Trop J Nat Prod Res, May 2023; 7(5):2969-2973 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Potential of Red Seaweed (*Dichotomania obtusata*) on Immune Response and Histopathology of Rat Testis Exposed to Nanoplastics

Hery Triwahyudi[1], Listijani Soehargo[1], Liwaul Muniroh[1], Rosalia N. Qolbi[1], Tazkia Q. 'Aini[1], Rifdah F. Z. Kurnia[1], Primadika A.D. Putra[1], Manikya Pramudya[1], Bayyinatul Muchtaromah[2], Alfiah Hayati[1]*

[1]*Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Campus C, Mulyorejo, 60115, Surabaya, Indonesia*
[2]*Biology Study Program, Faculty of Science and Technology, Universitas Islam Negeri Maulana Malik Ibrahim, Gajayana Street 50, 65114, Malang, Indonesia*

ARTICLE INFO

Article history:
Received 09 April 2023
Revised 07 May 2023
Accepted 08 May 2023
Published online 01 June 2023

ABSTRACT

Polystyrene nanoplastics (NP) are often found in aquatic environments. The accumulation of nanoplastics in aquatic biota such as fish, shrimp, squid, shellfish, etc. can reduce the health of humans who consume them. The purpose of this study was to observe the red seaweed's potential as a source of antioxidants in improving the immune system and reproductive health of rats (*Rattus novergicus*) exposed to nanoplastics. Twenty-five rats (*Rattus norvegicus*) were divided into five groups with various treatments, namely control, negative control (2 µL/kg BW NP), and three combined treatments of 2 µL/kg BW NP with varying concentrations of seaweed extract (50, 100, and 200 mg/kg BW), were treated to determine levels of TNF-α, IFN-γ, histopathology, size, and weight of the testes. The results showed that exposure to 2 µL/kg BW NP increased levels of cytokines (TNF-α and IFN-γ) and affected the histology and size of the rat testes. Administration of seaweed extract (50, 100, and 200 mg/kg BW) could improve cytokine levels by decreasing IFN-γ and repair rat testicular histology by increasing spermatid number and thickness of seminiferous tubule epithelium.

Keywords: reproductive health, nanoplastic, seaweed, immunology, testis

Introduction

There is a high accumulation of plastic waste but public understanding regarding the impact of these plastic materials is still lacking.[1] Due to their insolubility in water and resistance to biodegradation over extended periods, plastics persist in the environment for long durations.[2] In the long time, plastic waste can cause pollution due to degradation through mechanical stress or ultraviolet radiation into microplastics (MP) and nanoplastics (NP). Microplastics are particles that measure less than 5 mm in size, while nanoplastics are even smaller with a size range of 100 nm (0.001-0.1 µm).[3,4]

Along with the increase of NP pollution in the environment, more organisms and ecosystems were badly affected. NP can enter the body of organisms in various ways including the digestive system, skin exposure, and respiratory system. Several studies have stated that polystyrene plastic (53 – 180 nm in size) can be found in the brain of the *Carassius Carassius*,[5,6] microplastic particles (70 – 90 µm in size) are found in the brain of *Oreochromis niloticus*,[7] and particle with 5 µm in size can penetrate into rat brain through blood capillaries.[8] Fish is one of the animal protein sources which is needed to improve human health and nutrition. If humans consume fish contaminated with polystyrene nanoplastics (NP), these compounds can accumulate in the body, thereby reducing health.[9]

*Corresponding author. E mail: alfiah-h@fst.unair.ac.id.
Tel: +62 813-3095-0399

Citation: Triwahyudi H, Soehargo L, Muniroh L, Qolbi RN, 'Aini TQ, Kurnia RFZ, Putra PAD, Pramudya M, Muchtaromah B, Hayati A. Potential of Red Seaweed (*Dichotomania obtusata*) on Immune Response and Histopathology of Rat Testis Exposed to Nanoplastics Trop J Nat Prod Res. 2023; 7(5):2969-2973 http://www.doi.org/10.26538/tjnpr/v7i5.20

