Assessment of the Nephrotoxic Effect of Sodium Benzoate, Ascorbic Acid, and their Combined Administration in Albino Rats

Abstract

Background: The increasing use of preservatives like sodium benzoate and vitamin C in food and beverages has raised concerns about potential health risks, including the formation of benzene, a carcinogenic compound. This study evaluated the nephrotoxic effect of sodium benzoate, ascorbic acid, and their combined administration in albino rats
Methods: Thirty-six rats were divided into six groups: a control group, two groups receiving sodium benzoate (120 mg/kg and 240 mg/kg), a vitamin C group (100 mg/kg), and two combination groups receiving sodium benzoate (120 mg/kg or 240 mg/kg) with vitamin C (100 mg/kg). Treatments were administered orally for 28 days.Following the study period, blood samples were analyzed for electrolytes (potassium, sodium, chloride, bicarbonate), urea, and creatinine. Kidney tissues were examined histologically using H&E staining.
Results:There were no significant changes in potassium ($p=0.145$) or sodium ($p=0.147$) levels. However, there were significant increases in chloride ($p=0.010$), bicarbonate ($p=0.001$), creatinine ($p=0.007$), and urea ($p=0.000$) in experimental groups. Histological examination showed tissue distortions in treated groups compared to controls.
Conclusion: These findings indicate that sodium benzoate, vitamin C, and their combination, especially at higher doses, may compromise kidney function by disrupting biochemical parameters and causing tissue damage. Further research, including human studies, is recommended to explore the implications of these effects.

Keywords: Sodium Benzoate, Vitamin C, Kidney Function, Albino Rats, Benzene Formation, Histological Analysis, Biochemical Parameters, Preservatives

1.0 INTRODUCTION

The increasing demand for food and beverage preservation to extend shelf life has led to the widespread use of preservatives, which are essential in controlling enzymatic reactions and microbial activity during packaging, storage, distribution, retail, and consumption to prevent spoilage [1]. Food preservatives are generally categorized into two main groups: antioxidants and antimicrobials [2]. Antioxidants prevent or slow the oxidative degradation of food, helping to maintain its quality and nutritional content. In contrast, antimicrobial agents inhibit the growth of spoilage-causing and pathogenic microorganisms, ensuring food safety and prolonging shelf life [3]. Two common examples of such preservatives are sodium benzoate and ascorbic acid.

Sodium benzoate (SB), a widely used preservative, inhibits microbial growth even at low concentrations and is recognized as safe for food preservation [4]. Known as E211 in Europe, it is a tasteless, odorless, water-soluble salt with antifungal and antibacterial properties [5]. Approved by the FDA and classified as generally recognized as safe (GRAS) at concentrations up to 0.1%, it is commonly found in products like carbonated drinks, sauces, and jams [6], whereas, ascorbic acid (vitamin C) is a natural preservative with antioxidant properties, preventing oxidative spoilage by inhibiting oxygen reactions with food, thus preserving its

quality [7]. It extends shelf life by reducing harmful compounds and is effective in preserving fruits, vegetables, and meats. Additionally, ascorbic acid plays a vital role in collagen production, immune function, and neutralizing reactive oxygen species (ROS), protecting against oxidative damage [8].

Sodium benzoatein processed foods and beverages has been scrutinized for its potential adverse effects. When metabolized, sodium benzoate forms benzoic acid, which is further processed in the liver through glycine conjugation to produce hippuric acid, subsequently excreted by the kidneys. This metabolic pathway places a significant burden on renal excretory functions, especially with prolonged or excessive exposure.It has been also been reported to cause cancer and genotoxicity [9]. These harmful effects may be particularly observed when consumed in high doses or in combination with other substances.

Some foods and beverages in the market contain both sodium benzoate and ascorbic acid as preservatives (as shown in Figure 1), and the interaction between these two compounds has raised concerns due to the potential formation of benzene, a known carcinogen, when exposed to light or high temperature [10]. This reaction could lead to damage across various organs, particularly the kidneys, which are responsible for eliminating toxins. Due to their role in detoxification, the kidneys are particularly vulnerable to damage from prolonged exposure to toxic substances, including environmental pollutants and food additives[11].

A **B**

Figure 1A and 1B: Labels of Carbonated Drinks Indicating the Presence of Sodium Benzoate and Ascorbic Acid

Research on the combined effects of sodium benzoate and ascorbic acid on renal function is limited but crucial, given the widespread use of these compounds in the food industry. While the

antioxidant properties of vitamin Care believed to counteract oxidative stress, its interaction with sodium benzoate may lead to conflicting outcomes depending on dose, duration of exposure, and underlying physiological conditions. Hence, this study aimed at assessing the nephrotoxic effect of sodium benzoate, ascorbic acid, and their combined administration in albino rats.

