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Research Article

Potential of *Cinnamomum burmanni* Leaf Extract as an Exogenous Antioxidant and Spermatoprotective for *Rattus norvegicus* L. Exposed to Polystyrene Nanoplastics

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ABSTRACT

Polytyrene nanoplastics (NPs) (<1 µm) have high toxicity when entered and accumulated in cells. NPs accumulation causes oxidation stress, thus increasing reactive oxygen species (ROS) production, resulting in necrosis or apoptosis, as well as affecting endogenous antioxidant activity, such as superoxide dismutase (SOD) and catalase (CAT). *Cinnamomum burmanni* plant contains flavonoids, cinnamaldehyde, phen, and olic acid, potentially exogenous antioxidants. The study aims to analyze the potential of *C. burmanni* leaf extract for SOD and CAT levels, sperm quality, epithellia tubulus thickness, tubulus seminiferus diameter, and number of spermatogenic *Rattus norvegicus* cells exposed to NPs. Twenty-five male *R. novergicus* are divided into five groups (n = 5): two controls (without and with NPs) and three groups (combination of NPs and variations in extract concentrations of 100, 200, and 400 mg/kg. The SOD and CAT levels were measured with an ELISA kit. The histology was observed by counting the spermatogenic cells, measuring the epithellia thickness, and tubulus seminiferus diameter. The sperm motility, viability, and sperm count were observed to determine the sperm quality. Leaf extract of *C. burmanni* treatment with different concentrations not significantly increased SOD levels but significantly decreased the levels of CAT ($P<0.05$). The addition of *C. burmanni* leaf extract significantly increased the spermatogenic cell count, epithelia tubulous thickness, sperm viability, and sperm count ($P<0.05$), while sperm motility and tubulous seminiferous diameter not significantly increased. According to the results, *C. burmanni* leaf extract has antioxidant potential against the toxic effects of NPS.

1. Introduction

Plastic production has increased over the past 70 years from 1.7 million tonnes in the 1950s, and annual plastic production has continued to grow to 368 million tonnes by 2019; that number is expected to increase by 2050 (Sangkham *et al.* 2022; Cunningham *et al.* 2023). Based on the size, plastics are classified into three groups: macroplastics (>5 µm), microplastics (1-5 µm), and nanoplastics (<1 µm) (Hayati *et al.* 2022; Masseroni *et al.* 2022). Nanoplastics (NPs) are more toxic than microplastics (MPs) because they can enter the body through respiration, digestion, and skin (Lai *et al.* 2022; Haldar *et al.* 2023). Due to their small size, NPs can penetrate the biological barrier, enter the cells through the endocytosis pathway, and accumulate in cells (Yee *et al.* 2021; Joksimovic *et al.* 2022; Lai *et al.* 2022). The accumulation of NPs in the body and cells can cause disturbances in the reproductive system because they provoke the blood-testis barrier (BTB) damage in the testis (Xu *et al.* 2023). Nanoplastics that succeed in penetrating the BTB can cause a decrease in

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sperm quality and testosterone hormone levels so that the number of sperm cells and whole spermatid cells also decreases (Song *et al.* 2023; Xu *et al.* 2023). The toxicity of NPs to the reproductive system is caused by the excessive presence of reactive oxygen species (ROS) (Liu *et al.* 2020).

Reactive oxygen species (ROS) are free-radical molecules consisting of anion superoxide (O_2^-), hydroxyl (HO^*), hydroperoxyle (HO_2^*), and hydrogen peroxide (H_2O_2) (Chelombitko 2018). Normally, ROS are produced by mitochondria due to a biochemical reaction in the mitochondrial respiration involving electron transport chain processes (Checa and Aran 2020; Juan *et al.* 2021). Increased levels of ROS due to exposure to NPs can cause an imbalance of endogenous antioxidant enzymes, namely superoxide dismutase (SOD) and catalase (CAT). Superoxide dismutase is an enzyme that converts O_2^- to H_2O_2 and O_2, while CAT is an enzyme that converts H_2O_2 to water and O_2 (Ighodaro and Akinloye 2018). SOD and CAT levels will decrease because they can not balance high ROS levels (Awang Daud *et al.* 2022).