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Due to polystyrene nanoplastics (NP) toxicity, NP could cause oxidative stress, decreasing mitochondrial membrane potential, cell survival, membrane integrity, and fluidity. The condition of decreasing cell viability depends on the size and concentration of NPs.[10] Related to oxidative stress, NP increases Reactive Oxygen Species (ROS). The presence of ROS in cells, among other things, causes disturbances in protein/enzyme structures that affect their enzymatic functions. Another study using polystyrene in the testes of male Sprague Dawley rats showed that polystyrene decreased the activity of antioxidant enzymes and increased lipid peroxidation and superoxide levels. In addition, the expression of steroidogenic enzymes, follicle-stimulating hormone levels, luteinizing hormone levels, intra-testicular and plasma testosterone are found to decrease.

The immune system responds to NP exposure through the skin or mucosal secretions in the form of sweat or mucus. If NP is able to penetrate the skin barrier and enter the body's cells in high concentrations, it will damage the plasma membrane and even cause programmed cell death. High concentrations of NP disrupt the phospholipid bilayer of the plasma membrane structure. Moreover, the accumulation of NPs on the plasma membrane surface can impede the cellular signaling process, thereby disrupting the interaction of cell surface receptors with membrane ligands. NPs can penetrate the phospholipid bilayer in several ways including endocytosis (by the mediation of clathrin and caveolin) and penetration with the help of energy. NPs are considered more dangerous than microplastics because their small size allows them to penetrate biological membranes.[11] NPs in the cytoplasm will be responded to by cellular cytolytic lymphocytes CD4+. An increase in CD4+ cell ratio indicates that helper T cells (Th1) have been active in inducing TNF-α and IFN-γ levels after exposure to NPs.[12]

The mechanism of reproductive toxicity also occurs due to oxidative stress from polystyrene NP exposure. If polystyrene NP exposure occurs in the testes, the production of ROS increases in the testes, which can cause reproductive toxicity through lipid peroxidation or DNA damage. Polystyrene NPs exposure resulted in adverse effects on male reproductive health, including irregular spermatogonia, inflammation of the testes, decreased sperm quality, reduced testosterone levels,

impaired blood circulation in the testes, and presence of multinucleated gonadotrophic cells in the germinal tubules.[13]
Cell health problems caused by polystyrene NP can be prevented by materials with antioxidant properties. One of the ingredients that contain anti-oxidants is red seaweed (*Dichotomaria obtusata*). Red seaweed is a source of antioxidants that are thought to be able to inhibit cell and tissue damage due to the oxidant properties caused by NPs. Red seaweed has the potential as a source of antioxidants that can protect against risks to the immune system (levels of pro-inflammatory cytokines were raised). The bioactive content of red seaweed is a new complex of polysaccharides, polyphenols, fatty acids, flavonoids, etc.[14] Red seaweed contains flavonoids and phenolic compounds which act as natural antioxidants that can counteract various free radicals and have an effect on the immune system (immunostimulator). The content of flavonoids and phenolic compounds in red seaweed has an effect on inhibiting TNF-α production and increasing the number of B cells to produce antibodies. TNF-α is a group of cytokines produced by monocytes and macrophages that have a great ability to have pro-inflammatory effects by exposure to antigens including polystyrene NP. The content of flavonoids in red seaweed is a stimulus that can decrease IFN-γ. Th1 cells produce a pro-inflammatory cytokine known as IFN-γ that acts as a modulator of the immune system.[15]
There is still few information on the potential of red seaweed (*D. obtusata*) as an antioxidant to prevent damage against micro-nanoplastic contamination. Therefore, this study was conducted to analyze the potential of red seaweed as a source of antioxidants in improving the immune system (level of TNF-α and IFN-γ) and reproductive health (histopathology, size, and testicular weight) of rats (*Rattus novergicus*) exposed to polystyrene NPs.