2.0 MATERIALS AND METHODS

2.1 Procurement of Materials

Sodium benzoate, sterile bottles, lithium heparin, plain bottles, and filter paper were procured from Nexidon Nigeria Limited reagent store, while syringes, hand gloves, and cotton wool were obtained from Lloyd's Pharmacy in Port Harcourt.

2.2 Ethical Considerations

The internationally accepted National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals were observed.

2.2 Experimental Animals

Thirty-six (36) albino rats, weighing between 140-250 g, were randomly selected for the study. The animals were sourced from the Department of Anatomy, College of Medical Sciences, Rivers State University. They were transported in a well-ventilated wire cage to the animal house at the Department of Animal and Environmental Sciences, Rivers State University, Port Harcourt. The rats were housed under a 12-hour light/dark cycle with free access to solid poultry chow and water. Prior to the study, they were acclimatized for two weeks under standard conditions before being divided into six groups.

2.3 Acute Toxicity Study

The Fixed Dose Procedure [12] was employed for the study, conducted in two phases. In the first phase, three rats were administered sodium benzoate at a dose of 700 mg/kg body weight via oral gavage and monitored for signs of toxicity over 14 days. In the second phase, another group of three rats received sodium benzoate at a lower dose of 300 mg/kg body weight via oral gavage, with observations for toxicity conducted over the same 14-day period.

2.4 Dose Determination

Following the results of the acute toxicity study, two doses of sodium benzoate were selected: a low dose (120 mg/kg) and a high dose (240 mg/kg). Both doses were below the threshold that caused observable acute toxicity.

2.4.1 Low Dose of Sodium Benzoate

To determine the low dose of sodium benzoate, a dosage of 120 mg/kg body weight was calculated. For instance, for a rat weighing 234 g, the calculation proceeded as follows:

Since 120 mg of sodium benzoate is required for a 1 kg (1000 g) rat, the dose for a 234 g rat was calculated using the formula:

Dose = $\frac{120mg}{1000g} x\ 234g$ = **28.08mg**

Following the Organization for Economic Co-operation and Development (OECD) guidelines for volume selection [12], this 28.08 mg of sodium benzoate was dissolved in 2.34 ml of distilled water, ensuring accurate administration.

2.4.2 High Dose of Sodium Benzoate

To determine the low dose of sodium benzoate, a dosage of 240 mg/kg body weight was calculated. For instance, for a rat weighing 234 g, the calculation proceeded as follows:

Dose =$\frac{240mg}{1000g} x\ 234g$ = **56.16 mg**

Following the OECD guidelines for volume selection [12], this 56.16 mg of sodium benzoate was dissolved in 2.34 ml of distilled water, ensuring accurate administration.

2.4.3 Dose Calculation for Vitamin C

The dosage of vitamin C used in this study was 100 mg/kg, as adopted from the methodology of Kumar et al. [13]. For a rat weighing 234 g, the dosage was calculated as follows:

Since 100 mg of vitamin C is required for a 1 kg (1000 g) rat, the dose for a 234 g rat was determined using the formula:

Dose = $\frac{100mg}{1000g} x\ 234g$ = **23.4 mg**

Following the OECD guidelines for volume selection [12], this 23.4 mg of vitamin C was dissolved in 2.34 ml of distilled water, ensuring accurate administration.

2.5 Experimental Study Design

After a 14-day acclimatization period, 36 rats were divided into six groups of six rats each based on their body weight. Group 1 served as the control and received food, water, and 1.8 ml of distilled water orally once daily for 28 days. Group 2 received sodium benzoate at a low dose of 120 mg/kg body weight through oral administration daily for 28 days, while Group 3 was given a high dose of sodium benzoate at 240 mg/kg body weight under the same conditions. Group 4

received vitamin C at a dose of 100 mg/kg body weight orally once daily for 28 days. Group 5 was administered a combination of the low dose of sodium benzoate (120 mg/kg) and vitamin C (100 mg/kg) daily for 28 days, while Group 6 was treated with the high dose of sodium benzoate (240 mg/kg) combined with vitamin C (100 mg/kg) following the same daily regimen.

