WEB OF SCIENTIST: INTERNATIONAL SCIENTIFIC RESEARCH JOURNAL
ISSN: 2776-0979, Volume 3, Issue 12, Dec., 2022

EFFECT OF POLYETHYLENE TEREPHTHALATE AS POLLUTANT OF DRINKING WATER ON SOME PHYSIOLOGICAL PARAMETERS IN MALE RATS

AbdulSalam Tawfeeq Dawood
Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq

Haitham Oudeh Mohammed Alhilfi
Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq

*Corresponding email: haitham.alhilfi@nahrainuniv.edu.iq

Munqith Abdulmaged Alwan
Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq

Salah Mahdi Mohsin Al Chalabi
Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq

Abstract

Polyethylene terephthalate (PET) utilized in fabrics, containers for packaging of food stuffs, water and other beverages. This polymer is recently participating in a wide-range of environmental pollution. The current study investigate the effect of polyethylene terephthalate (PET) as pollutant of drinking water on some physiological parameters in male rats, including liver and kidney enzymes (ALT, AST, Urea, Creatinine); Oxidative stress biomarkers (MDA, CAT, GSH); reproductive hormones (FSH, LH, Testosterone) and thyroid hormones (T3, T4, TSH). Total of 18 male albino rats were divided into three groups which kept along 60 days of the study: Control; GI (gavage daily 0.12 mg kg^{-1} b.wt of PET; and GII (Gavage daily 0.6 mg kg^{-1} b.wt of PET). The results showed a significant increase in the level of ALT, AST, Urea and Creatinine for both PET groups. Also, a significant increase ($p<0.05$) in the level of MDA, CAT and GSH were observed. On the other hand, there were significant decreases ($p<0.05$) in the level of FSH, LH and Testosterone in PET groups. Finally, significant decreases in T3, T4 and TSH hormones in PET groups were monitored.

Keywords: polyethylene terephthalate (PET), environmental, pollution, ALT, GSH, FSH

Introduction

People are subjected to inhale or ingest plastic microparticles (MPs) from air, food and drinking water. Whereas, 5 - 13 million tons of plastic waste enters the oceans every year, these consumed by aquatic animals such as fish and crustaceans, then passed into human diet (Jambeck et al., 2015; Ragusa et al., 2021).

It has been reported that human were routinely inhale polyethylene terephthalate microplastics (PET-MPs). The median intake of these MPs estimated to be 184 ng/person/ day for children and 583 ng/ person/day for adults. Also, MPs intake can irreversibly accumulate to 6.4 µg/ person by the age of 18 years, and 40.7 µg/ person by the age of 70 years. Moreover, people who drink only plastic bottled water may consume additional MPs per year compared with those who drink only tap water (Zhang et al., 2022).

Another concern is that Antimony (Sb), a heavy metals used in the production of PET, might transfer from PET containers into mineral water depending on the capacity, time and temperature (Chapa-Martinez et al., 2016; Dhaka et al., 2022). Moreover, the recycled PET are the most sources for catalysts of heavy metals especially Sb that considered a vital source for pollution (Whitt, et al. 2016). It was demonstrated that water stored in plastic bottles below 20°C was not exposed to Sb transfer (Dhaka et al., 2022). In contrast, at 40 °C temperature, a remarkable rise in the concentration of Sb was observed. However, the maximum limit of 5 µg L^{-1} provided by the European Union was not exceeded. Also, the Sb migration was seen in the samples stored at 60 °C for 30 days and was above the permissible limits of Sb (Carneado et al. 2015).

The effect of PET or its components on human or animal health seems to be dose and or exposure time dependent. It has been demonstrated that the uptake of PET or plastic particles exhibit their cytotoxic effect only if accumulated in a higher concentration in human intestinal epithelial cells in vitro (Stock et al., 2020).