The prevention of oxidative stress effects that caused by the increasing levels of ROS can be prevented by adding exogenous antioxidant from plants, such as cinamon. *Cinnamomum burmanni* leaves extract has a potential as an antioxidant with IC_{50} values 95.52- 97.88 ppm (Kuspradini *et al.* 2016). That antioxidant potential is caused by the bioactive compounds contained in *C. burmanni* extract, such as cinnamaldehyde, flavonoids, and phenolic acid (Ervina *et al.* 2019; Muhammad *et al.* 2021a). The antioxidant in *C. burmanni* leaf extract acts as a scavenger to the free radicals so that the levels of free radicals decrease (Lee *et al.* 2023). Besides, the antioxidant activities of *C. burmanni* include metal ion chelation to prevent more reactive ROS from forming such a lipid peroxide and DNA damage do not occur. Antioxidants of *C. burmanni* also can inhibit the enzymes that produce ROS so that they can suppress the levels of ROS (Kumar and Pandey 2013; Davaatseren *et al.* 2017). Based on the explanations above, this study aims to analyze the antioxidant potential of *C. burmanni* leaf extract *in vivo* in rats exposed to NPs. This study was done to know the potential of *C. burmanni* leaves extract to SOD levels, CAT levels, and the testis function disturbance caused by NPS.

2. Materials and Methods

2.1. *Cinnamoum burmanni* Extraction

The extraction of 3 kilograms of *C. burmanni* leaves obtained from Kebun Raya Purwodadi, Purwodadi, Indonesia, was carried out by maseration method using 96% ethanol (Merck Millipore, Darmstadt, Germany). The leaves of *C. burmanni* were washed and then dried for 14 days without direct sunlight. Dried leaves are ground and soaked in ethanol for 3 × 24 hours by mixing every 24 hours. The result of the maceration is filtered using filter paper and inserted into a rotary evaporator (at a temperature of 40-55°C) to obtain a thick extract.

2.2. Animals and Ethical Statement

Rattus norvegicus male strain Wistar (150-200 grams) is 3 months old and was obtained from the Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia. The rats were kept at the Animal Laboratory, Faculty of Science and Technology, Airlangga University, at a temperature of 20°C with a 12-hour light/12-hour dark cycle. Rats are provided free access to food and drink. The use of *R. norvegicus* as a trial animal has been approved by the Ethical Clearance Commission of the Faculty of Dental Medicine, Airlangga University (Number: 381/HRECC.FODM/IV/2023).

2.3. Experimental Design

Twenty-five male *R. novergicus* are divided into five groups: (1) the control group without treatment, (2) 10 µL/kg BW NPs, (3) 10 µL/kg BW NPs + *C. burmanni* leaf extract 100 mg/kg BW, (4) 10 µL/kg BW NPs + *C. burmanni* leaf extract 200 mg/kg BW, and (5) 10 µL/kg BW NPs + *C. burmanni* leaf extract 400 mg/kg BW. The treatment is given for 42 days with the administration of NPs for 14 days and leaf extracts for 28 days. Nanoplastics were obtained from Sigma-Aldrich Inc. (Buchs, Switzerland).

2.4. Biochemical Assay

Blood obtained from *R. norvegicus* is certified to obtain blood serum. Blood serum is used to measure SOD and CAT levels. SODs and CATs are measured using ELISA SOD kits (BT Lab E0290Mo, Shanghai, China) and ELISA CAT kits (BT Lab E0076Mo, Shanghai, China).

2.5. Sperm Count, Motility, and Viability

Spermatozoa were obtained from cauda epididymis *R. norvegicus* in five research groups and then placed in the NaCl 0.9% solution. The sperm count was calculated using the Improved Neubauer Chamber (Assistant, Germany) with a light microscope (Olympus CX23). The sperm motility was observed using OptilabViewer4 software with a light microscope (Olympus CX23) by measuring the mileage of sperm cells for 10 seconds. The viability of spermatozoa was observed with eosin (1%) and nigrosin (10%) coloring with a light microscope at 10x10 magnification (Olympus CX23) by counting the colored and non-colored sperm cells.

2.6. Spermatogenic Cells Count

Testicles obtained from *R. norvegicus* in five research groups were inserted into a 10% NBF solution, which would subsequently go into the histological preparations phase of the paraffin method. The testicles embedded in paraffin were cut at a thickness of 4 µm and given hematoxylin-eosin coloring. Observations were performed under a light microscope (Olympus CX23) on a 10 × 10 zoom and OptilabViewer4 software. Spermatogenic cell count, tubular epithelial thickness, and tubular diameter were calculated using ImageRaster software.