2.6 Specimen Collection and Preparation

At the conclusion of the 28-day experimental period, the animals were fasted overnight and anesthetized in a jar containing chloroform-soaked cotton wool. Blood samples were then collected aseptically via cardiac puncture using 2 ml sterile syringes. A total of 2 ml of whole blood was drawn and transferred into lithium heparin bottles. The blood was centrifuged at 3000 rpm for 5 minutes to separate plasma, which was then transferred into plain bottles for kidney function analysis, including electrolytes (sodium, potassium, bicarbonate, and chloride ions), urea, and creatinine levels. Additionally, the kidneys were carefully excised and processed for histological examination.

2.7 Sample Analysis

The Spectrum and Biobase test kits were purchased and utilized for the analysis. The Spectrum test kit was employed for the measurement of sodium ion, potassium ion, chloride ion, urea, and creatinine, while the Biobase test kit was used for bicarbonate analysis. Additionally, the excised kidneys were prepared for histological examination using the hematoxylin and eosin (H & E) staining technique.

2.8 Statistical Analysis

The data generated from the analysis were expressed as Mean ± standard deviation, and analysed using the Statistical Package for Social Science (SPSS) version 24. Comparison of the mean and standard deviation values were made for the various parameters for the various groups using the one-way ANOVA and Tukey test. Results were considered statistically significant at 95% confidence interval ($p \leq 0.05$).

3.0 RESULTS

3.1 AcuteToxicity Study of Sodium Benzoate

The acute toxicity study results for sodium benzoate are shown in Tables 1a and 1b. Table 1a demonstrates that administering 700 mg/kg of sodium benzoate to rats led to visible signs of toxicity, such as wounds, lesions, and decreased activity, although no fatalities were observed. In contrast, Table 1b reveals that treatment with 300 mg/kg of sodium benzoate showed no signs of toxicity or mortality in the rats.

Table 1: Results of Acute Toxicity Study

1a. Phase I

Dose (mg/kg)	Observation
700	Presence of signs of toxicity such as wounds andlesions, and reduced activity. No mortality

Figure 2: Photographs Showing Wounds and Lesions on the Skin of Rats Administered 700 mg/kg of Sodium Benzoate During Phase I of the Acute Toxicity Study

1b. Phase II

Dose (mg/kg)	Observation
300	No signs of toxicity. No mortality

3.2 Comparison of the Levels of Serum Electrolytes of the Control and Test Groups

Table 2 compares the serum electrolyte levels of groups I to VI. The mean potassium levels ranged from 6.4 ± 1.38 mmol/L to 8.5 ± 0.46 mmol/L, with no significant differences (p=0.145). Sodium levels ranged from 135.98 ± 7.16 mmol/L to 147.66 ± 6.99 mmol/L, with no significant differences (p=0.147). Chloride levels varied from 82.46 ± 2.31 mmol/L to 111.16 ± 7.90 mmol/L, with group IV showing a significantly higher level (p=0.010) compared to the others. Bicarbonate levels ranged from 18.10 ± 1.17 mmol/L to 23.10 ± 1.62 mmol/L, with group V

having a significantly higher value (p=0.001), while group III had a significantly lower value. There were no significant differences between groups I, II, IV, V, and VI for bicarbonate levels.

Table 2: Comparison of Mean Serum Electrolyte Levels Across Groups I, II, III, IV, V, and VI

	Potassium (mmol/L)	Sodium (mmol/L)	Chloride (mmol/L)	Bicarbonate (mmol/L)
GROUP I	6.7 ± 1.23	135.98 ± 7.16	95.38 ± 14.42[a]	21.86 ± 1.09[a]
GROUP II	7.1 ± 1.78	147.66 ± 6.99	82.46 ± 2.31[a]	23.10 ± 1.62[a]
GROUP III	8.5 ± 0.46	147.00 ± 2.65	87.50 ± 13.67[a]	18.10 ± 1.17[b]
GROUP IV	7.6 ± 1.06	141.34 ± 7.49	111.16 ± 7.90[b]	21.70 ± 1.28[a]
GROUP V	6.4 ± 1.38	141.04 ± 11.30	86.72 ± 7.43[a]	22.66 ± 2.82[a]
GROUP VI	7.7 ± 1.37	145.34 ± 5.99	96.70 ± 17.36[a]	22.42 ± 1.15[a]
F-value	1.832	1.819	3.877	6.097
***P*-value**	0.145	0.147	0.010	0.001
Remark	NS	NS	**S**	**S**

Key:NS = not significant, S = significant. Values with different superscripts are significantly different (p<0.05)

3.3 Comparison of the Levels of Plasma Creatinine and Urea of the Control and Test Groups

Table 3 compares plasma creatinine and urea levels across groups I to VI. The mean creatinine levels ranged from 0.82 ± 0.08 mg/dL to 1.05 ± 0.11 mg/dL, with group VI showing a significantly higher level (p=0.007) compared to the others. No significant differences were observed between groups I, II, III, IV, and V. The mean urea levels ranged from 4.21 ± 1.97 mmol/L to 8.34 ± 0.39 mmol/L, with groups IV and VI showing significantly higher levels (p=0.000) than groups I, II, III, and V. No significant differences were found between groups I, II, III, and V, nor between groups IV and VI.

