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Research Article

Impact of Polystyrene Exposure on Hepatorenal Responses in Male and Female Albino Wistar Rats

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Abstract

Microplastics have become a major health concern because of its potential adverse influences on marine, wildlife and public health. In this study, pristine polystyrene and Styrofoam microplastics particles of diameter < 5mm were used to investigate the toxic effects of polystyrene microplastic (PS-MP) exposure on hepatic and renal function of male and female Wistar rats. The rats were divided into seven groups for both male and female, with one control group and six test groups each. The two forms of polystyrene microplastics were incorporated into the feed of the test groups in varying quantities (1, 5 and 10 % of the feed), and exposure lasted for a period of 90 days. Results showed that aspartate aminotransferase (AST), serum albumin (ALB), total bilirubin (TB), conjugated bilirubin (CB) were significantly ($p < 0.05$) decreased compared to control male and female rats. Histological analysis provided further insights, indicating that despite mild alterations in liver enzymes, albumin and total protein levels in specific test groups, microplastics did not compromise the structural integrity of hepatocytes in male and female rats. However, kidney function parameters exhibited significant ($p < 0.05$) increases in serum urea, creatinine, K+, and Cl- levels in test rats of both sexes compared to controls. Regardless of sex, the trends of elevated renal markers were similar. These findings suggest that exposure to polystyrene microplastics may adversely affects renal functional capacity even at low doses.

Keywords

Kidney, Liver, Microplastics, Polystyrene, Styrofoam

1. Introduction

Plastic pollution has emerged as a pressing environmental issue, raising concerns about its potential adverse impacts on marine ecosystems, wildlife, and public health. Despite global efforts, the prevalence of particulate plastics—ranging from small fragments to beads, spanning sizes from 5 mm down to the nanometer range—continues to increase in the environment [1, 2]. Highlighting the severity of the issue, the second United Nations conference on the environment in Rio de Janeiro, Brazil in 2015 identified microplastic pollution as one of the foremost scientific challenges in the field of envi-

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ronment and ecology [3].

The convergence of public health and environmental concerns underscores the importance of investigating the potential effects of microplastic exposure (MPs—fragments ≤ 5 mm) on human health, which presents a significant challenge for the future. Of particular concern are micro-sized plastic particulates, given their potential interaction with living organisms, their propensity to traverse biological barriers, accumulate in organs, and disrupt cellular function, leading to systemic exposure [8, 9].

Compounding the issue is the argument that microplastic contamination in terrestrial environments may be 4 to 23 times greater than in marine environments [10]. Moreover, mammals at high trophic levels are regularly exposed to microplastics through multiple routes, including drinking water, inhalation, and the food chain, highlighting the widespread nature of the issue [11, 12].

The liver serves as the primary target for ingested xenobiotics, playing a crucial role in their metabolism [13]. Conversely, the kidney is susceptible to these foreign chemicals due to its filtration of a substantial volume of toxins, which can accumulate in the kidney tubules, leading to nephrotoxicity as blood passes through [14]. Microplastics (MPs) pose a significant threat to these vital organs, potentially impairing the body's ability to eliminate waste, maintain fluid and electrolyte balance, and produce essential hormones.

Therefore, there is an urgent need for toxicity studies on the accumulation of MPs in mammalian models to assess the potential risks they may pose to human health. This study aimed to address this gap by investigating the effects of polystyrene microplastic exposure on the hepatic and renal function of albino Wistar rats. The results obtained from this study provide valuable insights into the potential health hazards associated with exposure to microscopic plastic particulates, highlighting the importance of understanding and mitigating the risks posed by microplastic pollution to mammals, including humans.

2. Material and Methods

2.1. Preparation of Polystyrene Microplastics (PS-MPs)

Polystyrene microplastics (pristine polystyrene pellets and Styrofoam plate) were obtained from chemical market Aba, Abia State, Nigeria. The pristine polystyrene pellets and Styrofoam plates were respectively crushed to obtain less than 5mm particles which are ingestible by organisms [15].

2.2. Animals and Experimental Design

Adult male and female albino rats weighing between 120 and 150 grams were procured from the Animal House Unit in the Department of Biochemistry at the University of Port Harcourt, Nigeria. Upon arrival, the animals underwent a seven-day acclimatization period during which they were provided with ad libitum access to feed and water. They were housed in well-ventilated cages maintained at room temperature (28 °–30 ℃) and subjected to controlled light cycles (12 hours light/12 hours dark).

All experimental procedures involving animals were conducted in accordance with the guidelines set forth by the Institutional Animal Care and Use Committee at the University of Port Harcourt, Rivers, Nigeria, and adhered to the National Institute of Health Guide for the Care and Use of Laboratory Animals.

The rats were randomly assigned to one of seven experimental groups, each comprising ten rats (five males and five females), housed separately in individual cages.

Group 1: Control group, consisting of animals fed with standard rat feed and distilled water only.

Group 2: Rats fed with standard rat feed supplemented with 1% polystyrene pellets (PSP).

Group 3: Rats fed with standard rat feed supplemented with 5% polystyrene pellets (PSP).