Majority of reports agree that acetaldehyde, formaldehyde, phthalate esters and antimony are related to PET water bottle containers and can migrate into water depending on storage and types of drinking water (Bach et al., 2012; Chapa-Martinez et al., 2016; Xu et al., 2020). Also, few researchers reported that water stored in PET bottles especially recycled ones contains endocrine disrupters that might lead to many health problems, including hormonal imbalances and cancers (Guo et al., 2018; Chaisupakitsin et al., 2019; Margetaki, 2021).

Dietary supplementation of less than 5% concentration of nonwoven fabric of polyethylene and PET for 13 weeks has no apparent toxic effect on rats, despite the detectable levels of antimony in blood samples of these animals (Merski et al., 2008).

However, Zoran et al., 2021 reported that single dose of PET 125 mg kg^{-1} increases the weight of adrenal and testicular tissue in rats.

It has been noted that heavy occupational exposure to Sb might lead to health problems such as dermatitis, respiratory tract irritation and gastritis. Also, chronic inhalation of this metal increases the incidence of carcinoma in rats (NTP, 2005). However, this effect was interpreted likely to be as a result of overloading the lung with this heavy inert metal (Merski et al., 2008). Preheating of food placed in plastic containers, in the microwave daily for 2 minutes along 40 days, is decreasing the level of LH, FSH and testosterone in male rats (Ibama et al., 2020).

There were scarce evidences, regarding the effect of direct oral intake of PET on human or animal. The current study highlighted the effect of direct oral gavage of PET on different biochemical parameters in male rats.

Materials and Methods

Experimental Animal

A total of 18 male albino rats 5-6 weeks old (average weight of 170±10mg) at animal house in Biotechnology research center at Al-Nahrain University were used. Polyethylene terephthalate powder was utilized. Animals were divided into three equal groups that kept for two weeks into separate cages for acclimatizing before starting of the study. Control group, Group I (GI) (Given a daily dose of 0.12 mg kg^{-1} PET by gavage tube). Group II (GII) (Given a daily dose of 0.6 mg kg^{-1} PET by gavage tube). Animals were kept for 60 days along study period and received diet and water ad libitum. The experiments and procedures were approved by the scientific committee of the Biotechnology research center.

Blood Collection

At the end of the experimental study, rats were fasted overnight for blood collection. First, animals were placed in a closed jar with cotton soaked with chloroform for anesthesia. Then 5ml syringe with 21 gage needle were utilized to collect blood via cardiac puncture and the collected blood were placed in a sterile tube and allowed to clot in upright position for 20 min at RT. The serum was separated immediately after spin with centrifuge at 3000 rpm for 15 min, and then stored at -20 ^{o}C for further biochemical tests.

Liver and Kidney Functions Assays

Serum liver enzymes Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were used to evaluate hepatic function, whereas serum urea

and Creatinine was used to assess renal function. All reagents were purchased from BioMerieux-France unless otherwise indicated. Serum level of ALT and AST were assayed based on a coupling calorimetric method. The kinetic determination of ALT is based on it catalyzes of L-alanine into pyruvate that catalyzed in the presence of lactate dehydrogenase into lactate. Whereas, AST catalyzing L-aspartate into oxaloacetate that catalyzed by malate dehydrogenase into malate (Huang et al., 2006). Serum Urea and Creatinine was analyzed by the enzymatic kinetic reaction method and as previously described by Elewal, H., 2014.

Oxidative Stress Assays

For determination of oxidative damage, lipid peroxidation of malondialdehyde (MDA), reduced Glutathione (GSH) and Catalase (CAT) were assessed. Reagents were supplied by Abcam, UK, unless indicated.

For determination of MDA levels, reagents were purchased from MyBiosource (San Diego, USA). Lipid peroxidation refers to the oxidative degradation of lipids especially of cell membrane that lead to cell damage. In lipid peroxidation assay, MDA react with Thiobarbituric acid (TBA) forming MDA-TBA that is quantified calorimetrically under OD=532nm.