Table 3: Comparison of Mean PlasmaCreatinine and Urea Levels Across Groups I, II, III, IV, V, and VI

	Creatinine (mg/dL)	Urea (mmol/L)
GROUP I	0.87 ± 0.05^{a}	5.42 ± 0.80^{a}
GROUP II	0.83 ± 0.14^{a}	5.23 ± 0.98^{a}
GROUP III	0.82 ± 0.08^{a}	4.21 ± 1.97^{a}
GROUP IV	0.98 ± 0.12^{a}	8.18 ± 0.40^{b}
GROUP V	0.94 ± 0.07^{a}	6.06 ± 1.98^{a}
GROUP VI	1.05 ± 0.11^{b}	8.34 ± 0.39^{b}
F-value	4.211	8.661
***P*-value**	0.007	0.000
Remark	**S**	**S**

Key:S = significant. Values with different superscripts are significantly different ($p<0.05$)

3.4 Histological Analysis Results of Kidney Tissues from the Different Rat Groups

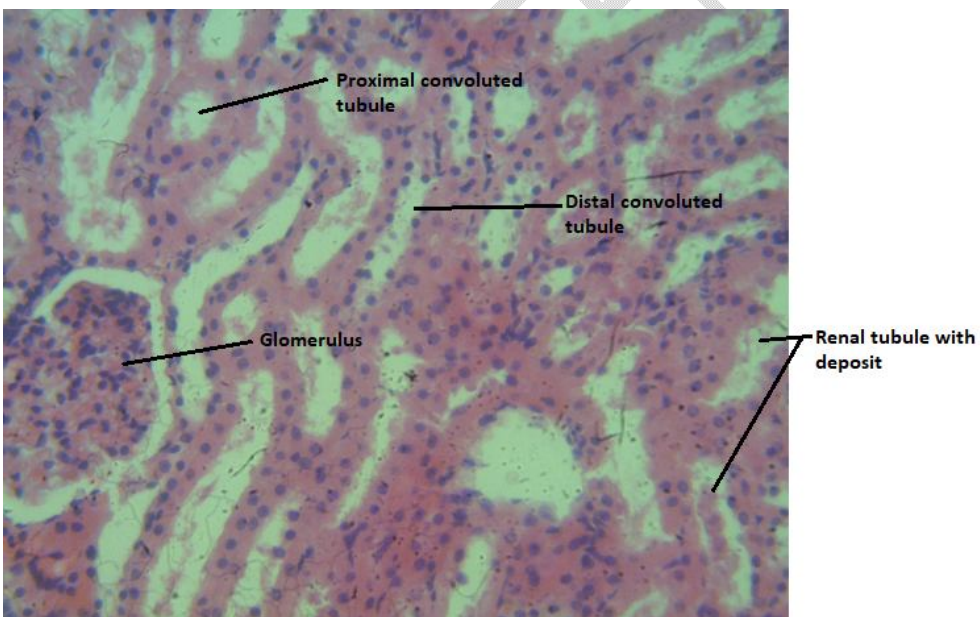

Figure 3: Photomicrograph of the kidney tissue showing well delineated glomerulus,proximal and distal convoluted tubules. Tissue shows normal microstructural appearance. H & E, X400