Materials and Methods

Material and Chemicals
Red seaweed was obtained from Ketapang Beach, Banyuwangi, Indonesia (8º05'32"S; 144º24'55"E) on May 2021. The red seaweed was identified by the taxonomist (Dr. Moch. Affandi) of the Department of Biology, Faculty of Science and Technology, Universitas Airlangga A voucher of specimen with number LTBUA.00344.20 was deposited at Department of Biology, Faculty of Science and Technology, Universitas Airlangga as reference. Ethanol pro-analyze was purchased from Merck (Merck Millipore, Darmstadt, Germany). Nanoplastics were purchased from Sigma-Aldrich Solution (Merck Millipore, Darmstadt, Germany). TNF-α and IFN-γ Enzyme-linked Immunosorbent Assay (ELISA) kit was purchased from Bioassay Technology Laboratory (Shanghai Korain, Shanghai). All the remaining chemicals and solvents in the experiments were of analytical reagent grade.

Red Seaweed extraction
After being rinsed with water, the fresh red seaweed was left to dry in the air for 14 days. During the air-drying process, red seaweed was not exposed directly to sunlight. After that, dried red seaweed was cut into small pieces and reduced to obtain a fine powder. 2 kg of red seaweed was macerated with 2 L of ethanol overnight, at room temperature (24-29ºC) with constant stirring every 3 hours. Subsequently, the extract was filtered and macerated again twice. Then, the extract was subjected to a rotary evaporator (temperature 25-30ºC).

Animal
Male Winstar rats (*Rattus norvegicus*), aged 6-8 weeks, 150 ± 50 grams in weight were purchased from Faculty of Veterinary, Universitas Airlangga, Surabaya, Indonesia. The rats were housed at ~20 °C, with12-h a light/12-h dark cycle. The rats were provided with unrestricted access to food and water throughout the duration of the experiments. Each rat was placed in box with a size of 30 × 40 × 15 cm with a large wire cover All procedures involving animal care were approved by the Ethical Clearance Commission, Faculty of Dental Medicine, Universitas Airlangga, Indonesia (Certificate no. 440/HRECC.FODM/VII/2022).

Experimental design
After being adapted to the environment for two weeks, twenty-five rats were divided into five groups randomly: (1) control group without any treatment, (2) negative control group exposed to 2 µL/kg BW NP without red seaweed administration, (3) treatment group with NP exposure and red seaweed 50 mg/kg BW, 4) treatment group with NP exposure and red seaweed 100 mg/kg BW, and (5) treatment group with NP exposure and red seaweed 200 mg/kg. Administration of NP and red seaweed were done for 35 days by oral gavage. Then, rats were sacrificed.

TNF-α and IFN-γ assay
Whole blood was collected and then subjected to centrifugation at 3000 rpm and 4ºC for 10 mins to separate the serum from other component The serum samples were used to determine the levels of TNF-α and IFN-γ using a commercial Enzyme Linked Immuno Sorbent Assay (ELISA) kit (Bioassay Technology Laboratory, Shanghai, China) as per the manufacturer's protocol. The absorbance was measured at 450 nm using an ELISA reader, and the OD value was utilized to calculate the TNF-α levels by comparing it to a standard curve.

Histology testis processing
On the 36th day, testicular tissue samples were collected from all groups for histopathological examination. 10% neutral buffered formalin solution was used for testes sample fixation. After 24 hours of fixation, they were prepared for serial sectioning using standard techniques. Tissue sections of approximately 4 µm thickness were obtained using a semi-automated microtome, and then embedded in paraffin, mounted in DPX, and stained with hematoxylin and eosin. The sections were analyzed under a light microscope (Olympus)
Testicular histopathological changes were assessed qualitatively by comparing the normal control group, and negative control with variations in seaweed concentration. In addition, the diameter and circumference measured using a digital camera microscope (OPTILAB) included the diameter of the seminiferous tubules and the number of spermatogenic cells (spermatogonia and spermatids) in the seminiferous tubules.[16]

Statistical analysis
Normality and homogeneity of the data were assessed by performing Kolmogorov-Smirnov normality test and Levene's homogeneity test. The test was continued with the Two-way ANOVA variant test ($\alpha = 0.05$) followed by Duncan test using Windows Statistical Package for Social Science (SPSS) to determine the mean difference between sampling locations and Duncan's follow-up test using the SPSS version 24 program. Data on the histological structure of the gonads of rats were analyzed descriptively by presenting them in the form of pictures or photographs.