Glutathione peroxidase (GPx) plays an important role in the protection of cell from oxidative damage. GPx oxidize reduced glutathione (GSH) to produce glutathione (GSSG). Then, in the same reaction Glutathione reductase reduces oxidized glutathione to GSH. This reaction consumes NADPH, and decrease NADPH, that quantified under OD=340nm, is proportional to GPx levels.

Catalase (CAT), is an enzyme that degrade free radicals H_2O_2 and eventually protect cells from oxidative damage produced by these radicals. Calorimetric method with OD=570nm of plate reader was used to detect CAT activity (Catalase assay kit, BioAssay systems, USA).

Hormonal Assays

For the detection of hormonal level of Luteinizing Hormone (L.H), Follicle stimulating Hormone (FSH), Enzyme linked Immunosorbent assay were utilized (ELISA assay Kit, CUSABIO, USA). And for determination of serum levels of Testosterone, ELISA assay kit were utilized (Diagnostic Automation Inc., USA) and following the manufacturer instructions.

Estimation of serum Triiodothyronine (T3), total serum Thyroxin (T4) and serum thyrotropin (TSH) were performed utilizing ELISA assay kit (Labor Diagnostika Nord GmbH, Nordhorn, Germany) and following the manufacturer instructions.

WEB OF SCIENTIST: INTERNATIONAL SCIENTIFIC RESEARCH JOURNAL
ISSN: 2776-0979, Volume 3, Issue 12, Dec., 2022

Statistical Analysis

All collected data were analyzed using T test. Data were expressed as Mean±SEM. The Statistical significance was set at P≤0.05. The Statistical Packages for the Social Sciences program (SPSS- V.14) were used.

Results

Liver and Kidney Functions

The current results revealed a significant increase (P≤0.05) in the level of ALT enzyme in PET groups for both concentration 0.6 and 0.12 mg dl^{-1} b.wt (34.21±4.92 and 36.30±1.54 IU L^{-1} respectively) compared with control (23.54±1.38 IU L^{-1}). Also, there were significant increase (P≤0.05) in AST enzyme in PET groups (30.67 ±2.47 and 40.20±1.60 IU L^{-1} respectively) compared with control (25.10±1.20 IU L^{-1}), (Fig.1a and b).

On the other hand, blood urea level were elevated significantly (P≤0.05) for both PET groups (30.53 ±1.35 and 32.45 ±1.97 mg dl^{-1} respectively), compared with control (20.00 ±3.05 mg dl^{-1}). Moreover, there were significant increase (P≤0.05) in serum Creatinine in PET groups (0.57 ±0.17and 0.86 ±0.12 mg dl^{-1} respectively), compared with control (0.36 ±0.08 mg dl^{-1}), (Fig.1c and d).