2.7. Statistical Analysis

Statistical analysis using GraphPad Prism 10.1.0. The obtained data were tested for normality and homogeneity using the Shapiro-Wilk test ($P>0.05$). The data were then analyzed using One Way Analysis of Variance (ANOVA) ($P<0.05$) and the Kruskal-Wallis test ($P<0.05$) to determine the presence of significant differences in the data. Statistical tests were followed by Tukey's and Dunn's tests ($P<0.05$) to identify differences between treatment groups.

3. Results

3.1. *C. burmanni* Leaves Extract Influences SOD and CAT Levels in Rats Exposed by NPs

SOD levels increase after the addition of *C. burmanni* extract, although there is no significant difference between groups. The *C. burmanni* leaf extract also influences CAT by decreasing its levels. A significant decrease in CAT levels occurs when adding 100 mg/kg and 400 mg/kg *C. burmanni* leaves extract (Table 1).

Table 1. SOD and CAT levels on study groups

Treatment	SOD levels (ng/ml)	CAT levels (ng/ml)
Control	14.35±3.23	3.61±0.086
10 µL/kg NPs	8.654±4.28	3.98±0.26
10 µL/kg NPs & 100 mg/kg extract	9.79±1.66	3.3±0.23*
10 µL/kg NPs & 200 mg/kg extract	8.26±3.78	3.61±0.01
10 µL/kg NPs & 400 mg/kg extract	14.32±2.72	3.39±0.26*

Values show as means ± SD of 5 experiments. *P<0.05; compared to 10 µL/kg NPs group

3.2. *C. burmanni* Leaves Extract Repairs The Reproductive System

Sperm viability increased in all groups of *C. burmanni* leaves extract treatment, but a significant increase occurred in the 400 mg/kg group. Sperm motility also increased due to adding *C. burmanni* extract, although there was no significant difference between groups. The sperm count also increased dramatically after the *C. burmanni* extract treatment in all concentrations (Table 2).

The *C. burmanni* extract significantly increased the number of spermatogenic cells in the 400 mg/kg *C. burmanni* extract group, both in the number of spermatogonium, primary spermatocyte, and round spermatid. The epithelium thickness significantly increased in all *C. burmanni* extract treatment groups (100 mg/kg, 200 mg/kg, and 400 mg/kg). However, although there was an increase in diameter, there was no significant difference between groups in tubule seminiferous diameter (Table 2).

4. Discussion

In this study, NPs exposure to *R. norvegicus* decreased SOD levels significantly. The results of this study are similar to those of Li *et al.* (Li *et al.* 2020), in which SOD levels decreased after NPs exposure to freshwater shrimp. A decrease in SOD levels was also in zebrafish exposed to NPs for 14 days at concentrations of 10 µg/L and 100 µg/L (Umamaheswari *et al.* 2021). The endogenous antioxidant SOD is the body's first defense against ROS by converting anion superoxide (O_2^-) into hydrogen peroxide (H_2O_2) with low reactivity (Hu and Palić 2020). At the first exposure to NPs, the SOD levels will increase to fight the ROS production in the body, but as the exposure time increase, the SOD levels will decrease due to the excessive exposure of NPs, which can cause an increase in ROS production that will decrease SOD synthesis in the body (Feng *et al.* 2022). The addition of *C. burmanii*

Table 2. Effect of *C. burmanni* leaves extract on rat reproductive system

Parameter	Treatment				
	Control	10 µL/kg NPs	10 µL/kg NPs & 100 mg/kg extract	10 µL/kg NPs & 200 mg/kg extract	10 µL/kg NPs & 400 mg/kg extract
Viability (%)	81±2	35±1.64	56±1.51	68±3.39	77±0.54*
Motility (µm/s)	3.94±8.5	26.8±10.2	29.7±8.3	34.6±5	36.1±8.6
Sperm count (U/L)	88775±23603	43662±299	84736±527****	91776±909****	91960±1220****
Spermatogonium	110.3±5.2	85.4±2.7	92.3±4.6	96.3±7.9	106.5±8.8***
Primary spermatocyte	107.7±3.7	84.4±1.5	91.5±5.5	94.2±7.4	103.1±7.6***
Round spermatid	110.6±6	88.1±3.9	96.5±6.63	95.7±13.5	111.1±3.62***
Tubulus seminiferus diameter (µm)	335.6±20.2	284.1±27.6	302.7±9.6	319.2±38.7	330±21.5
Epithellium thickness (µm)	73.9±5.3	55.2±2	62.6±3.6*	67±2***	70.2±3.7****