Group 4: Rats fed with standard rat feed supplemented with 10% polystyrene pellets (PSP).

Group 5: Rats fed with standard rat feed supplemented with 1% processed polystyrene plate (FP).

Group 6: Rats fed with standard rat feed supplemented with 5% processed polystyrene plate (FP).

Group 7: Rats fed with standard rat feed supplemented with 10% processed polystyrene plate (FP).

2.3. Preparation of Blood for Biochemical Analyses

The experiment lasted for 90 days, on day 91st; the animals were sacrificed 24 hours after the last dose. Blood samples were collected and allowed to coagulate at room temperature. The clear, non-haemolysed supernatant sera were collected using Pasteur pipette after centrifugation at 3000rpm and stored at -20 ℃ for subsequent analysis [16].

2.4. Biochemical Parameters Determination

The activities of aspartate transaminase AST, alanine transaminase ALT, alkaline phosphatase ALP, gamma glutamyl transferase GGT and total protein were determined by using a chemistry auto-analyzer (LISA 200, France). Serum albumin concentration was determined according to Doumas et al. [17] while the method of Evelyn and Malloy [18] was used to determine the serum bilirubin content of the samples. The concentration of creatinine was determined using the method of Tietz et al. [19] while the method of Kaplan [20] was employed to determine the serum urea concentration. Serum electrolytes concentrations were measured using the flame photometer as described by Bassir [21].

2.5. Statistical Analysis

All data were subjected to statistical analysis. Values were reported as mean ± standard deviation (SD), while One-Way ANOVA was used to test for significance using statistical product service solution (SPSS). The results were considered significant at values (P) less than 0.05, which is 95% confidence level ($p < 0.05$).

3. Results

3.1. Effects of Microplastics Exposure on AST, ALT, ALP Activities and Total Protein

The effects of microplastics exposure on the liver transaminases, alkaline phosphatase and total protein levels of female and male rats are depicted in Figures 1 and 2. Serum aspartate transferase (AST) in the female rats exposed to varying levels of PSP and FP were significantly ($p<0.05$) decreased when compared with the control whereas alanine aminotransferase (ALT) showed significant ($p<0.05$) increase in 10% PSP exposure female rat group and insignificant ($p>0.05$) increase in other PSP and FP exposure groups compared to control. Similarly, significant ($p<0.05$) decreases in AST levels of all male exposure groups except in 5% PSP exposure were recorded when compared to normal control group. Alkaline phosphatase (ALP) showed no significant ($p>0.05$) change in all exposure groups compared to the control. A different trend was observed when ALP activity in the male rats exposed to 10% FP was compared with normal control. Total protein (TP) showed no significant ($p>0.05$) change in female and male microplastics exposure groups compared to the control.

3.2. Effects of Microplastics Exposure on ALB, Conjugated Bilirubin (CB), Total Bilirubin and GGT Activity

Figure 3 and 4 respectively show the effects of microplastics exposure on serum ALB, GGT and bilirubin levels in female and male rats. Exposing female rats to 1, 5, 10 % PSP and FP resulted in significantly ($p<0.05$) decreased conjugated and total bilirubin levels compared with the control animals. ALB recorded decrease in 5% PSP exposure female rat group while the other PSP and FP exposure female rat groups had no significant ($p>0.05$) difference compared to the control. Gamma-glutamyl transferase (GGT) showed no significant ($p>0.05$) change in all exposure female groups compared to the control. CB reduced significantly ($p<0.05$) in 10% FP exposure male group when compared with the control animals whereas similar to the trend observed in the female rat groups, GGT in the male rats showed no significant ($p>0.05$) change in the 1, 5, 10% PSP and FP exposure groups compared to the control.