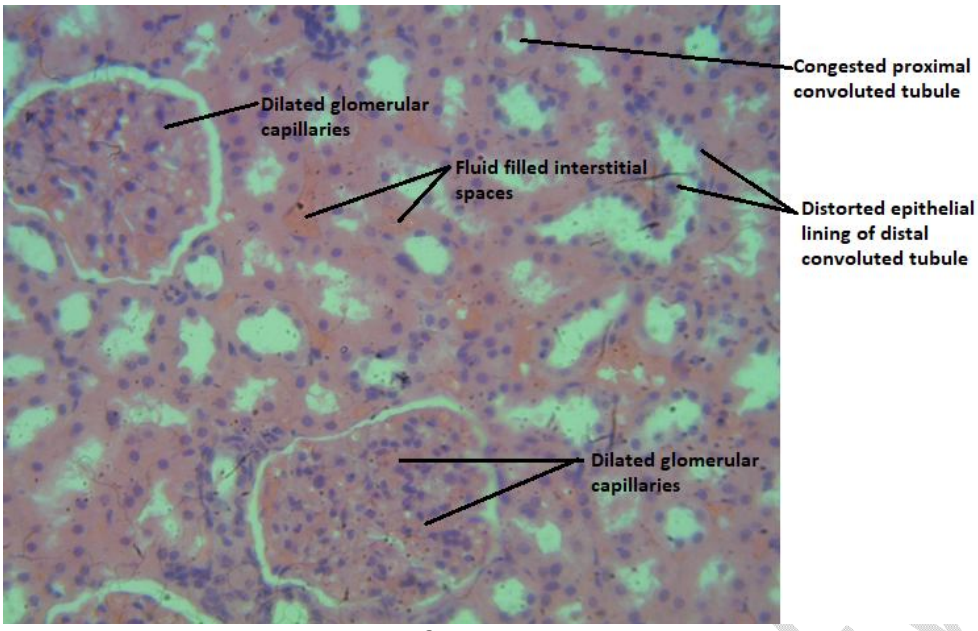

Figure 4: Photomicrograph of the kidney tissue showing dilated glomerular capillaries, fluid-filled interstitial spaces (oedematous) and distorted epithelial lining of distal convoluted tubules. Distortion of microstructure is indicated. H & E, X400

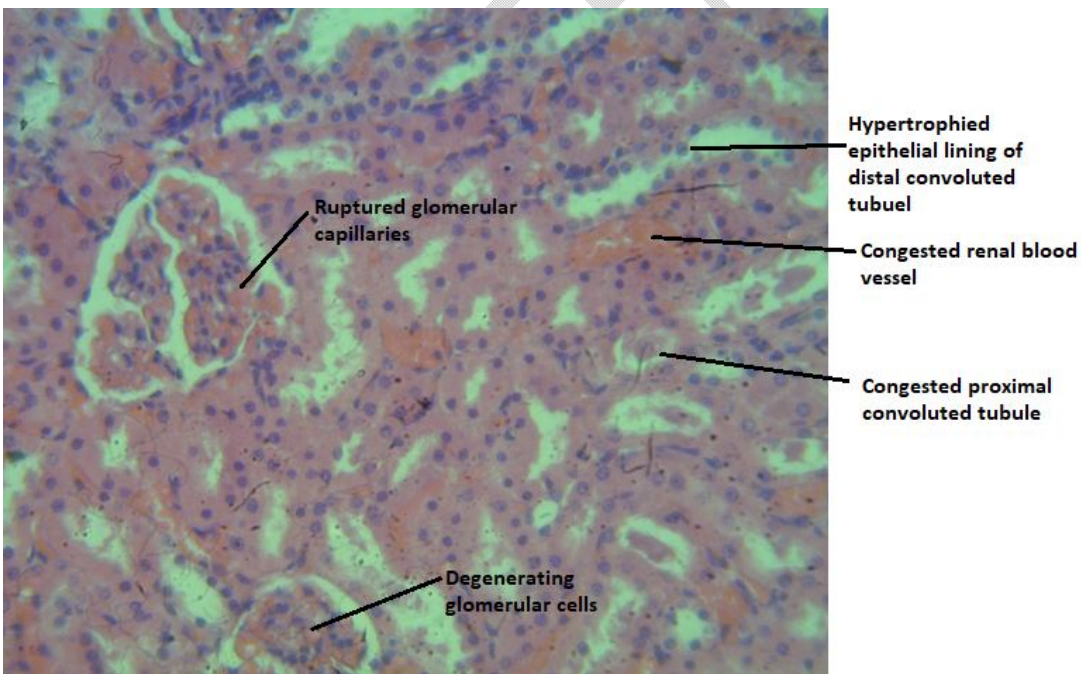

Figure 5: Photomicrograph of the kidney tissue showing ruptured glomerular capillaries, congested proximal and distal convoluted tubules and distorted epithelial lining of distal convoluted tubules. Distortion of microstructure is indicated. H & E, X400