Values show as means ± SD of 5 experiments. *P<0.05; ***P<0.001; ****P<0.0001 compared to 10 µL/kg NPs group

extract increased the SOD levels, although there were no significant differences. The same results were also in another study where the addition of *C. cassia* extract increased SOD levels (Liao *et al.* 2012). Increased levels of SOD by *C. burmanni* extract due to the contents of a secondary metabolite compound, cinnamaldehyde. Cinnamaldehyde will trigger upregulation of the nuclear factor erythroid 2-related factor 2 (NRF-2) which will subsequently trigger SOD synthesis so that SOD levels increase (Susilowati *et al.* 2022).

Observations of CAT levels differ from the results obtained from SOD levels. In this study, CAT levels after NP exposure increased, although not significantly. The research results on NP's exposure to increased CAT levels were also in some other studies (Babaei *et al.* 2020, 2022; Sincihu *et al.* 2023). The increase in CAT levels due to NPS exposure is likely because CAT is the primary defense in splitting ROS H_2O_2, which increased because of NPs exposure to H_2O and O_2 (Babaei *et al.* 2020, 2022) . The addition of *C. burmanni* extract decreased the levels of CAT in the blood serum of mice. A decrease in CAT levels with the addition of Cinnamomum extract was also in a study conducted by El-Baz et al (El-Baz *et al.* 2023). The reduction in CAT levels due to the addition of *C. burmanni* extract occurs because the antioxidant content in the extract can neutralize ROS by donating one of its electrons to free radicals (El-Houssiny *et al.* 2012).

Sperm quality includes motility, viability, and sperm count decreased due to NPs exposure based on observations. A decrease in sperm quality was also in other studies using PS-NPs of different sizes, 25 nm, 50 nm, and 100 nm (Xu *et al.* 2023). Other research also showed similar results in which the quality of sperm decreased as a result of oral NPs exposure to male rats (Huang *et al.* 2022). The decrease in sperm quality is due to increased ROS levels because of NPs exposure. Increased levels of ROS result in lipid peroxidation of the lipid structures of the cell membrane containing polyunsaturated fatty acids (PUFA) (Mattioli *et al.* 2022). Damage to cell membranes results in an influx of Ca^{2+} ions in the cell so that the cell swells and necrosis occurs (Zhou *et al.* 2019). The decrease in sperm quality can be suppressed with the administration of *C. burmanni* extract, as done in this study. A similar study was also conducted by other researchers where the administration of *C. zeylanicum* extract at 75 mg/kg increases the motility, viability, and number of sperm cells (Khaki 2015). A significant increase also occurs in viability and sperm count in the result. There was also an increase in motility, although there were no significant differences. An improvement in sperm quality due to the addition of *C. burmanni* extract occurs because *C. burmanni* extract contains bioactive compounds, flavonoids, and cinnamaldehyde (Chegini *et al.* 2019). Flavonoids and cinnamaldehyde act as antioxidants that neutralize free radicals by donating one of its electrons (Speisky *et al.* 2022). Free radical neutralization by flavonoids and cinnamaldehyde will disrupt the reduction in ROS levels so that sperm quality improves.

Increased levels of ROS due to NPs exposure also resulted in a significant decrease in the number of spermatogenic cells, resulting in a reduction in epithelial thickness and tubulous diameter. Other studies have similar results: a decreased spermogenic cell count in mice exposed to NPs (Triwahyudi *et al.* 2023). A reduction of tubules epithelium thickness in mice was also in other studies using PS-NPs at concentrations of 0.2 mg/kg, 1 mg/kg, and 10 mg/kg (Zhou *et al.* 2022). A decrease in the number of spermatogenic cells occurs because high levels of ROS can trigger apoptosis or cell death through the intrinsic pathway. High levels of ROS

may trigger the p53 protein to stimulate the activity of the BCl-2 homology 3 (BH3) protein. The BH3 protein will inhibit the Bcl-2 protein, which is antiapoptosis, and boost the Bax protein, which is proapoptosis. Bax will then head to the mitochondria and damage the outer membrane, causing the release of cytochrome C (cytC) into the cytoplasm. The presence of cytC in the cytoplasm will form an apoptosome complex with caspase nine and apoptotic protease-activating factor 1 (APAF-1) which will then activate caspace three and therefore occur apoptosis (Franklin 2011; Patel *et al.* 2020).