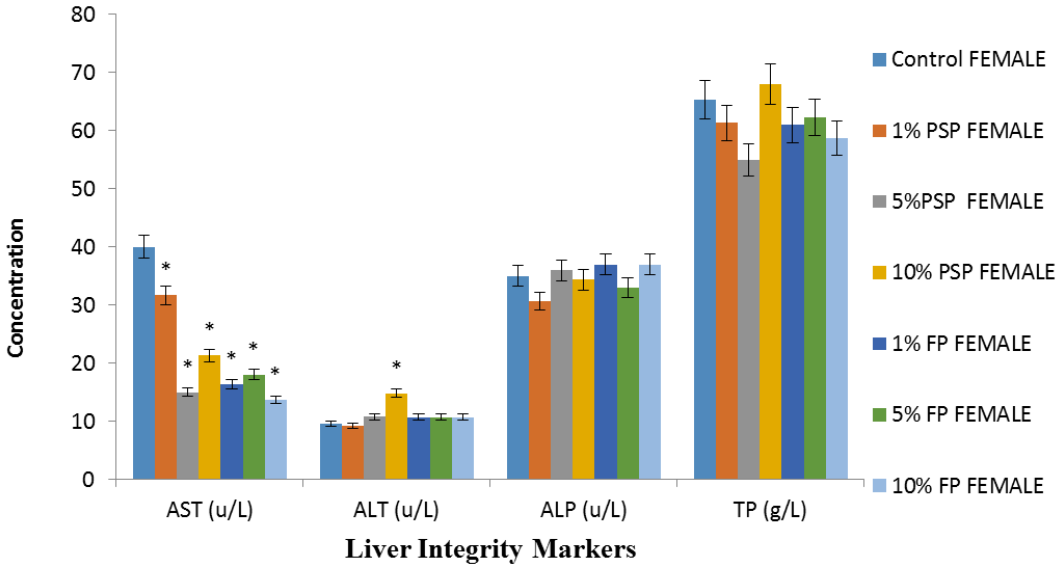

Figure 1. *Effects of polystyrene exposure on serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total protein (TP) of Female Wistar rats.*

Values are Mean ± SD, n=5.
*Values are significantly different from the normal control group at ($p<0.05$)
PSP = Polystyrene pellets, FP = Styrofoam plate

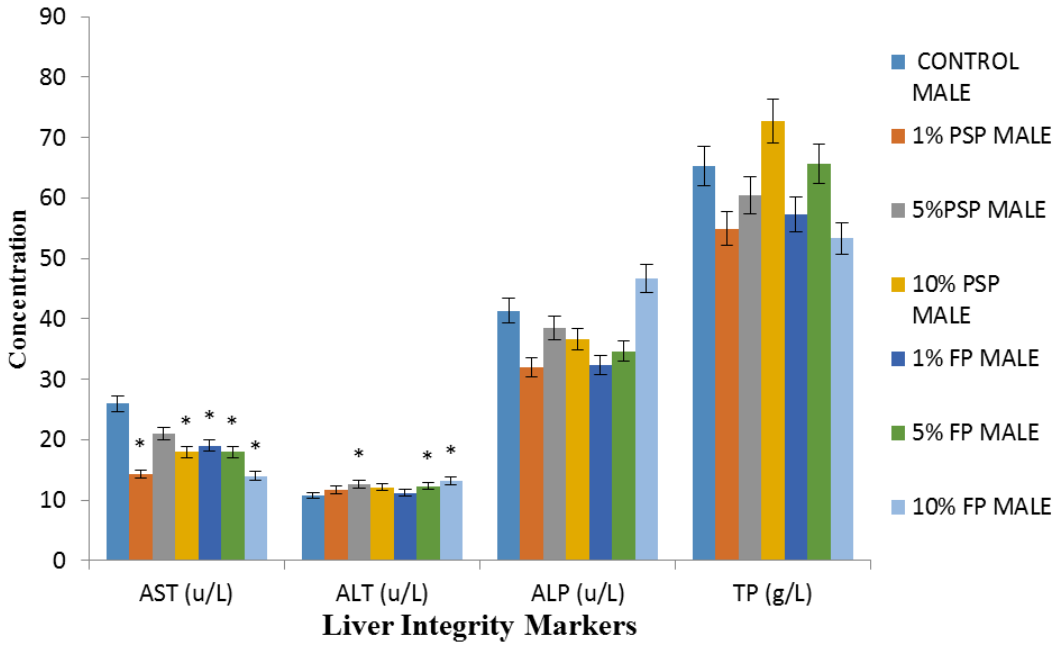

Figure 2. *Effects of polystyrene exposure on serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total protein (TP) of Male Wistar rats.*

Values are Mean ± SD, n=5.
*Values are significantly different from the normal control group at ($p<0.05$)
PSP = Polystyrene pellets, FP = Styrofoam plate