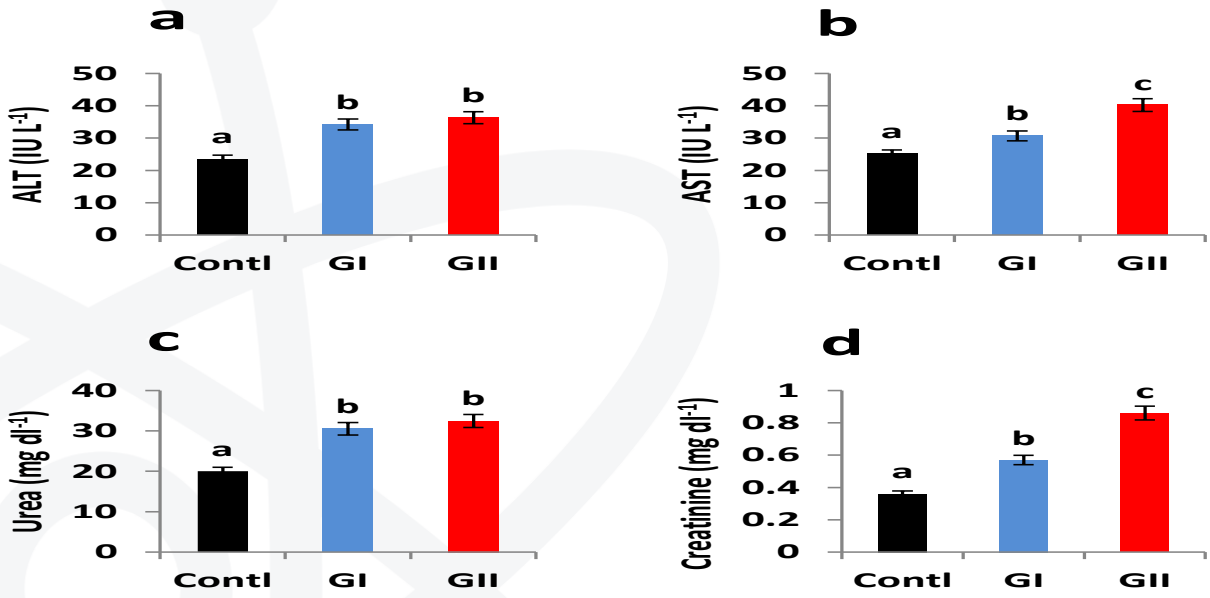

Fig.1: Effect of 60 days gavage of male rats with polyethylene terephthalate (PET) at 0.12 and 0.6 mg kg^{-1} on the level of Liver enzymes , **a** (Alanine aminotransferase, ALT) and **b** (Aspartate aminotransferase, AST), also on kidney function, **c** (Urea) and **d** (Creatinine), in male rats

* Data are presented as the mean ± SEM and different letters indicate significant differences (P≤0.05).

WEB OF SCIENTIST: INTERNATIONAL SCIENTIFIC RESEARCH JOURNAL
ISSN: 2776-0979, Volume 3, Issue 12, Dec., 2022

Oxidative stress

The malondialdehyde level was increase significantly (P≤0.05) in both PET groups (3.70 ±0.44 and 4.9 5 ±0.37 umol L^{-1} respectively) compared with control (1.06 ±0.16 umol L^{-1}), (Fig.2a). In regard with levels of Catalase, the results showed significant increase in both PET (237.34±10.53 and 310.53 ±13.67 pg ml-1 respectively) compared with control (166.28 ±8.52 pg ml-1), (Fig.2b). Also, there was a significant decrease in Glutathione level for PET groups (12.63±2.74 and 9.36 ±0.91 ng ml^{-1}) compared with control (20.42±4.50 ng ml^{-1}), (Fig.2c).

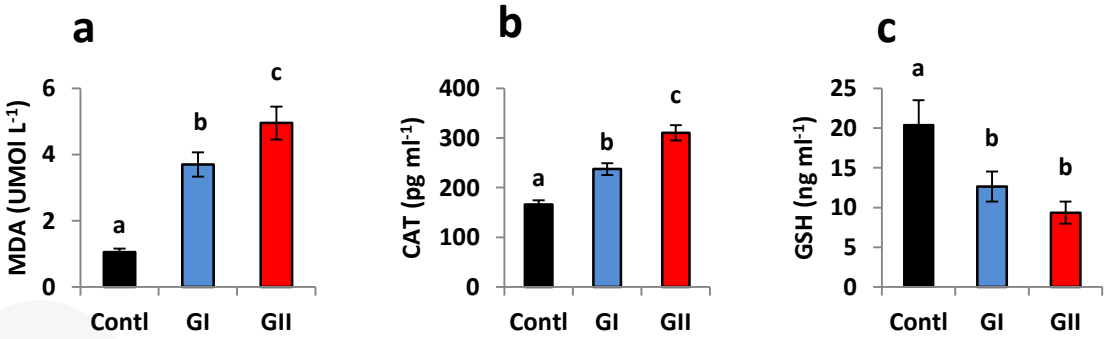

Fig.2: Effect of 60 days gavage of male rats with polyethylene terephthalate (PET) at 0.12 and 0.6 mg kg^{-1} on oxidative stress biomarkers in male rats, **a** (Malondialdehyde, MDA); **b** (Catalase, CAT) and **c** (Reduced Glutathione, GSH).