In this study, adding *C. burmanni* extracts improved the thickness of the epithelium by significantly increasing the number of spermatogenic cells. The increasing number of spermatogenic cells also increased the diameter of tubulus, although there were no significant differences. The *C. burmanni* extract can prevent the apoptosis pathway, as explained in the previous paragraph. The findings are similar to those obtained by Arisha *et al.* (2023), where the addition of *C. zeylanicum* extract prevents apoptosis in spermatogenic cells, resulting in a decrease in the thickness of the epithelium and low tubules diameter (Arisha *et al.* 2023). *C. burmanni* extract contains secondary metabolite compounds such as phenolic acid, cinnamaldehyde, and flavonoids that are potentially antioxidant (Ervina *et al.* 2019; Muhammad *et al.* 2021b). The secondary compound can neutralize free radicals by donating one of its electrons, thereby reducing free radical reactivity and preventing apoptosis in spermatogenic cells (Speisky *et al.* 2022). Based on the discussion above, *C. burmanni* extract has the potential to be an antioxidant against NPs exposure by improving homeostasis or the balance of endogenous antioxidants, SOD, and CAT. *C. burmanni* leaf extract also has the potential to prevent infertility by enhancing the quality of spermatozoa and spermatogenic cells.

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References

Arisha, S.M., Sakr, S.A. and Abd-Elhaseeb, F.R., 2023. *Cinnamomum zeylanicum* alleviate testicular damage induced by high fat diet in albino rats; histological and ultrastructural studies. *Heliyon*. 9, e17025. https://doi.org/10.1016/j.heliyon.2023.e17025

Awang Daud, D.M., Ahmedy, F., Baharuddin, D. M. P., & Zakaria, Z. A., 2022. Oxidative stress and antioxidant enzymes activity after cycling at different intensity and duration. *Appl. Sci.* 12, 9161. https://doi.org/10.3390/app12189161

Babaei, A.A., Rafiee, M., Khodagholi, F., Ahmadpour, E., & Amereh, F., 2020. Physiological stress response of the Wistar albino rats orally exposed to polystyrene nanoparticles. *Research Square* [Preprint]. https://doi.org/10.21203/rs.3.rs-23042/v1

Babaei, A.A., Rafiee, M., Khodagholi, F., Ahmadpour, E., & Amereh, F., 2022. Nanoplastics-induced oxidative stress, antioxidant defense, and physiological response in exposed Wistar albino rats. *Environmental Science and Pollution Research*. 29, 11332-11344. https://doi.org/10.1007/s11356-021-15920-0

Checa, J., Aran, J.M., 2020. Reactive oxygen species: drivers of physiological and pathological processes. *Journal of Inflammation Research*. 13, 1057-1073. https://doi.org/10.2147/JIR.S275595

Chegini, R., Soleimani, P., Sadeghi, M., Yosef, R. M., & Zafari, F., 2019. Investigating the effect of fennel and cinnamon combined extract on spermatogenesis and testis tissues in busulfan induced infertile rats. *Journal of Applied Biotechnology Reports*. 6, 96-100. https://doi.org/10.29252/JABR.06.03.03

Chelombitko, M.A., 2018. Role of reactive oxygen species in inflammation: a minireview. *Moscow University Biological Sciences Bulletin*. 73, 199-202. https://doi.org/10.3103/S009639251804003X

Cunningham, B.E., Sharpe, E.E., Brander, S.M., Landis, W.G., Harper, S.L., 2023. Critical gaps in nanoplastics research and their connection to risk assessment. *Frontiers in Toxicology*. 5, 1-16. https://doi.org/10.3389/ftox.2023.1154538

Davaatseren, M., Jo, Y.J., Hong, G.P., Hur, H.J., Park, S., Choi, M.J., 2017. Studies on the anti-oxidative function of trans-cinnamaldehyde-included β-cyclodextrin complex. *Molecules: A Journal of Synthetic Chemistry and Natural Product Chemistry*. 22, 1-12. https://doi.org/10.3390/molecules22121868