Results and Discussion

TNF-α and IFN-γ Levels
Nanoparticles are polymer particles that contain toxic substances capable of inducing oxidative stress, inflammation, immune dysfunction, neurotoxicity, neoplasia, metabolic alterations, and disruption of energy homeostasis.[17,18,19] The particles are capable of significantly altering the secretion of both proinflammatory cytokines, such as IL-6 and TNF, and anti-inflammatory cytokines, such as IL-10).[20] Increasing both pro and anti-inflammatory cytokine levels indicates a contra mechanism with the aim of balancing the body's physiology.
The results of TNF-α and IFN-γ levels are presented in Figure 1. The results of TNF-α level showed that negative control group (NP group) with the administration of 2 µL/kg BW NP increased TNF-α levels significantly ($p<0.05$). Meanwhile, all treatment groups with the administration of seaweed extract (50, 100, and 200 mg/kg BW) decreased TNF-α levels but did not show significant differences compared to control group and negative control group.
Negative control group (NP group) also showed rising of IFN-γ levels significantly after exposure to NPs ($p<0.05$) Administration of 50, 100, and 200 mg/kg BW seaweed extract reduced IFN-γ levels significantly

compared to negative control group ($p<0.05$). Seaweed extract with concentration of 100 and 200 mg/kg BW showed highest decrease in IFN-γ levels.

Figure 1. Effect of seaweed extract on TNF-α and IFN-γ in rats exposed to NP.

This study found that induction of 2 µL/kg BW NP (100 nm) can increase the serum levels of TNF-α and IFN-γ in rats exposed for 35 days. This is also supported by several other studies which have documented the effect of NP on various levels of cellular toxicity and pathology. Nanoparticles (NPs) with a size of 20 nm can penetrate monocytic cells easily and have a significant cytotoxic effect. On the other hand, larger NPs (ranging from 100 to 1000 nm) can trigger the secretion of proinflammatory cytokines, such as IL-6 and IL-8, by monocytes and macrophages, as well as stimulate the production of ROS, induce genotoxic stress, and cause DNA damage as assessed by the micronucleus-blocking cytokinesis assay.[21,22] While other researchers stated that low levels of toxicity were observed for microplastics (MP) measuring 0.1 and 5 µm. These particles can induce mitochondrial depolarization and inhibit the activity of the toxicant efflux pump.[23] Cytotoxicity also appears in 20 µm microplastics at high concentrations which induces ROS production. Thus, MP and NP particles can stimulate the production of reactive oxygen species (ROS) when they enter the cell. Furthermore, both MP and NP can lead to cytotoxicity in Hela cells, trigger the release of pro-inflammatory cytokines such as IL-6 and TNF-α from human peripheral blood mononuclear cells (PBMCs), and promote histamine release from the mast cell pathway.[24,25]

Diameter of Seminiferous Tubules and Epitellium Thickness

The results of histological observations and measurements of the rat testes showed that in general there were no changes in the structure of the testes (Figure 2). The result of diameter of seminiferous tubules and thickness of seminiferous tubules epitelium were presented in Figure 3. The result showed no significant difference in diameter of the seminiferous tubules in all groups after NPs exposure and red seaweed administration. Meanwhile, exposure to NP in negative control group (NP group) reduced thickness of the seminiferous tubule epithelium significantly ($p<0.05$). Administration of red seaweed (50, 100, and 200 mg/kg BW) showed a significant difference in thickness of the seminiferous tubule epithelium compared to NP group. Red seaweed administration could restore the thickness of the seminiferous tubule (Figure 3).

