7th Workshop

SPECIFIC METHODS FOR FOOD SAFETY AND QUALITY

September 22nd 2021, Vinča Institute of Nuclear Sciences - National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia

PROCEEDINGS

SPECIFIC METHODS FOR FOOD SAFETY AND QUALITY

7th WORKSHOP: SPECIFIC METHODS FOR FOOD SAFETY AND QUALITY

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A SINGLE DOSE OF MICROPLASTIC PARTICLES INDUCES CHANGES IN ORGAN WEIGHT OF MALE WISTAR RATS

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ABSTRACT

Increased plastic usage creates environmental problems due to its accumulation and degradation into microplastic particles (MP), less than 5 mm in diameter. Particles from various sources like food and beverages contaminated with polyethylene terephthalate (PET) could accumulate in different tissues exerting adverse effects. Since data regarding potential acute toxicity in doses that represent realistic/daily ingested MP doses are entirely lacking, male Wistar rats were exposed to a single dose (1.4, 35 or 125 mg/kg) of PET-generated MP by oral gavage. Changes in wet organ weight to body weight ratio of testicles, adrenal glands and kidneys were examined 24 h following the treatment. Results point to increased organ/body weight ratio of testicles in rats exposed to two higher MP doses, while adrenal glands were augmented only after treatment with 125 mg/kg. Further research is needed to gain insight into the underlying mechanisms of the observed phenomenon.

INTRODUCTION

Excessive production, usage and discharge of various pollutants in our environment arose due to increased industrialization. Lately, particular attention has been drawn to plastic since large amounts of its waste have been accumulated in all ecosystems [1]. While this polymer is generally perceived to pose minimum risk to humans and other living beings, it is noticed that pieces smaller than 5 mm, generally termed as microplastic particles (MP), could greatly impact health. Humans could accumulate MP generated from polyethylene terephthalate (PET), present (due to contamination) in various sources such as marine food, salt, milk, tea, coffee, and drinking water [2]. Within the body, these particles could act as endocrine-disrupting chemicals mimicking activities of endogenous steroid hormones and interfering with

organs' structure and function [1]. For instance, chronic fishes' exposure to MP induces impacts to the endocrine system in a sex-dependent manner, increases cortisol levels, induces hyperactivity and initiates histological alterations in kidneys [2]. Up to date, there are no data regarding the effects of acute MP exposure on hormone-producing organs such as testicles, adrenal glands and kidneys of mammals.

The current study tested whether a single ingested dose of PET-originated MP, representing realistic/daily ingested dose in humans [3], induces a shift in the organ weight/body weight ratio (relative organ weight) of testicles adrenal glands and kidneys, 24 h following the application. The obtained data support the speculations of potential MP acute toxicity effects.

EXPERIMENTAL

Polyethylene terephthalate bottles of worldwide famous soft drink brand were thoroughly washed with distilled and Milli-Q water. After filing, produced MP sawdust was sifted through a laboratory sieve with a mesh size of 160 μm. Laser diffraction analysis showed that specimens are composed of MP with a median diameter of 85 μm (d10 = 35 μm and d90 = 170 μm).

For the purpose of the experiment, adult male Wistar rats (300 – 350 g, n= 4 per group) were randomly divided into five groups: intact animals (I); rats that received 2.5 ml of Milli-Q (Q); rats subjected to either 1.4 or 35 or 125 mg/kg of MP dissolved in 2.5 ml of Milli-Q (P1, P2, P3, respectively). The doses were calculated by multiplying human doses to correction factor (Km, 6.2 for rats) [3], [4]. All treatments were applied in a single dose by oral gavage.

24 h after treatment administration, rats were weighed and sacrificed. Testicles, kidneys and adrenal glands were carefully isolated and rinsed in phosphate-buffered saline (PBS). Excessive PBS was removed, and the organs were weighed. The relative organ weights were calculated as the ratio of organ (wet, mg) weight to body weight (g). Obtained data were analyzed by One-way analysis of variance followed by Tukey's *posthoc* test using GraphPad Prism 6 Software (San Diego, USA). Results are presented as a percentage of I group ± SEM. Results were considered significant at p value < 0.05.

All experimental procedures were approved by the Ethical Committee for the Use of Laboratory Animals of VINČA Institute of Nuclear Sciences - National Institute of the Republic of Serbia, University of Belgrade (the authorization number 323-07-03460/2021-05) and were in accordance with European Communities Council Directive (2010/63/EU).

RESULTS AND DISCUSSION

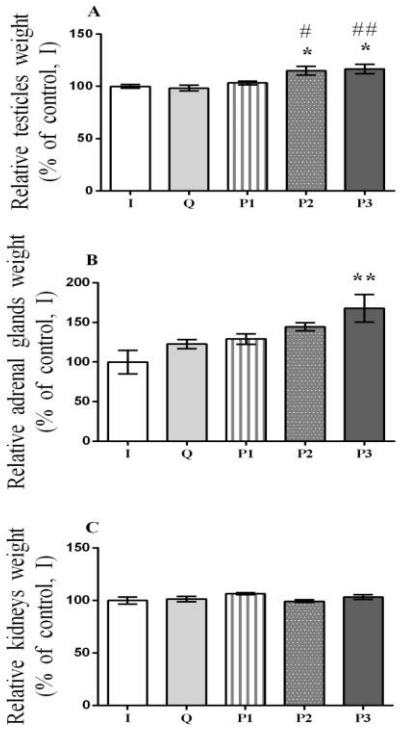

Figure 1. Relative organ weights of male rats 24 h after receiving a single dose of microplastic **A)** Testicles, **B)** Adrenal glands, and **C)** Kidneys. Data are presented as % of I. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test (* represents difference to I group, # represents difference to Q group).

Significant differences in organ weight between treated and control animals may occur in the absence of any morphological change, often preceding them. Thus, organ weight can be one of the indicators of substance toxicity [5]. In a current study, treatment with two higher MP doses induced an increase in the relative weight of testicles compared to both the I and Q groups ($p < 0.05$ for P2 and $p < 0.01$ for P3, respectively). At the same time, P3 animals showed an increase in the relative weight of adrenal glands regarding animals in the I group ($p < 0.01$ for P3) (Figure 1A and 1B).

Observed enlargement can generally reflect increased interstitial or tubule fluid content due to impairment of fluid flow in an organ and inflammation, etc. In opposite, no changes were observed in the relative weight of organs following MP treatments: in testicles upon 1.4 mg/kg MP treatment, in adrenal glands after 1.4 mg/kg or 35 mg/kg MP treatments, and all MP treated groups of kidneys. Such absences of changes following MP treatments suggest that either these organs are not affected by proposed doses of MP or have different sensitivity to MP (Figure 1C). Some other mechanisms that do not impact organ weight but damage its structure and function could be employed.

Since organs of interest in this study are hormone-producing, any substance that affects their structure and functions, such as MP, could induce overall endocrine system disbalance and might act as an endocrine chemical disruptor leading to a variety of disorders.

CONCLUSION

Based on the presented data, the concern should be raised regarding daily MP intake through food and beverages since even a single MP dose could impair the physiological structure and function of endocrine organs.

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