El-Baz, Y.G., Moustafa, A., Ali, M.A., El-Desoky, G.E., Wabaidur, S.M., Faisal, M.M., 2023. An analysis of the toxicity, antioxidant, and anti-cancer activity of cinnamon silver nanoparticles in comparison with extracts and fractions of cinnamomum cassia at normal and cancer cell levels. *Nanomaterials*. 13, 1-19 . https://doi.org/10.3390/nano13050945

El-Houssiny, A.S., Ward, A.A.M., Mansour, S.H., Abd- El- Messieh, S.L., 2012. Biodegradable blends based on polyvinyl pyrrolidone for insulation purposes. *Journal of Applied Polymer Science*. 124, 3879-3891. https://doi.org/10.1002/app.35483

Ervina, M., Lie, H.S., Diva, J., Caroline, Tewfik, S., Tewfik, I., 2019. Optimization of water extract of *Cinnamomum burmannii* Bark to ascertain its *in vitro* antidiabetic and antioxidant activities. *Biocatalysis and Agricultural Biotechnology,* 19, 101152. https://doi.org/10.1016/j.bcab.2019.101152

Feng, M., Luo, J., Wan, Y., Zhang, J., Lu, C., Wang, M., Dai, L., Cao, X., Yang, X., Wang, Y., 2022. Polystyrene nanoplastic exposure induces developmental toxicity by activating the oxidative stress response and base excision repair pathway in Zebrafish (*Danio rerio*). *ACS Omega*. 7, 32153-32163. https://doi.org/10.1021/acsomega.2c03378

Franklin, J.L., 2011. Redox regulation of the intrinsic pathway in neuronal apoptosis. *Antioxidants and Redox Signaling*. 14, 1437-1448. https://doi.org/10.1089/ars.2010.3596

Haldar, S., Muralidaran, Y., Míguez, D., Mulla, S.I., Mishra, P., 2023. Eco-toxicity of nano-plastics and its implication on human metabolism: current and future perspective. *Science of the Total Environment*. 861, 160571. https://doi.org/10.1016/j.scitotenv.2022.160571

Hayati, A., Pramudya, M., Soepriandono, H., Astri, A.R., Kusuma, M.R., Maulidah, S., Adriansyah, W., Dewi, F.R.P., 2022. Assessing the recovery of steroid levels and gonadal histopathology of tilapia exposed to polystyrene particle pollution by supplementary feed. *Veterinary World*. 15, 517-523. https://doi.org/10.14202/vetworld.2022.517-523

Hu, M., Palić, D., 2020. Micro- and nano-plastics activation of oxidative and inflammatory adverse outcome pathways. *Redox Biology*. 37, 1-16. https://doi.org/10.1016/j.redox.2020.101620

Huang, T., Zhang, W., Lin, T., Liu, S., Sun, Z., Liu, F., Yuan, Y., Xiang, X., Kuang, H., Yang, B., Zhang, D., 2022. Maternal exposure to polystyrene nanoplastics during gestation and lactation induces hepatic and testicular toxicity in male mouse offspring. *Food and Chemical Toxicology*. 160, 112803. https://doi.org/10.1016/j.fct.2021.112803

Ighodaro, O.M., Akinloye, O.A., 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine*. 54, 287-293. https://doi.org/10.1016/j.ajme.2017.09.001

Joksimovic, N., Selakovic, D., Jovicic, N., Jankovic, N., Pradeepkumar, P., Eftekhari, A., & Rosic, G., 2022. Nanoplastics as an invisible threat to humans and the environment. *Journal of Nanomaterials*. 2022, 1-15. https://doi.org/10.1155/2022/6707819

Juan, C.A., de la Lastra, J.M.P., Plou, F.J., Pérez-Lebeña, E., 2021. The chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. *International Journal of Molecular Sciences*. 22, 1-21. https://doi.org/10.3390/ijms22094642

Khaki, A., 2015. Effect of *Cinnamomum zeylanicumon* on spermatogenesis. *Iranian Red Crescent Medical Journal*. 17, 1-4. https://doi.org/10.5812/ircmj.18668

Kumar, S., Pandey, A.K., 2013. Chemistry and biological activities of flavonoids: an overview. *The Scientific World Journal*. 2013, 1-16. https://doi.org/10.1155/2013/162750

Kuspradini, H., Putri, A.S., Sukaton, E., Mitsunaga, T., 2016. Bioactivity of essential oils from leaves of *Dryobalanops lanceolata, Cinnamomum burmannii, Cananga odorata*, and *Scorodocarpus borneensis*. *Agriculture and Agricultural Science Procedia*. 9, 411-418. https://doi.org/10.1016/j.aaspro.2016.02.157