Number of Spermatogonia and Spermatid

The number of spermatogonia and spermatid were presented in Figure 3. Exposure to NP decreased the number of spermatogonia. Administration of red seaweed extract to a concentration of 200 mg/kg has not been able to recover the number of spermatogonia like the control group. A different result was obtained for the number of spermatids. Exposure to NP reduced the number and administration of seaweed extract starting at 100 mg/kg could restore the number of spermatids like the control group.

The presence of ROS that causes cytotoxicity by NPs can be mitigated by adding antioxidants from red seaweed extracts. In this study, it was proven that 50 mg/kg of seaweed extract was able to suppress IFN-γ levels in rats exposed to NP. Changes in the size of the seminiferous tubules (epithelial thickness) and the number of spermatid cells indicate that the NPs are cytotoxic, but the addition of seaweed extract which acts as an antioxidant can neutralize the oxidants from the NPs so that the amount can be restored. It is suspected that seaweed extract can restore this number to stimulate the process of primary spermatocyte cell division to become secondary spermatocytes and cell division end to form spermatids. A previous study also reported the potential antioxidant properties of red seaweed.[26] Red seaweed is abundant in alkaloids, flavonoids, glycosides, polyphenols, protein, reducing sugar, saponin, steroids, and tannins.[26] Most seaweed including red seaweed and brown seaweed contain polyphenols with potential antioxidant properties against cell damage due to oxidative stress in rats.[27] Thus, cytotoxicity of NP can be prevented by administration of red seaweed antioxidants.

Figure 2. Histological structure of rat testes. (A) Control, (B) negative control (2 µL/kg BW NP), and (C, D, and E) combination treatments of 2 µL/kg BW NP and variations in seaweed extract concentrations of 50, 100, and 200 mg/kg BW

Figure 3. Diameter and thickness of the testicular seminiferous tubule epithelium and the number of spermatogenic cells after exposure to various concentrations of NP and seaweed extract.

Conclusion

Exposure to 2 µL/kg BW of polystyrene nanoplastics increased cytokines (TNF-α and IFN-γ) and affected the histology and size of the rat testes. Administration of red seaweed extract can improve cytokine levels, restore epithelium thickness of seminiferous tubule and restore the number of spermatids. This study suggests that the red seaweed (*Dichotomania obtusata*) extract could effectively improve immune response and protect testes tissue against nanoplastics.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