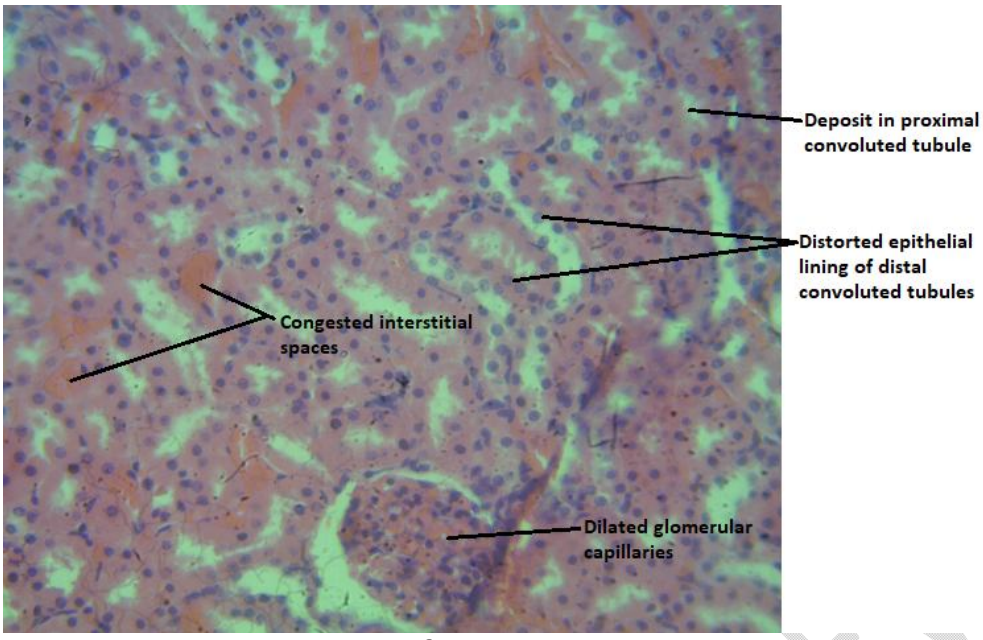

Figure 6: Photomicrograph of the kidney tissue showing dilated glomerular capillaries, congested or fluid-filled interstitial spaces, congested proximal and distal convoluted tubules and distorted epithelial lining of distal convoluted tubules. Distortion of microstructure is indicated. H & E, X400

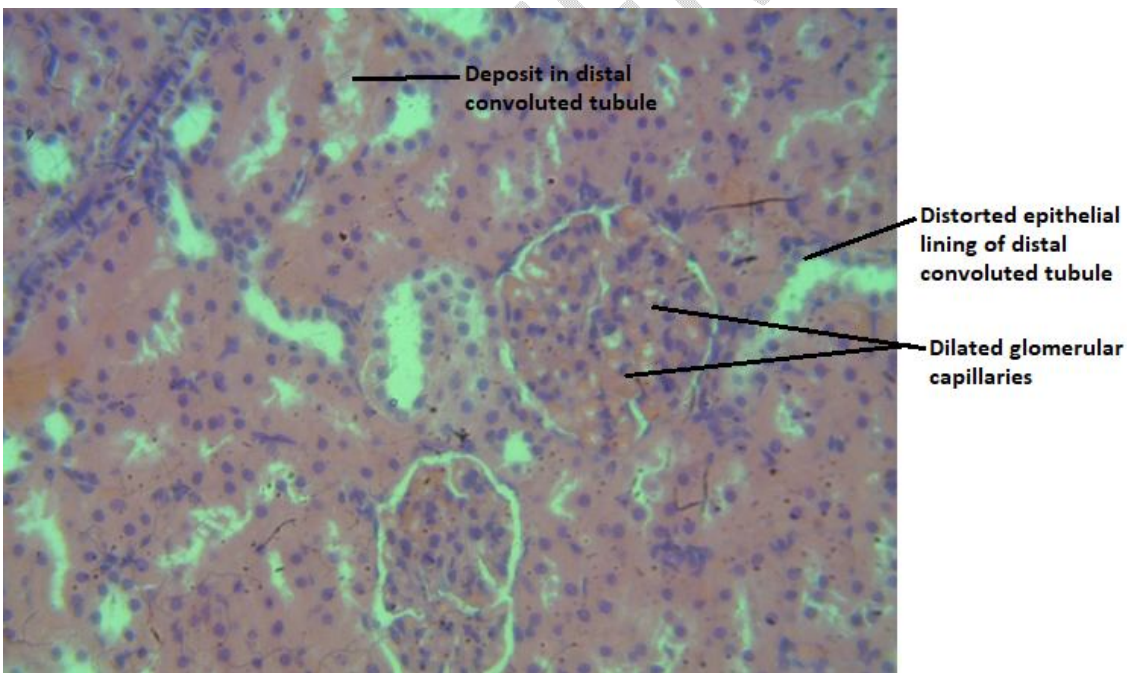

Figure 7: Photomicrograph of the kidney tissue showing dilated glomerular capillaries, distorted epithelial lining of distal convoluted tubule with deposit in lumen. No fluid congestion in interstitial space. Tissue shows distortion of microstructure. H & E, X400