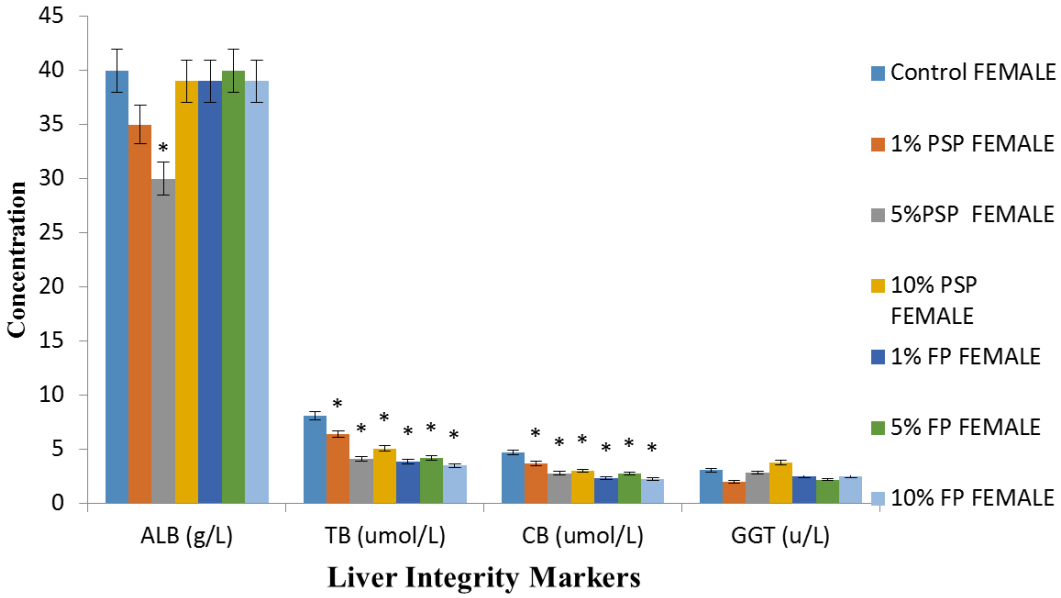

Figure 3. *Effects of polystyrene exposure on serum serum albumin (ALB), total bilirubin (TB), conjugated bilirubin (CB) and gamma-glutamyl transpeptidase (GGT) of Female Wistar rats.*

Values are Mean ± SD, n=5.
*Values are significantly different from the normal control group at ($p<0.05$)
PSP = Polystyrene pellets, FP = Styrofoam plate

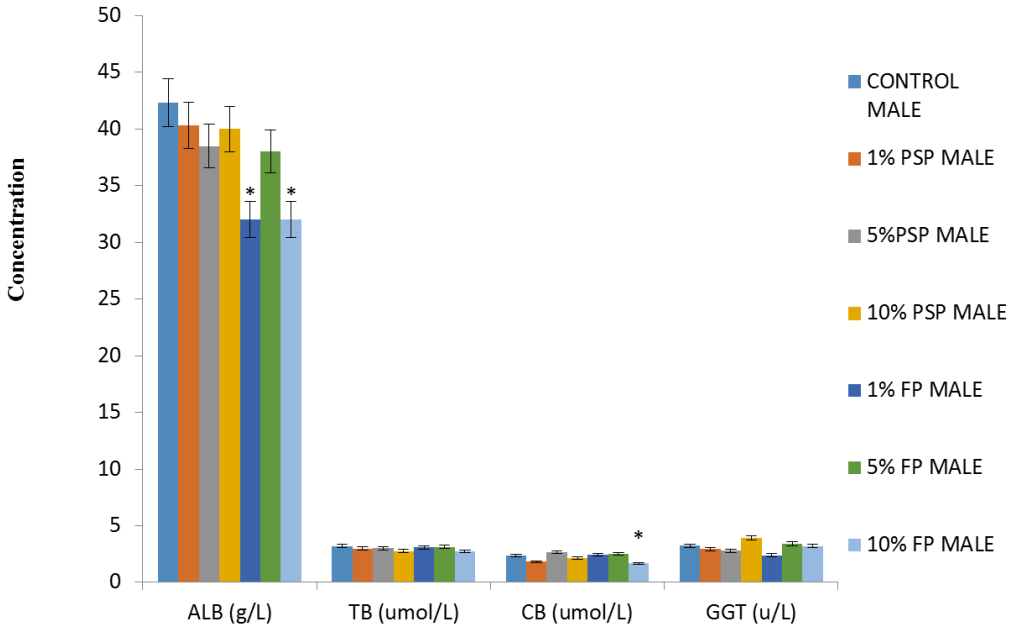

Figure 4. *Effects of polystyrene exposure on serum serum albumin (ALB), total bilirubin (TB), conjugated bilirubin (CB) and gamma-glutamyl transpeptidase (GGT) of Female Wistar rats.*

Values are Mean ± SD, n=5.
*Values are significantly different from the normal control group at ($p<0.05$)
PSP = Polystyrene pellets, FP = Styrofoam plate