* Data are presented as the mean ± SEM and different letters indicate significant differences (P≤0.05).

Reproductive hormones

There were significant decrease (P≤0.05) in the level of L.H in both PET groups (0.425±4.23 and 0.491±6.29 mIU ml^{-1} respectively) compared with control (0.964±12.34 mIU ml^{-1}), (Fig.3a). Also, the results showed significant decrease in the level of FSH in PET groups (1.632±17.32 and 1.515±14.76 mIU ml^{-1} respectively) compared with control (2.426±23.45 mIU ml^{-1}), (Fig.3b).

Furthermore, there was significant decrease in the level of testosterone in both GI and GII groups (1.30 ±0.1 2 and 2.02 ±0.42 ng ml^{-1} respectively) compared with control (3.09 ±0.84 ng ml^{-1}), (Fig.3c).

WEB OF SCIENTIST: INTERNATIONAL SCIENTIFIC RESEARCH JOURNAL
ISSN: 2776-0979, Volume 3, Issue 12, Dec., 2022

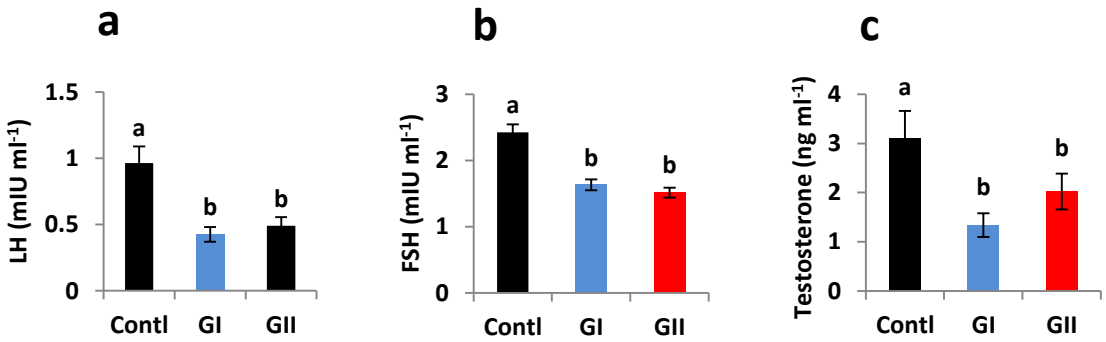

Fig.3: Effect of 60 days gavage of male rats with polyethylene terephthalate (PET) at 0.12 and 0.6 mg kg^{-1} on reproductive hormones, **a** (Follicle stimulating hormone, FSH); **b** (Luteinizing hormone, L.H) and **c** (Testosterone).

* Data are presented as the mean ± SEM and different letters indicate significant differences (P≤0.05).

Thyroid hormones

The current study revealed a significant decrease (P≤0.05) in T3 hormone in GI PET group (55.1 1 ±2.86 ng dl^{-1}) compared with group received 0.6 mg kg^{-1} b. wt. PET (67.43 ±3.45 ng dl^{-1}), and control group (88.09 ±3.24 ng dl^{-1}), (Fig.4a).

Also the results showed a significant decrease in T4 hormone in PET groups (7.32 ±0.56 and 6.54 ±0.39 ug dl^{-1} respectively) compared with control (11.54 ±0.4 9 ug dl^{-1}), (Fig.4b). Moreover, there were significant increases in TSH levels for both PET groups (3.42 ±0.17 and 3.88 ±0.32 mIU ml^{-1} respectively) compared with control (1.68 ±0.33 mIU ml^{-1}), (Fig.4c).