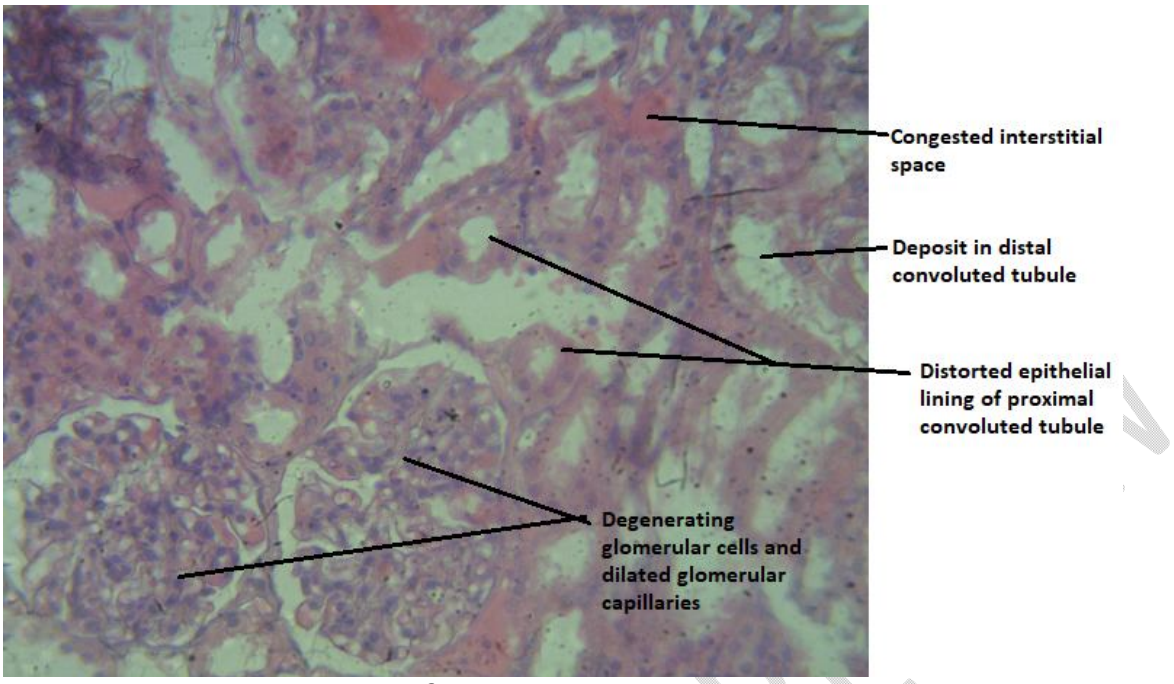

Figure 8: Photomicrograph of the kidney tissue showing dilated glomerular capillaries (with cellular degeneration), deposit in distal convoluted tubules and localized congestion of interstitial spaces. Distortion of microstructure is indicated. H & E, X400

4.0 DISCUSSION

This study aimed to evaluate the nephrotoxic effect of sodium benzoate, ascorbic acid, and their combined administration in albino rats. The acute toxicity study results in this research reveal the effects of sodium benzoate on the health of albino rats at different doses. The administration of 700 mg/kg of sodium benzoate led to visible signs of toxicity, including wounds, lesions, and reduced activity in the rats, although no fatalities occurred. In contrast, a lower dose of 300 mg/kg did not result in any observable toxicity or mortality. The lesions and reduced activity observed in the higher dose group indicate that sodium benzoate may induce a stress response or organ-specific damage, such as skin and muscle toxicity, although the absence of fatalities suggests that the overall survival of the rats was not compromised at this dosage.

The serum electrolyte levels (Table 2) of the rats across different experimental groups, show no significant differences in potassium and sodium levels among all groups, which suggests that neither sodium benzoate nor vitamin C had a measurable impact on electrolyte balance at the administered doses. This finding is not consistent with Hasson et al. [14], whose study reported an increase in the levels of potassium and sodium in the serum due to administration of sodium benzoate. Choudhary and Rathinasamy[15] noted that disruption of Na+/K+ ATPase activity can adversely affect electrolyte regulation and balance. Hence, the non-significant differences in sodium and potassium levels observed in this study may have stemmed from the possibility that sodium benzoate and vitamin C did not impact Na+/K+ ATPase activity. Similarly, the findingfrom this study contrasts with the findings of Ibekwe et al. [16], who reported significant

increase in serum potassium and sodium levels, indicating that sodium benzoate may disrupt electrolyte homeostasis. This discrepancy could stem from differences in study design, such as animal strain, duration of exposure, or the dosages used.