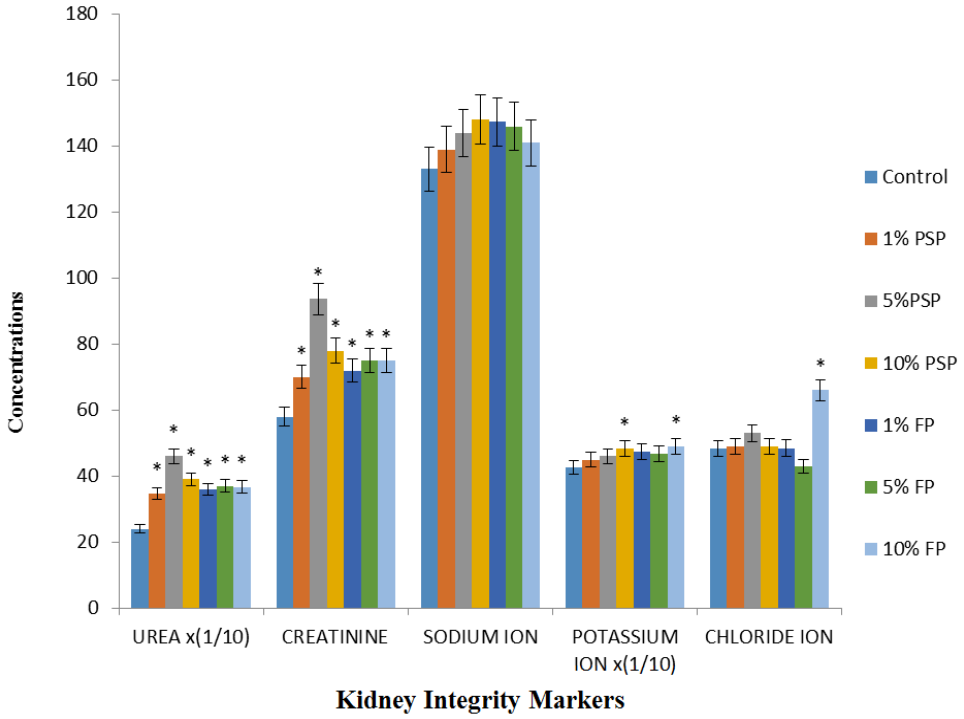

Figure 5. *Concentrations of serum urea, creatinine and electrolytes of female Wistar rat exposed to different percentage of polystyrene MP compared to the control.*

Values are Mean ± SD, n=5.
*Values are significantly different from the normal control group at ($p<0.05$)
PSP = Polystyrene pellets, FP = Styrofoam plate

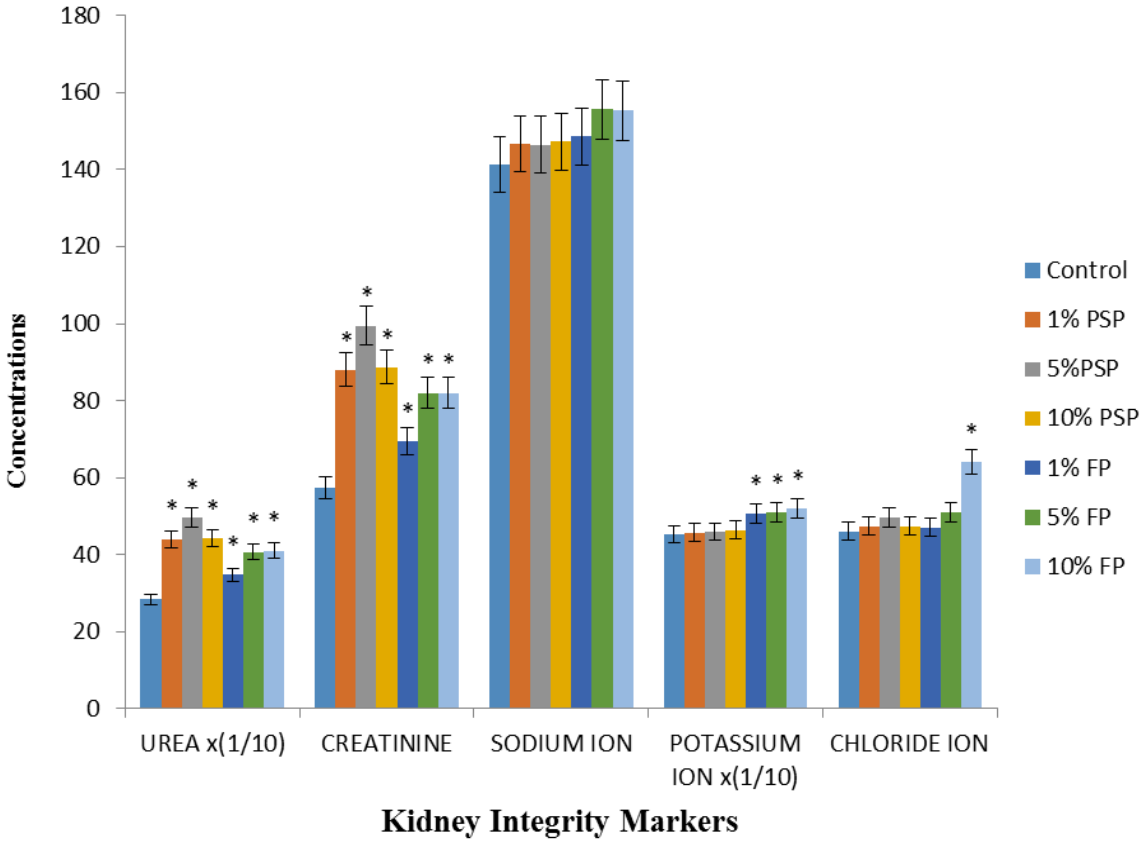

Figure 6. *Concentrations of serum urea, creatinine and electrolytes of male Wistar rat exposed to different percentage of polystyrene MP compared to the control.*

Values are Mean ± SD, n=5.
*Values are significantly different from the normal control group at ($p<0.05$)
PSP = Polystyrene pellets, FP = Styrofoam plate

3.3. Effects of Microplastics Exposure on Serum Urea, Creatinine and Electrolytes

The effects of polystyrene microplastics PSP and FP on serum urea, creatinine and electrolytes of male and female rats are respectively presented in Figures 5 and 6. A significant ($P<0.05$) increase in serum urea concentration associated with a corresponding increase in the levels of creatinine levels of female rats in all exposure groups compared to the control. A similar trend was also observed in the male rats. Serum sodium (Na^+) showed non-significant ($P>0.05$) increase in male and female rats from PSP and FP exposure groups compared to the control. Potassium (K^+) showed significant ($P<0.05$) increase in female rats exposed to 10% PSP and 10% FP compared to control while Cl^- showed significant ($P<0.05$) increase only in 10% FP exposure groups compared to the control (Figure 2a). K^+ levels in the male rats exposed to 1% FP, 5% FP and 10% FP were significantly ($P<0.05$) elevated compared to control.