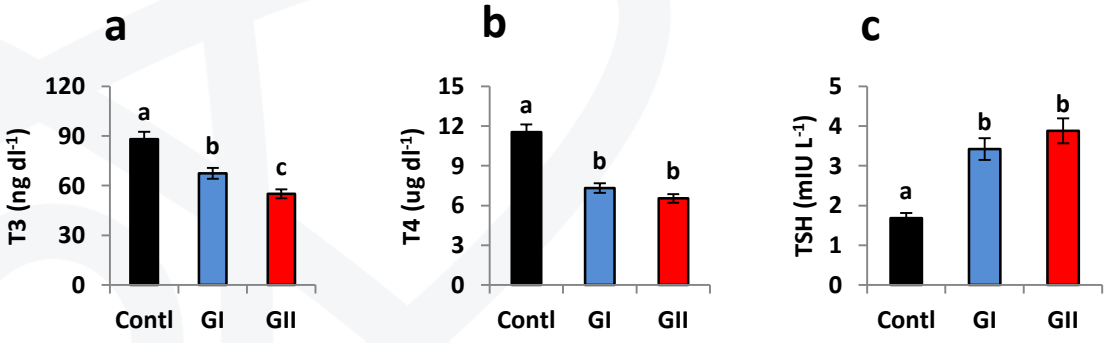

Fig.4: Effect of 60 days gavage of male rats with polyethylene terephthalate (PET) at 0.12 and 0.6 mg kg^{-1} on Thyroid hormones in male rats, **a** (Triiodothyronine, T3); **b** (Thyroxin, T4) and **c** (Thyrotropin, TSH)

* Data are presented as the mean ± SEM and different letters indicate significant differences (P≤0.05).

Discussion

Liver enzymes (ALT & AST) were increased in male rats for both PET concentrations. It was reported previously that feeding of rats with Polyethylene (PE) lead to liver damage, enlargement of hepatic tissues and increasing in ALT and AST enzymes (Al-Harbi et al., 2020). On the other hand, serum urea and Creatinine were also increased in both PET groups. Terephthalic acid (TSA) is utilized in PET production, and two weeks dietary supplementation of this acid leads to increase incidence of bladder calculi, aciduria and excretion of Ca and Mg in urine (Bang et al., 2011). The above mentioned scenarios might explain the impairment of liver and kidney function after PET supplementation in the current study.

Oxidative damage in the current study is indicated by increase of MDA and catalase and reduction of GSH in both PET groups. It was reported that PET might exert its oxidative damage effect in marine organisms if supplemented in higher concentration or in case of long term exposure (Parolini et al., 2020). However, the process of preparation of degraded PET powder (DPET) from recycled plastic containers might explain the disruption of oxidative stressor biomarkers. This is due to the presence of either magnesium oxide or zinc citrate in the process of DPET powder preparation (Alzuhairi et al., 2016; Hussein et al., 2018).

There were changes in reproductive and thyroid hormones for both PET groups. Whereas, Bisphenol A (BPA), an endocrine disruptor, is widely used in plastic manufacturing, PET products contain no such component (Pant et al., 2022). In their studies, Guo et al., 2018 and Margetaki, 2021 showed that the concentrations of thyroid hormone were inversely related to the levels of blood and urinary antimony. In the current study it might be possible that antimony released from PET interrupt the production of pituitary and thyroid hormones. However, further study to pursuit the signaling pathways that PET components affects hormonal imbalances is proposed.

Conclusion

The results of this study revealed that oral intake of polyethylene terephthalate (PET) at a concentration of 0.12 or 0.6 mg kg^{-1} b.wt disrupt liver and kidney functions, as well as reproductive and thyroid hormones in male rats.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgements

This study was conducted in the Biotechnology research center- Department of Environmental Biotechnology. Thanks to all our colleagues in the department for their support. Special thanks to animal house worker Omer Rahim Jassim for his great assistance during the study period.

