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A PILOT STUDY OF THE EFFECT OF POLYSTYRENE MICROPLASTICS ON OXIDATIVE STATUS IN MICE

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Microplastics environmental pollution and their impact on organisms' health are currently widely debated. This study aimed to determine the effects of polystyrene microplastics (PS–MPs) on lipid peroxidation (LPO) and glutathione (GSH) levels in the brain, liver, ovaries/ testes, kidneys, and lungs of mice. Sexually mature male and female albino mice were divided into control groups and groups exposed to 1 μm PS–MPs at a dose of 0.1 mg/24h, administered orally for 14 days. The results showed that in female mice PS–MPs administration led to significant increase of LPO in the kidneys and lungs. In contrast, in male mice, LPO was significantly decreased in the brain, liver, and kidneys. PS–MPs administration also led to significantly increased GSH concentrations in the liver in both male and female mice, and a decrease in the brain and testes in males. In conclusion, PS–MPs induced varying degrees of oxidative stress in male and female mice.

Key words: polystyrene microplastics, oxidative stress, organs, mice.

Introduction

Plastic production is steadily increasing globally with a large part of already–used plastics ending up in the environment as pollutants (Issifu et Sumaila, 2020; Lau *et al.*, 2020; Yan *et al.*, 2024). One of the most widespread plastics in the world is polystyrene (PS) (Wagner *et al.*, 2014; Sen *et al.*, 2018; Gelbke *et al.*, 2019). It is widely used in personal care products, thermal insulation, food packaging, and meat/poultry trays (Sharma *et al.*, 2023; Rybak *et al.*, 2023). The fragmentation of larger plastic objects caused by various weathering effects leads to the formation of smaller particles in different shapes (Gigault *et al.*, 2018; Anagnosti *et al.*, 2020). Those with sizes of 100 nm–5 mm are referred to as microplastics (MPs) and those <100 nm as nanoplastics (NPs) (Avio *et al.*, 2017). The incorporation of MPs into various products such as textiles, car tires, artificial surfaces, exfoliants in cosmetics, toothpaste, etc., further increases their distribution in the environment (Anik *et al.*, 2021). The presence of MPs and NPs has been found in air, soils, rivers, lakes, and marine environments worldwide (Zhang *et al.*, 2020; Landrigan *et al.*, 2023). Pollution with MPs is a growing environmental problem. Due to their small size, they become a serious problem, as they can get into and accumulate in the body of organisms (plants, animals, and humans). The main routes of exposure to these particles in living organisms are through air, food, and water (Lett *et al.*, 2021; Salthammer, 2022). The accumulation of MPs in tissues can have various side effects on health, such as growth or reproduction problems, oxidative stress, inflammation, physical stress, weakened immunity, histological damage, or even death (Avio *et al.*, 2015; Ferreira *et al.*, 2019; Li *et al.*, 2020). Some studies found that the damage caused by the absorption of MPs was largely induced by oxidative stress (OS) (Hu et Palić, 2020; Ding *et al.*, 2023). The OS effects caused by

MPs involve not only those by primary generated reactive oxygen species (ROS) but also by their subsequent generation in cells and tissues. Upon absorption of MPs, the integrity of the cell membrane is disrupted, the lipid bilayer is altered, pores are formed, and the generation of intracellular ROS is increased. In turn, ROS generation leads to mitochondrial dysfunction, the release of pro–inflammatory cytokines, and cell damage (Jeong *et al.*, 2016; Cui *et al.*, 2023). In recent years, research on the effect of MPs on the function of various organs through OS has been intensified. However, there is still no definitive data on the harmful impact of MPs in various organs and tissues. The gender differences in OS response to MPs exposure has not been thoroughly investigated. Therefore, this experimental study aimed to assess the effect of polystyrene microplastics (PS–MPs) on oxidative status, as measured by lipid peroxidation (LPO) and total glutathione (GSH) levels, in male and female mice organs.