3.4. Histological Examination of Liver and Kidney Sections

Histological examinations revealed that microplastics-exposure in the female and male rats did not result in severe hepatic damage as indicated by intact hepatocytes and normal portal triads (Figures 7 and 8). The FP-treated female rats however showed renal damage in the degree of various distortion patterns presented in (Figure 9). Similarly, microplastics exposure resulted in renal damage associated with infiltration of interstitial tissues with inflammatory cells (Figure 10).

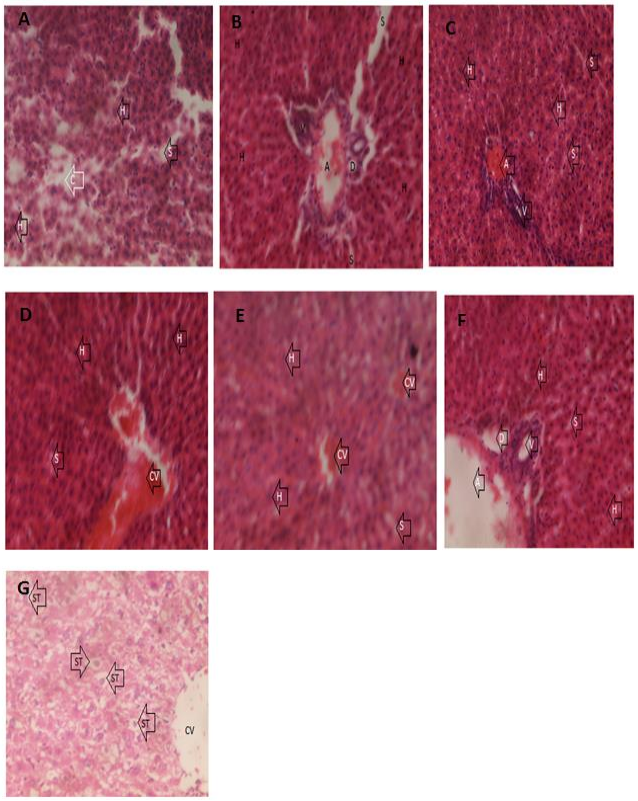

Figure 7. *(A-G) Histological assessment of female rat liver sections stained with hematoxylin and eosin (x400). (A) Group 1 (Control): showing no lesions or abnormality; (B) Group 2 (1% PSP): showing normal hepatocytes; (C) Group 3 (5% PSP): showing intact hepatocytes (H) and sinusoids (S); (D) Group 4 (10% PSP): showing congested central vein, intact hepatocytes (H) and sinusoids (S); (E) Group 5 (1% FP): showing intact hepatocytes (H) and sinusoids (S); (F) Group 6 (5% FP): showing intact hepatocytes (H) and sinusoids (S); (G) Group 7 (10% PSP): showing intact hepatocytes (H) radiating away from central vein (CV) and sinusoids (S).*

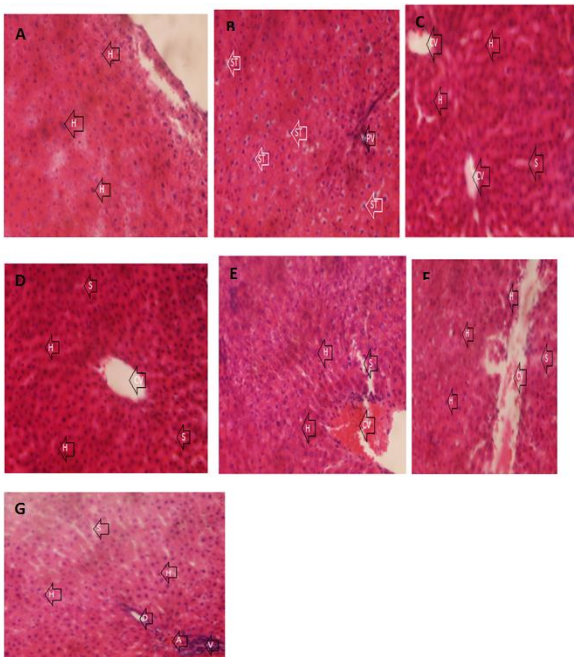

Figure 8. *(A-G) Histological assessment of male rat liver sections stained with hematoxylin and eosin (x400). (A) Group 1 (Control): showing histologically normal liver with intact hepatocytes; (B) Group 2 (1% PSP): showing mildly distorted liver with microvesicular steatosis; (C) Group 3 (5% PSP): showing intact hepatocytes (H) and sinusoids (S); (D) Group 4 (10% PSP): showing patent central vein, intact hepatocytes (H) and sinusoids (S); (E) Group 5 (1% FP): showing intact hepatocytes (H) and sinusoids (S); (F) Group 6 (5% FP): showing congested central vein, intact hepatocytes (H) and sinusoids (S); (G) Group 7 (10% PSP): showing intact hepatocytes (H), sinusoids (S) and portal triad (hepatic artery (A), Portal vein (V) and bile duct (D)).*