References

1. Alzuhairi, M., Al-Ghaban, A.M., & Almutalabi, S.N. (2016). Chemical Recycling of Polyethylene Terephthalate (PET) as Additive for Asphalt. *ZANCO Journal of Pure and Applied Sciences*, 28(2), s675-67.
2. Al-Harbi, H., Al-Hasawi, Z., Binnaser, Y., Al-Hasawi, R., Al-Solami, F., & Al-Ghamdi, A. (2020). Effect of Polyethylene and Butylated Hydroxytoluene on the histological structure and some enzymes of rat liver. *Journal of Bioscience and Applied Research*, 6(2), 68-82. https://doi:10.21608/jbaar.2020.116133
3. Bach, C., Dauchy, X., Chagnon, M., & Etienne, S. (2012). Chemical compounds and toxicological assessments of drinking water stored in polyethylene terephthalate (PET) bottles: a source of controversy reviewed. *Water Res.*, 46, 571–583. https://doi.org/10.1016/j.watres.2011.11.062
4. Bang, D.Y., Lee, I.K. , & Lee, B.M. (2011). Toxicological Characterization of Phthalic Acid. *Toxicol. Res.*, 27(4), 191-203. https://doi.org/10.5487/TR.2011.27.4.191
5. Chapa-Martínez, C.A., Hinojosa-Reyes, L., Hernández-Ramírez, A., Ruiz-Ruiz, E., Maya-Treviño, L., & Guzmán-Mar, J.L. (2016). An evaluation of the migration of antimony from polyethylene terephthalate (PET) plastic used for bottled drinking water. *Science of The Total Environment*, 565, 511-518. https://doi.org/10.1016/j.scitotenv.2016.04.184
6. Carneado, S., Hernndez-Nataren, E., López-SJnchez, J.F., & Sahuquillo, A. (2015). Migration of antimony from polyethylene terephthalate used in mineral water bottles. *Food Chem.*, 166, 544–550. https://doi.org/10.1016/j.foodchem.2014.06.041
7. Chaisupakitsin, M., Chairat-Utai, P., & Jarusiripot, C. (2019). Degradation of polyethylene terephthalate bottles after long sunlight exposure. *Songklanakarin J. Sci. Technol.*, 41, 259–264. https://doi.org/10.14456/sjst- psu.2019.32
8. Dhaka, V., Singh, S., Anil, A., Naik, T., Garg, S., Samuel, J., Kumar, M. Ramamurthy, P., & Singh, J. (2022). Occurrence, toxicity and remediation of polyethylene terephthalate plastics, A review. *Environ. Chem. Lett.*, 20, 1777–1800. https://doi.org/10.1007/s10311-021-01384-8

9. Elewal, H. A. (2014). Study the nephro-protective effects of losartan on rats. *Az. J. Pharm Sci.,* 49, 202-210.
10. Guo, J., Lv, N., Tang, J., Zhang, X., Peng, L., Du, X., Li, S., Luo, Q., Zhang, D., & Chen, G. (2018). Associations of blood metal exposure with thyroid hormones in Chinese pregnant women: a cross-sectional study. *Environment International*, 121, 1185-1192.
11. Huang, X.J., Choi, Y.K., Im, H.S., Yarimaga, O., Yoon, E., & Kim, H.S. (2006). Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) Detection Techniques. *Sensors (Basel),* 31, 6(7), 756–82.
12. Hussein, A.A., Alzuhairi, M., & Aljanabi, N.H. (2018). Chemical and Biological Treatment of Plastic Wastes by Bacteria Isolated from Contaminated Soils in Baghdad, Iraq. *Al-Nahrain Journal of Science*, 21 (2), 130-37. https://doi:10.22401/JNUS.21.2.19
13. Ibama, O., Chioma, U., Konne, J. L., & Konne, F. (2020). Effect of Ingested Foods Preheated (With Microwave) in Plastic Containers on the Reproductive Profile of Male Albino Rats. *Journal of Advances in Medicine and Medical Research*, 32(24), 71-75. https://doi.org/10.9734/jammr/2020/v32i2430753
14. Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., & Law, K.L. 2015. Plastic waste inputs from land into the ocean. *Science,* 347(6223), 768–771. https://doi:10.1126/science.1260352
15. Margetaki, K., Vafeiadi, M., Kampouri, M., Roumeliotaki, T., Karakosta, P., Daraki, V., Kogevinas, M., Hu, H., Kippler, M., Chatzi, L. (2021). Associations of exposure to cadmium, antimony, lead and their mixture with gestational thyroid homeostasis. *Environmental Pollution*, 289, 117905.
16. Merski, J.A., Johnson, W.D., Muzzio, M., Lyang, N.L., & Gaworski, C.L. (2008). Oral toxicity and bacterial mutagenicity studies with a spunbond polyethylene and polyethylene terephthalate polymer fabric. *Int. J. Toxicol.*, 27(5), 387-95. https://doi:10.1080/10915810802408729
17. NTP (National Toxicology Program), (2005). Antimony trioxide (cas no. 1309- 64-4). Brief review of toxicological literature. Research Triangle Park, North Carolina: NTP/NIEHS. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr590_508.pdf?utm_source=direct&utm_medium=prod&utm_campaign=ntpgolinks&utm_term=tr590
18. Pant, M.K., Ahmad, A.H., Naithani, M., & Pant, J. (2022). Plastic bottle feeding produces changes in biochemical parameters in human infants - A pilot study. *Clin Exp Pediatr*, 65(9), 459-465. https://doi:10.3345/cep.2022.00234.19