Materials and methods

Experimental animals

A total of 24 sexually mature male and female SWISS albino mice, aged 3 months, with body weights of 30–35 g for females and 40–45 g for males, were used in the study. The mice were housed under controlled laboratory conditions (22 ± 1°C, 50 ± 5% humidity, and a 12–hour light/dark cycle). They were kept in cages with free access to food and water, under the experimental protocol and the requirements of the European Communities Council Directive (86/609/EEC). The animals were obtained from a specialized breeding facility, Experimental breeding base for experimental animals (EBBEA), Slivnitsa, Bulgaria. The study was approved by the ethics committee.

Experimental design

The mice were divided into four groups of 6 animals per group: 1) Control ♀, 2) PS–MPs ♀, 3) Control ♂, and 4) PS–MPs ♂. The control groups (male and female) received purified water, while the experimental groups (male and female) were given water containing 1 μm PS–MPs at a dose of 0.1 mg per 24 hours for 14 days. Every three days, the body weight of the experimental animals and the amount of water consumed were recorded.

Methods

Tissue preparations

At the end of the experiment, the animals under ketamine/xylazine narcosis were decapitated, and the studied organs – brain, liver, ovaries, **t**estes, kidneys, and lungs, were dissected and immediately frozen at –25°C for later biochemical analysis. After thawing, the tissue samples were homogenized using a Teflon pestle in 0.15 M KCl and 10 mM potassium phosphate buffer (pH 7.4). The homogenates were centrifuged at 3000 rpm for 10 minutes, and biochemical parameters (LPO and GSH) were measured spectrophotometrically in the resulting post–nuclear fraction.

Biochemical Analyzes

- ***Determination of protein content*** Protein content was conducted by the method of Lowry *et al.* 1951. Protein content was read at 700 nm, and determined using a calibration curve obtained with bovine serum albumin – Pentex USA.
- ***Lipid peroxidation* (LPO)** was measured with MDA Assay Kit, Cat. № MAK085, Sigma–Aldrich Co. LLC, USA

- ***Total glutathione*** **(GSH)** was determined with Assay Kit CS0260, Sigma–Aldrich Co. LLC, USA

Statistical analysis

Data analysis was performed using the Paired–Samples T–test to compare the control groups versus the PS–MPs–treated groups. The specialized package IBM SPSS Statistics, version 26 (SPSS Inc, Chicago, USA) was used. Results were presented as mean±SD in the tables. The accepted statistically significant differences were: *$p \leq 0.05$, ***$p \leq 0.001$

Results and discussion

Environmental pollution, including with MPs, can induce excess free radical production, and changes in non–enzymatic and enzymatic antioxidants, with oxidative stress (OS) induction (Ahmed *et al.*, 2018). PS–MPs have been found to cause OS in mice liver, kidney, and gut (Deng *et al.*, 2017). One of OS's most frequently studied markers is lipid peroxidation (LPO) since unsaturated fatty acids are highly susceptible to oxidative changes. The end–product of LPO, malondialdehyde (MDA) is an important parameter that can indirectly reflect the degree of oxidative tissue injury (Liu *et al.*, 2022). In our study, the oral exposure of mice to PS–MPs led to different patterns of OS in studied organs depending on the gender. In female mice, a statistically significant increase in LPO was observed in the kidneys and lungs, and an increase in the brain and liver, although statistically insignificant (Table 1). In male mice, the PS–MPs administration led to a significant decrease in LPO in the brain, liver, and kidney (Table 1). Published research demonstrated that the accumulation of MPs in tissues led to lipid metabolism disturbance with a notable reduction in total cholesterol and triglyceride levels (Deng *et al.*, 2017). In serum of mice treated with MPs, there was an increase in metabolites related to lipid metabolism, such as taurine, ethanol, and various lipids, while choline levels decreased (Wright *et al.*, 2013). Histological analysis showed a formation of lipid droplets in the liver of MPs–treated mice (Deng *et al.*, 2017; Lu *et al.*, 2018). Lipid droplets are usually seen under inflammatory conditions and serve as markers for inflammatory responses. The observed in this study different patterns of LPO in male and female mice may be a result of sex–dependent differences in the lipid profiles (Zhu *et al.*, 2023), which can be influenced by factors such as hormones, genetics, and age. The observed in this study, increase in LPO in testes after administration of PS–MP (although statistically insignificant) has also been reported by other authors (Fang *et al.*, 2024), who suggested that PS–MPs toxicity in male mice reproductive system was associated with activating spermatogonial mitochondrial oxidative stress and apoptosis.