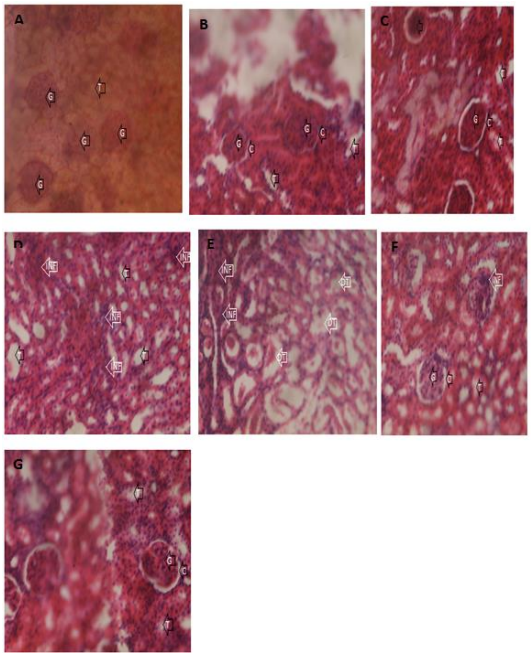

Figure 9. *(A-G) Histological assessment of female rat kidney sections stained with hematoxylin and eosin (x400). (A) Group 1 (Control): showing no lesions or abnormality; (B) Group 2 (1% PSP): showing normal kidney with intact Glomeruli (G) and patent Bowman's capsule (C); (C) Group 3 (5% PSP): showing intact kidney glomerulus (G) and Bowman's capsule (C); (D) Group 4 (10% PSP): showing distorted kidney showing interstitial tissues filled with inflammatory cells (INF); (E) Group 5 (1% FP): showing distorted kidney with damaged renal tubule (DT); (F) Group 6 (5% FP): showing distorted kidney with interstitial tissues filled with inflammatory cells; (G) Group 7 (10% PSP): showing distorted kidney.*

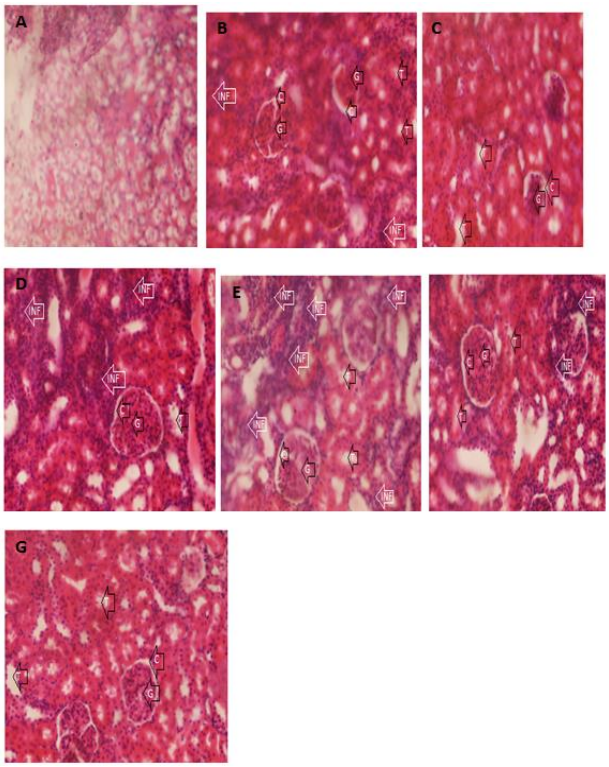

Figure 10. *(A-G) Histological assessment of male rat kidney sections stained with hematoxylin and eosin (x400). (A) Group 1 (Control): showing no lesions or abnormality; (B) Group 2 (1% PSP): showing normal kidney with intact Glomeruli (G) and patent Bowman's capsule (C); (C) Group 3 (5% PSP): showing normal kidney glomerulus (G) and Bowman's capsule (C); (D) Group 4 (10% PSP): showing distorted kidney showing interstitial tissues filled with inflammatory cells (INF); (E) Group 5 (1% FP): showing kidney with interstitial tissues filled with inflammatory cells (INF); (F) Group 6 (5% FP): showing distorted kidney with interstitial tissues filled with inflammatory cells; (G) Group 7 (10% PSP): showing distorted kidney.*

4. Discussion

Microplastics, synthetic particles with sizes less than 5 mm, have become pervasive environmental contaminants, entering mammals through direct ingestion, inhalation, and dermal contact, and accumulating in various organs over time [22]. The intracellular accumulation of microplastics has been associated with oxidative stress in exposed organisms, as demonstrated in previous studies [23, 24, 27].

