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EVALUATION OF URINARY AND SERUM RENAL BIOMARKERS FOLLOWING ACUTE MICROPLASTIC EXPOSURE IN MALE RATS

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ABSTRACT

Microplastic particles (MPs) smaller than 5 mm are ubiquitous environmental pollutants, with concerning potential impacts on human health. This study focused on early changes in renal function following a single oral dose (35 mg/kg) of mechanically generated irregular polyethylene terephthalate (PET) MPs of two different sizes (~41 μm and ~106 μm) in male *Wistar* rats. Renal function was evaluated 24 h post-exposure using serum biochemical parameters and urinalysis. Serum urea and creatinine were measured as indicators of glomerular filtration, while urinalysis assessed the presence of blood and leukocytes. Exposure to MPs resulted in a significant elevation of serum urea and creatinine levels compared with controls, but no blood or leukocytes were present in urine. These findings indicate that acute exposure to MPs may induce early functional changes in the kidney before overt signs of urinary tract injury and inflammation become apparent, suggesting early physiological responses.

INTRODUCTION

Plastic materials have widespread industrial benefits, but have recently come to light as a serious pollutant with potential risk to the ecosystem and human health. The term microplastics is used to describe polymeric matrices or synthetic solid particles of regular or irregular shape that can be categorized as either primary or secondary microplastics [1]. Microplastic particles (MPs) range in size from 1 μm to 5 mm, and are insoluble in water. They have been detected in a wide array of shapes and polymeric structures, and their distribution is vast - from drinking and marine water, agroecosystems and foods, to the atmosphere [1].

Consequently, human exposure to MPs is mainly through ingestion, inhalation or dermal contact. Multiple studies have reported accumulation of MPs in

different organs and tissues, as well as their cellular uptake, raising concerns about possible adverse biological effects of these polymers [2-3].

Although the kidney represents a crucial organ for the elimination of xenobiotics, information on its susceptibility to acute MPs exposure remains limited [4]. Due to the valuable role of the kidneys in the maintenance of overall physiological homeostasis, the need for assessment of renal function in the context of MPs exposure is a crucial part of any toxicological and biomedical research [5]. This is most commonly done using serum biomarkers such as urea and creatinine, while urinalysis parameters including the presence of blood and leukocytes can be additional indicators of structural damage and inflammation.

Accordingly, this study aimed to assess the effects of a single dose of MPs on renal function in male *Wistar* rats using serum biomarkers and urinalysis 24 h post - exposure.

EXPERIMENTAL

MPs were prepared from polyethylene terephthalate (PET) bottles that were mechanically filed, as previously described [6].

The approved experiments (protocol authorization number 323-07-03460/2021-05) were performed on young adult male *Wistar* rats (n = 12; body weight 300 - 350 g) maintained under standard laboratory conditions. The animals were randomly assigned into three experimental groups: the control group (Q), which received 2.5 mL of Milli-Q water; and two groups treated with different sizes of MPs dispersed in 2.5 mL of Milli-Q, in sizes of 41 μm (S) or 106 μm (L), both at doses of 35 mg/kg. All treatments were administered as a single dose by oral gavage. Urine samples were collected prior to sacrifice, 24 h post - exposure. Analysis was performed using visual colorimetric comparison with commercial reagent test strips (Insight Urinalysis Reagent Strips, Acon Laboratories, USA). The parameters assessed included hematuria, the presence of blood, and the presence of leukocytes, both monitored semi-quantitatively according to the manufacturer's colorimetric scale. Following trunk blood collection and clotting at room temperature, the samples were centrifuged (3000 rpm for 15 min utilizing Heraeus Labofuge GL (Heraeus, Germany)) to obtain serum, which was used for biochemical analysis. Renal function was evaluated by measuring urea and creatinine levels using standard enzymatic colorimetric assays, as indicators of glomerular filtration capacity.

Data, presented as mean ± SEM relative to the control, were analyzed using one-way ANOVA followed by appropriate post hoc tests for multiple comparisons vs. the control group in GraphPad Prism software (version 8.02, USA). Differences were considered statistically significant at $p < 0.05$. Urinary blood and leukocyte presence was assessed semi - quantitatively and analyzed descriptively.

RESULTS AND DISCUSSION

To assess the potential effect on renal physiological functions, we measured the serum levels of urea and creatinine. As shown in Figure 1., serum levels of urea were significantly elevated in both MPs groups compared to control ($p < 0.05$ for S and L vs. Q), suggesting that PET particles of both sizes similarly affect their clearance and metabolic processes associated with kidney function. A significant increase was also detected in creatinine levels compared to the control group ($p < 0.05$ for S and L vs. Q), further supporting an alteration in renal filtration - related processes. Together, these changes indicate an early functional response of the kidney following acute PET MPs exposure.

Table 1. Effects of acute oral exposure to polyethylene terephthalate microplastics on the presence of blood and leukocytes in urine of male *Wistar* rats 24 h after treatment. Data are presented descriptively (– indicates no blood or leukocytes detected).

Groups / Parameters	Q	S	L
Hematuria	–	–	–
Leukocytes	–	–	–

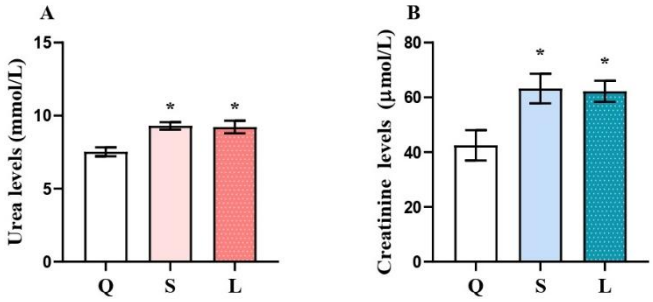


Figure 1. Effects of acute oral exposure to polyethylene terephthalate microplastics on serum A) urea and B) creatinine levels in male *Wistar* rats 24 h after treatment. Data are presented as the mean ± SEM relative to the control (*$p < 0.05$).

As shown in Table 1., neither hematuria nor leukocytes were present in the urine of experimental groups. This absence indicates that acute PET MPs exposure did not result in detectable urinary tract injury or inflammatory changes within the investigated time frame.

Taken together, the observed changes in serum renal biomarkers accompanied by the absence of urinary abnormalities suggest that acute PET MPs exposure primarily affects renal physiological processes during the early phase after exposure. The combined assessment of serum and urinary parameters provides a broader view of renal responses and indicates that functional alterations may occur before detectable manifestations of renal injury.

CONCLUSION

Acute oral exposure to PET MPs results in early alterations of renal - related parameters in male Wistar rats, highlighting the rapid physiological response of the kidney following microplastic intake. These findings provide further insight into the short - term effects of microplastic exposure and support the need for continued investigation of their biological consequences.

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