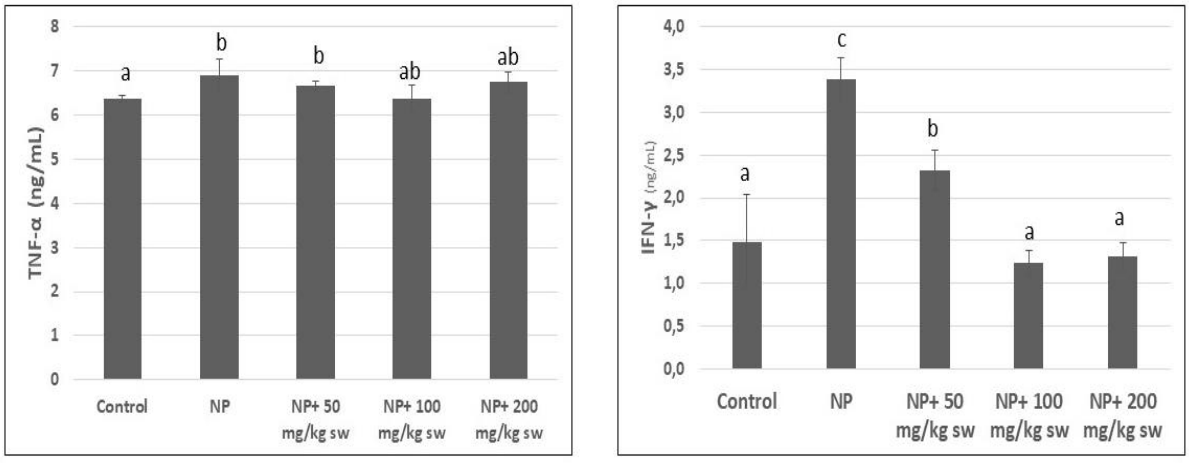

Figure 1: Effect of seaweed extract on TNF-α and IFN-γ in rats exposed to NP

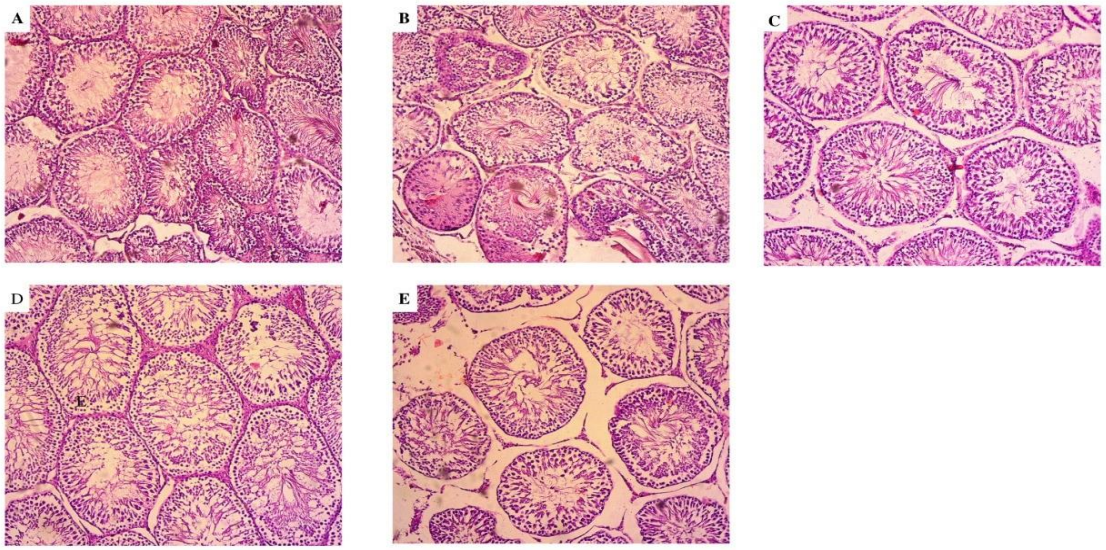

Figure 2: Histological structure of rat testes. (A) Control, (B) negative control (2 µL/kg BW NP), and (C, D, and E) combination treatments of 2 µL/kg BW NP and variations in seaweed extract concentrations of 50, 100, and 200 mg/kg BW

