum*, administered in low and high dosages (Groups 3 and 4), significantly reduced urea levels compared to the acetaminophen-induced group (Group 2) (Figure 1), thereby stabilizing the urea level. This effect was statistically significant ($P \leq 0.05$). Based on these results, it seems that the ethanolic leaf extract of *Bryophyllum pinnatum* can protect kidney function against some types of kidney disease. Zhu et al. [14] conducted research that aligns with this discovery.

The kidney contributes to the maintenance of body homeostasis by reabsorbing vital substances and excreting waste products. The muscles produce creatinine, a waste product, through the metabolism of creatine phosphate. The liver synthesizes creatine, which then enters the bloodstream and skeletal muscle primarily absorbs it for energy generation. Elevated levels of creatinine in the bloodstream indicate a decline in renal function [13]. In comparison to the normal control group,

this study found that creatinine retention increased significantly in the acetaminophen-induced renal toxic group (positive control group). Therefore, paracetamol intoxication negatively affects the kidney, leading to a change in its normal creatinine level [12]. Animals treated with *Bryophyllum pinnatum* extracts exhibited a significant reduction in creatinine levels (Figure 2), confirming the beneficial impact of *Bryophyllum pinnatum* extract on acetaminophen-induced renal damage. Alterations in urea and creatinine levels suggest that the kidney's excretory function may still be compromised even after the administration of acetaminophen. Other researches [15-18] also reported similar findings about the alterations in urea and creatinine levels.

The homeostasis of sodium, potassium, and chloride levels in the bloodstream serves as a reliable measure of the efficient performance of the kidneys and heart. Sodium is linked to blood pressure, and in numerous individuals with hypertension, reducing sodium consumption leads to a decrease in blood pressure. The group that received only acetaminophen showed an increase in sodium levels compared to the normal control group. However, in all groups that received both the leaf extract and acetaminophen, sodium levels fell significantly ($p \leq 0.05$) compared to the negative control group. Conversely, potassium, found in the fluid inside cells, has been identified as one of the electrolytes that can help prevent hypertension [19]. The reduction in serum potassium levels (in the groups receiving the leaf extract) compared to group two suggests that *Bryophyllum pinnatum* leaf extract may have a protective or stabilizing effect on membrane channels. The acetaminophen-induced kidney toxicity group experienced a substantial rise in sodium and potassium levels, as well as a decline in chloride levels. The ethanolic leaf extract of *Bryophyllum pinnatum* administered protected against these changes (Table 1). Substantial changes in the levels of these bodily electrolytes suggest inadequate kidney function or kidney damage. Therefore, the *Bryophyllum pinnatum* leaf extract likely exhibited antioxidative activity by effectively reducing the production of reactive oxygen species (ROS) caused by acetaminophen toxicity, thus minimizing oxidative stress [17].

CONCLUSION

The study provided sufficient evidence of the impact of *Bryophyllum pinnatum* leaf extract on renal function parameters in rats exposed to acetaminophen toxicity. The results clearly demonstrated that acetaminophen-induced toxicity had adverse effects on the kidneys of adult Wistar rats, as indicated by changes in renal function parameters such as serum urea, serum creatinine, serum Na^+, and K^+ levels. The study assessed the renal protective effects of *Bryophyllum pinnatum* leaf extract on serum urea, creatinine, and electrolytes (Na^+ and K^+) in rats exposed to acetamninophen, and found that the administration of *Bryophyllum pinnatum* leaf extracts reduced the acetaminophen-induced changes in kidney function parameters. Therefore, due to its significant impact on acetaminophen toxicity in the kidney, we recommend considering *Bryophyllum*

pinnatum leaf extract as part of acetaminophen toxicity treatments.

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Ethics approval

This study was approved by the Research and Ethics Committee of the Department of Biochemistry, Faculty of Basic Medical Sciences, Bayelsa Medical University, Yenagoa, Bayelsa State, Nigeria, with a Reference Number FBMS/AD/BCH/REC/29/02.

Conflict of Interest

The authors declare that there is no conflict of interest

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