Table 1: Levels of LPO (nmoles MDA/mg protein) in the studied organs after exposure of mice to 1 μm PS–MPs

Organs	female		male	
	Control ♀	PS-MPs ♀	Control ♂	PS-MPs ♂
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Brain	6.59±0.41	6.85±0.35	7.24±0.11	5.66±0.01***
Liver	1.57±0.40	2.06±0.34	2.59±0.06	1.56±0.04***
Ovaries/testes	3.47±1.55	3.00±0.78	3.50±0.25	4.10±0.10
Kidneys	1.05±0.05	1.35±0.04*	1.86±0.06	1.52±0.14*
Lungs	0.46±0.02	0.55±0.04*	1.00±0.01	0.41±0.01

*Note: *$p \leq 0.05$, ***$p \leq 0.001$*

Glutathione is one of the most important antioxidants involved in the antioxidant defense, interacting directly with ROS and indirectly as a coenzyme in essential antioxidant enzymes such

as glutathione peroxidases and transferases (Ramakrishnan *et al.,* 2017; Auguet *et al.,* 2022). The antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), together with GSH, are sensitive markers to assess early oxidative damage from environmental stressors, incl. MPs (Deng *et al.*, 2014). Our study indicated that the 1μm PS–MPs administration led to a significant increase in GSH concentration in the liver in both male and female mice after 14 days of intake (Table 2). This observation is in agreement with the results reported by Zou *et al.*, 2023 that after 5 μm and 0.5 μm PS–MPs exposure, the activities of antioxidant enzymes SOD and CAT, and GSH in the liver were highly significant compared with the controls. It is likely that under such treatment (1μm PS–MPs administration for 14 days) the antioxidant defense in the liver was activated to overcome the resulting OS. Glutathione is synthesized primarily in the liver, which is the body's main center for detoxification and antioxidant production. However, in all other studied organs in our experiment, GSH decreased especially statistically significant in male mice brain and testes (Table 2). Oxidative stress in tissues is often associated with reduced cellular antioxidants incl. overall GSH concentrations (Halliwell et Gutteridge, 2015). Specifically, it has been demonstrated that PS–MPs reduced the GSH level, mitochondrial membrane potential, endoplasmic reticulum calcium, and increased ROS in oocytes (Liu *et al.*, 2022).

Table 2: Levels of GSH (ng/mg protein) in the studied organs after exposure of mice to 1 μm PS–MPs

Organs	female		male	
	Control ♀	**PS-MPs ♀**	**Control ♂**	**PS-MPs ♂**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Brain	1287.73±254.8	1050.32±143.6	812.92±183.5	433.44±34.2*
Liver	2987.40±184.9	3409.59±135.7*	3762.57±424.5	4527.85±173.5*
Ovaries/testes	1050.99±315.6	802.42±145.4	388.12±5.4	183.50±7.6***
Kidneys	198.94±14.9	158.62±34.8	149.33±44.3	141.08±11.7
Lungs	228.59±50.2	213.80±59.1	297.58±61.3	227.63±19.7

*Note: *p ≤ 0.05, ***p ≤ 0.001*

Conclusion

In conclusion, the present pilot study demonstrated that the administration of PS–MPs of the same size, concentration, and treating period could induce a different degree and type of responses in terms of oxidative stress in male and female mice. However, further studies are needed to elucidate the specific role of oxidative stress in the mechanism of PS–MPs toxicity.

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