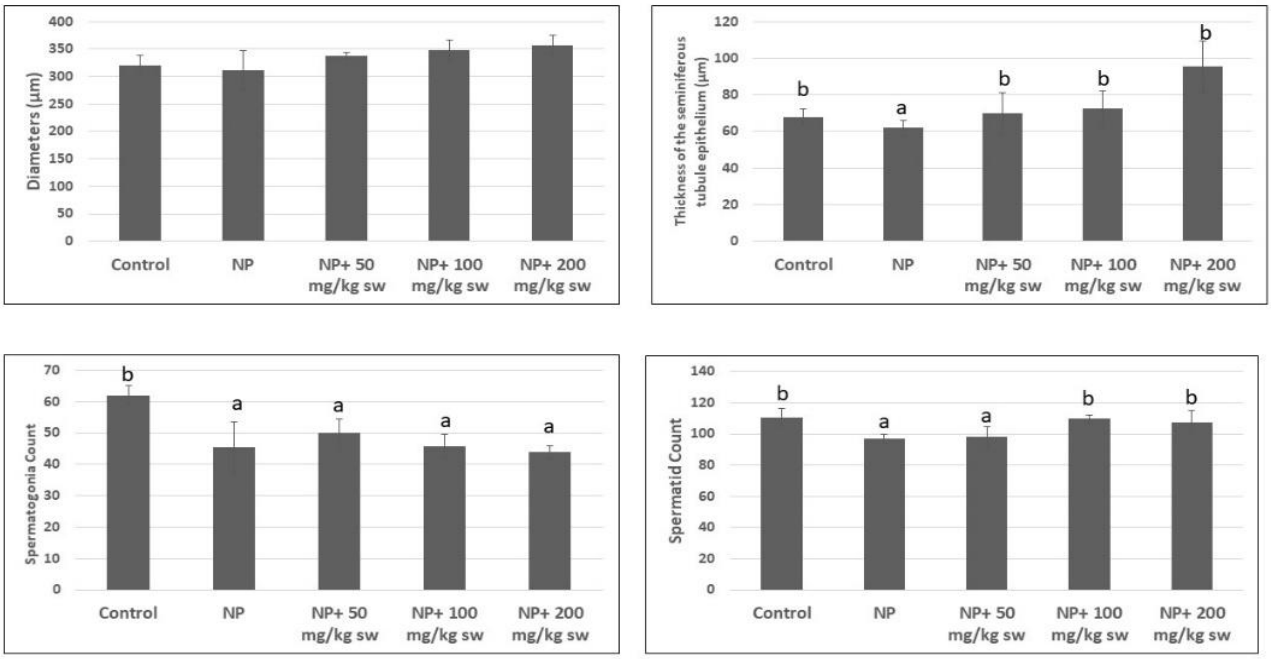

Figure 3: Diameter and thickness of the testicular seminiferous tubule epithelium and the number of spermatogenic cells after exposure to various concentrations of NP and seaweed extract.

Acknowledgements

The author would like to express gratitude to the Directorate of Research, Technology, and Community, Ministry of Education, Culture, Research and Technology, Universitas Airlangga, Indonesia which provides funding for Master Thesis Research Activities in 2022 Grant no. 1004/UN3/2022, May 11th 2022.

References

1. Chamas A, Moon H, Zheng J, Qiu Y, Tabassum T, Jang JH, Abu-Omar M, Scott SL, Suh S. Degradation Rates of Plastics in the Environment. ACS Sustainable Chem. Eng. 2020; 8:3494−3511.
2. Jiang X, Yang Y, Wang Q, Liu N, Li M. Seasonal variations and feedback from microplastics and cadmium on soil organisms in agricultural fields. Environ Inter J. 2022; 161: 1-11
3. Deng Y, Zhang Y, Lemos B, Ren H. Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. Sci Rep-UK. 2017; 7: 46687
4. Materic D, Kjaer HA, Vallelonga P, Tison J, Rockmann T, Holzinger R. Nanoplastics measurements in Northern and Southern polar ice. Environ Res. 2022; 208:112741.
5. Mattsson K, Johnson EV, Malmendal A, Linse S, Hansson LA, Cedervall T. Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. Sci Rep. 2017; 7:1–7.