19. Parolini, M., De Felice, B., Gazzotti, S., Annunziata, L., Sugni, M., Bacchetta, R., & Ortenzi, M. (2020). Oxidative stress-related effects induced by micronized polyethylene terephthalate microparticles in the Manila clam. *Journal of Toxicology and Environmental Health*, Part A, 83(4), 168-179. https://doi:10.1080/15287394.2020.1737852
20. Ragusa, A., Svelato, A., Santacroce, C ., Catalano, P., Notarstefano, V., Carnevali, O., Papa, F., Ciro Antonio Rongioletti, M., Baiocco, F., Draghi, S., D'Amore, E., Rinaldo, D., Matta, M., & Giorgini, E. (2021). Plasticenta: First evidence of microplastics in human placenta. *Environment International*, 146, 106274. https://doi.org/10.1016/j.envint.2020.106274
21. Stock, V., Laurisch, C., Franke, J., Dönmez, M., Voss, L., Böhmert, L., Braeuning, A., & Sieg, H. (2020). Uptake and cellular effects of PE, PP, PET and PVC microplastic particles. *Toxicol In Vitro*, 70, 105021. https://doi:101016/j.tiv.2020.105021
22. Whitt, M., Brown, W., Danes, J.E., & Vorst, K.L. (2016). Migration of heavy metals from recycled polyethylene terephthalate during storage and microwave heating. *J. Plast. Film Sheeting,* 32, 189–207. https://doi.org/10.1177/8756087915590190
23. Xu, X., Zhou, G., Lei, K., LeBlanc, G.A., & An, L. (2020). Phthalate Esters and Their Potential Risk in PET Bottled Water Stored under Common Conditions. *International Journal of Environmental Research and Public Health*, 17(1), 141. https://doi.org/10.3390/ijerph17010141
24. Zhang, C., Chen, J., Ma, S., Sun, Z., & Wang, Z. (2022). Microplastics May Be a Significant Cause of Male Infertility. *American Journal of Men's Health*, 16(3), 15579883221096549. https://doi:10.1177/15579883221096549
25. Zoran, S., Todorović, A., Nenad, F., Veljković, F., & Stojanović, G. (2021). A single dose of microplastic particles induces changed in organ weight of male Wistar rats. Proceedings / 7th Workshop Specific Methods for Food Safety and Quality, Belgrade, Serbia, 15th International Conference on Fundamental and Applied Aspects of Physical Chemistry - Physical Chemistry, 119-122. https://dais.sanu.ac.rs/handle/123456789/12381.