The results also showed a significant elevation in chloride levels in the group treated with vitamin C (group IV). This finding agrees with that of Otanwa et al. [17], who observed that vitamin C administered at certain doses can affect plasma chloride level. However, the finding contrasts with that ofEteng et al. [18], who observed no significant changes in chloride levels in albino rats following the administration of vitamin C. The mechanism behind this elevation from this present study could be related to the influence of vitamin C on acid-base balance. High doses of ascorbic acid (vitamin C) can cause metabolic acidosis, which may lead to compensatory chloride retention, as suggested by Biga et al. [19].Additionally, high sodium levels, as found in sodium benzoate, can also disrupt acid-base balance, potentially altering chloride levels due to compensatory mechanisms like chloride shift during metabolic acidosis [20]. This finding is in disagreement with Ibekwe et al. [16], whose result showed that there was no significant difference in the serum level of chloride.

The bicarbonate levels showed a significant decrease in group III (high-dose sodium benzoate), suggesting that sodium benzoate may induce metabolic acidosis, as supported by Zu et al. [21]. This study found that the metabolism of sodium benzoate to benzoic acid could lead to acid accumulation in the body, lowering bicarbonate levels as the body compensates for the acid load. The reduction in bicarbonate levels aligns with findings from Mohammad [22], who described how metabolic acidosis could lead to a decrease in bicarbonate levels through renal compensation. This result contrasts with Efekemo et al. [23], who did not observe changes in bicarbonate levels in similar studies. These differences could stem from the varying doses of sodium benzoate used, or differences in experimental animal models and methods of assessing acid-base balance.

The plasma creatinine and urea levels (Table 3) provide further evidence of kidney dysfunction. Group VI, which received a combination of sodium benzoate and vitamin C, exhibited significantly higher plasma creatinine levels compared to the other groups. Creatinine is a key marker for renal function, and elevated levels typically indicate impaired kidney filtration capacity. This elevation may be related to histopathological changes observed in the kidney tissue, such as glomerular damage, tubular congestion, and necrosis. Hasson et al. [14] reported similar findings, suggesting that sodium benzoate alters glycine metabolism, which can impair creatinine elimination and contribute to elevated serum creatinine levels. The significant increase in plasma urea levels observed in groups IV and VI also suggests kidney impairment, as urea is another biomarker of renal function. The presence of significant urea elevation in these groups is consistent with the findings of Soliman and Khalid [24], who suggested that metabolic byproducts resulting from the combination of sodium benzoate and vitamin C could contribute to kidney dysfunction.

Histological results further support the biochemical findings of kidney dysfunction. Group I, the control group, exhibited normal kidney architecture, with well-delineated glomeruli and tubules, which is consistent with typical kidney function. However, the histological sections of groups II to VI, which received various treatments with sodium benzoate and vitamin C, showed

significant distortions in the kidney microstructure. These included dilated glomerular capillaries, nephrocellular necrosis, and congested or fluid-filled interstitial spaces, all indicating severe damage to the kidney tissues. These changes are indicative of renal injury, which was also reflected in the elevated plasma creatinine and urea levels, suggesting compromised kidney function. The findings from this study are in agreement with Bakar and Aktac[25], who observed similar kidney damage following exposure to food preservatives. The combined effects of sodium benzoate and vitamin C may worsen these histopathological alterations, as both compounds can influence renal function through different mechanisms.

5.0 CONCLUSION

The findings from both biochemical analyses (including electrolyte levels, creatinine, and urea) and histopathological assessments emphasize the toxic effects on renal function and structure, particularly following treatment with vitamin C alone, and the combination of high-dose sodium benzoate with vitamin C. These treatments resulted in significant biochemical alterations and tissue damage, suggesting that both sodium benzoate and ascorbic acid may contribute to nephrotoxicity in rats. The observed elevations in creatinine and urea levels are consistent with impaired renal function, while histological changes, such as glomerular damage and tubular distortion, further support this conclusion. This may imply that preservatives such as sodium benzoate, when combined with antioxidants like ascorbic acid (vitamin C), may worsen renal damage due to their combined effects.

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