Our investigation focused on evaluating the impact of dietary exposure to polystyrene microplastics at concentrations of 1%, 5%, and 10% on hepatic and renal function in both male and female albino Wistar rats. Surprisingly, our data revealed no significant impairment in liver function at these concentrations. This contrasts with the findings of Moos et al. [25], who reported hepatocyte dysfunction in rat models exposed to polyethylene microplastics. The observed disparity may be attributed to variations in the nature and dosage of microplastic exposure, with our study employing lower dietary doses.

Histological analysis provided further insights, indicating that despite mild alterations in albumin and total protein levels in specific groups, microplastics did not compromise the structural integrity of hepatocytes in the male and female rats. Enzymatic activity assessment of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) suggested no serious hepatocellular damage. Decreased AST levels hinted at minimal enzyme leakage into the bloodstream. Liver enzymes activities are usually raised in acute hepatoxicity [26]. Moreover, there was no evidence of hepatobiliary obstruction, as ALP and GGT levels remained within normal ranges in both male and female rats.

Urea and creatinine, metabolic waste products excreted by the kidney, play pivotal roles in maintaining the body's homeostasis by regulating electrolyte balance in the renal tubules [28]. These non-protein nitrogenous metabolites undergo clearance via glomerular filtration, making the assessment of serum urea, creatinine, and electrolytes (such as Na+, K+, Cl-) crucial biochemical markers for diagnosing renal failure and damage [29]. In this study, exposure to polystyrene resulted in a significant increase in urea and creatinine levels compared to the control group. This finding suggests that polystyrene exposure may disrupt the urea cycle. Creatinine, produced endogenously in muscle through a non-enzymatic reaction with creatine phosphate, is closely related to muscle mass. Its serum level serves as an indicator of glomerular filtration rate, as creatinine is readily filtered and experiences minimal tubular reabsorption [30]. The significant increase in creatinine on exposure to polystyrene MP may have resulted from glomerular inflammation and interstitial nephritis [31], though the exact mechanism was not covered in this study. Histopathological assessment of the kidney of both male and female rats further confirmed renal dysfunction as indicated by the presence of inflammatory infiltrates in interstitial tissues following dietary exposure to polystyrene microplastics. Regardless of the sex of the animals in the study, the trends of elevated renal markers were similar.

The body's fluid compartments, including both extracellular and intracellular fluids, contain inorganic electrolytes. These electrolytes, in their dissociated forms, play a crucial role in facilitating the movement of water and ions between the various body fluid compartments [32, 33]. In this study, higher doses of microplastic exposure (10% PSP and 10% FP) led to increased potassium concentration in female rats while FP exposure caused significant increase in potassium concentration in male rats suggesting hyperkalemia. Chloride concentrations were significantly increased when compared to the control on exposure to 10% FP in male and female rats hence leading to hyperchloremia. Kang et al. [34] reported that hyperkalemia and hyperchloremia are rare but do occur when there is loss of body fluids containing less potassium and chloride respectively than plasma along with water intake restriction or if there is excessive potassium and chloride intake with limited liquid intake respectively. Nduka [35] concluded that hyperkalemia and hyperchloremia almost always indicates water depletion. Since water was not however restricted in this study, the elevated potassium and chloride levels in the rats may be attributed to the inability of the kidneys to excrete the electrolytes from the tubular fluid.

5. Conclusion

In conclusion, our findings provide valuable insights into the impact of polystyrene microplastic exposure on hepatic and renal function in albino male and female Wistar rats. Further research is warranted to elucidate the underlying mechanisms and potential long-term consequences of these observed effects, emphasizing the urgency of addressing the growing concerns surrounding microplastic pollution in our environment.

Abbreviations

PS-MP	Polystyrene Microplastics
PSP	Polystyrene Pellets
FP	Processed Polystyrene Plates
TP	Total Protein
AST	Aspartate Aminotransferase
ALB	Albumin
TB	Total Bilirubin
CB	Conjugated Bilirubin
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
GGT	Gamma Glutamyl Transferase

Author Contributions

Chinedu Joseph Okonkwo: Conceptualization, Validation, Supervision, Methodology, Project Administration

Udoka Chukwudubem Nnoruka: Writing - Original Draft Preparation, Project Administration

Chioma Joy Okonkwo: Conceptualization, Validation, Writing - Original Draft Preparation, Review and Editing

Ifenna Ilechukwu: Conceptualization, Methodology

Donatus Chuka Belonwu: Conceptualization, Supervision

Ethical Consideration

All animal experiments were approved by the Institutional Animal Care and Use Committee at the University of Port Harcourt, Choba, Nigeria, and all experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Statement of Human and Animal Right

Respecting the principles of ethical treatment of animals, this study exclusively utilized albino Wistar rats. The research protocol, focusing on the welfare of the animal subjects, received approval from the University of Port Harcourt Institutional Animal Care and Use Committee, ensuring adherence to established guidelines for the humane treatment of animals in research.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

Data Availability Statement

The data supporting the results of this study are available upon request to the corresponding author.

Conflict of Interest

The authors declare no conflicts of interest.

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