6. Guerrera MC, Aragona M, Porcino C, Fazio F, Laurà R, Levanti M, Montalbano G, Germanà G, Abbate F, Germanà A. Micro and nano plastics distribution in fish as model organisms: histopathology, blood response and bioaccumulation in different organs. Appl Sci. 2021; 11: 5768.
7. Ding J, Huang Y, Liu S, Zhang S, Zou H, Wang Z, Zhu W, Geng J. Toxicological effects of nano- and micro-polystyrene plastics on red tilapia: Are larger plastic particles more harmless? J Hazard Mater. 2020; 396: 122693.
8. Liu L, Xu K, Zhang B, Ye Y, Zhang Q, Jiang W. Cellular internalization and release of polystyrene microplastics and nanoplastics. Sci Total Environ. 2021; 779:146523
9. Piyawardhana. Occurrence of microplastics in commercial marine dried fish in Asian countries. J Hazard Mater. 2022; 423:127093.
10. Rozman U, Kalcikova G. Seeking for a perfect (non-spherical) microplastic particle – The most comprehensive review on microplastic laboratory research. J Hazard Mater. 2022; 424:127529.
11. Yee S, Hii L, Looi CK, Lim W, Wong S, Kok Y, Tan B, Wong C, Leong C. Impact of microplastics and nanoplastics on human health maxine. Nanomaterials-Basel. 2021; 11: 496.
12. Park E, Han J, Park E, Seong E, Lee G, Kim D, Son H, Han H, Lee B. Repeated-oral dose toxicity of polyethylene microplastics and the possible implications on reproduction and development of the next generation. Toxicol Lett. 2020; 324:75-85.
13. Zhang C, Chen J, Ma S, Sun Z, Wang Z. Microplastics may be a significant cause of male infertility. Am J Mens Health. 2022; 1–8.
14. Zavaglia A, Lage MAP, Lopez C, Mejuto CJ, Gandara J. The potential of seaweeds as a source of functional ingredients of prebiotic and antioxidant value. Antioxid. 2019; 8:406-436.
15. Chiang L, Teik Ng L, Chiang W, Chang M, Lin C. Immunomodulatory activities of flavonoids, monoterpenoids, triterpenoids, iridoid glycosides and phenolic compounds of plantago species. Plant Med. 2003; 69:600-604.
16. Hayati A, Pramudya M, Soepriandono H, Astri AR, Kusuma MR, Maulidah S, Adriansyah W, Dewi FRP. Assessing the recovery of steroid levels and gonadal histopathology of tilapia exposed to polystyrene particle pollution by supplementary feed. Vet World; 15:517-523
17. Chang X, Xue Y, Li J, Zou L, Tang M. Potential health impact of environmental micro- and nanoplastics pollution. J Appl Toxicol. 2019; 40:4-15
18. Hu M, Palić D. Micro- and nano-plastics activation of oxidative and inflammatory adverse outcome pathways. Redox Biol. 2020; Article 101620, 10.1016/j.redox.2020.101620
19. Prata JC, da Costa JP, Lopes I, Duarte AC, Rocha-Santos T. Environmental exposure to microplastics: an overview on possible human health effects. Sci Total Environ. 2020; 702: 134455,
20. Annkatrin A, Schwiebs A, Solhaug A, Stenvik J, Nilsen AM, Wagner M, Relja B, Radeke HH. Nanoplastics affect the inflammatory cytokine release by primary human monocytes and dendritic cells. Environ I. 2022; 163:107173
21. Prietl B, Meindl C, Roblegg E, Pieber TR, Lanzer G, Fröhlich E. Nano-sized and micro-sized polystyrene particles affect phagocyte function. Cell Biol. Toxicol. 2014; 30:1–16.
22. Poma A, Vecchiotti G, Colafarina S, Zarivi O, Aloisi M, Arrizza L, Chichiriccò G, Di Carlo P. In vitro genotoxicity of polystyrene nanoparticles on the human fibroblast Hs27 cell line. ACS Appl. Nanomat. 2019: 9:1299
23. Wu B, Wu X, Liu S, Wang Z, Chen L. Size-dependent effects of polystyrene microplastics on cytotoxicity and efflux pump inhibition in human Caco-2 cells. Chemosphere. 2019; 221: 333–341
24. Schirinzi GF, Pérez-Pomeda I, Sanchís J, Rossini C, Farré M, Barceló D. Cytotoxic effects of commonly used nanomaterials and microplastics on cerebral and epithelial human cells. Environ Res. 2017; 159:579–587
25. Hwang JD, Choi S, Han J, Choi J, Hong. An assessment of the toxicity of polypropylene microplastics in human-derived cells. Sci Total Environ. 2019; 684:657–669
26. Warsi W, Jaswir I, Khatib A, Ahmed Q, Nawi, MSBM, Rohman A, Narwanti I. Phytochemical screening, total phenolic, reducing sugar content, and antioxidant activities of *Gelidium spinosum* (S.G.Gmelin) P.C. Silva. Trop J Nat Prod Res. 2023; 7(3):2618-2623
27. Husni A, Fauzi DN, Nugroho AE. Anti-stress effect of brown seaweed *Sargassum hystrix* J. Agard ethanol extract on cold-induced stress in wistar rats. Trop J Nat Prod Res. 2021; 5(9):1547-1550