Lai, H., Liu, X., Qu, M., 2022. Nanoplastics and human health: hazard identification and biointerface. *Nanomaterials*. 12, 1-13. https://doi.org/10.3390/nano12081298

Lee, P.H., Lin, I.H., Hsieh, P.C., 2023. Measurement of bioactive compounds, antioxidants, and α-glucosidase and α-amylase inhibitory effects of *Cinnamomum osmophloeum* Kanehira extracted with various solvents. *Measurement: Food*. 9, 100069. https://doi.org/10.1016/j.meafoo.2022.100069

Li, Y., Liu, Z., Li, M., Jiang, Q., Wu, D., Huang, Y., Jiao, Y., Zhang, M., Zhao, Y., 2020. Effects of nanoplastics on antioxidant and immune enzyme activities and related gene expression in juvenile *Macrobrachium nipponense*. *Journal of Hazardous Materials*. 398, 122990. https://doi.org/10.1016/j.jhazmat.2020.122990

Liao, J.C., Deng, J.S., Chiu, C.S., Hou, W.C., Huang, S.S., Shie, P.H., Huang, G.J., 2012. Anti-inflammatory activities of *Cinnamomum cassia* constituents *in vitro* and *in vivo*. *Evidence-based Complementary and Alternative Medicine*. 2012, 1-12. https://doi.org/10.1155/2012/429320

Liu, Z., Huang, Y., Jiao, Y., Chen, Q., Wu, D., Yu, P., Li, Y., Cai, M., Zhao, Y., 2020. Polystyrene nanoplastic induces ROS production and affects the MAPK-HIF-1/NFkB-mediated antioxidant system in *Daphnia pulex*. *Aquatic Toxicology*. 220, 105420. https://doi.org/10.1016/j.aquatox.2020.105420

Masseroni, A., Rizzi, C., Urani, C., Villa, S., 2022. Nanoplastics: status and knowledge gaps in the finalization of environmental risk assessments. *Toxics*. 10, 1-22. https://doi.org/10.3390/toxics10050270

Mattioli, S., Dimauro, C., Cesarani, A., Dal Bosco, A., Bartolini, D., Galli, F., Migni, A., Sebastiani, B., Signorini, C., Oger, C., Collodel, G., Castellini, C., 2022. A dynamic model for estimating the interaction of ROS-PUFA-antioxidants in rabbit. *Antioxidants*. 11, 1-20. https://doi.org/10.3390/antiox11030531

Muhammad, D.R.A., Tuenter, E., Patria, G.D., Foubert, K., Pieters, L., Dewettinck, K. 2021a. Phytochemical composition and antioxidant activity of *Cinnamomum burmannii* Blume extracts and their potential application in white chocolate. *Food Chemistry*. 340, 127983. https://doi.org/10.1016/j.foodchem.2020.127983

Muhammad, D.R.A., Tuenter, E., Patria, G.D., Foubert, K., Pieters, L., Dewettinck, K., 2021b. Phytochemical composition and antioxidant activity of *Cinnamomum burmannii* Blume extracts and their potential application in white chocolate. *Food Chemistry*. 340, 127983. https://doi.org/10.1016/j.foodchem.2020.127983

Patel, M.Y., Stovall, K., Franklin, J.L., 2020. The intrinsic apoptotic pathway lies upstream of oxidative stress in multiple organs. *Free Radical Biology and Medicine*. 158, 13-19. https://doi.org/10.1016/j.freeradbiomed.2020.05.025

Sangkham, S., Faikhaw, O., Munkong, N., Sakunkoo, P., Arunlertaree, C., Chavali, M., Mousazadeh, M., Tiwari, A., 2022. A review on microplastics and nanoplastics in the environment: their occurrence, exposure routes, toxic studies, and potential effects on human health. *Marine Pollution Bulletin*. 181, 113832. https://doi.org/10.1016/j.marpolbul.2022.113832

Sincihu, Y., Lusno, M.F.D., Mulyasari, T.M., Elias, S.M., Sudiana, I.K., Kusumastuti, K., Sulistyorini, L., Keman, S., 2023. Wistar rats hippocampal neurons response to blood low-density polyethylene microplastics: a pathway analysis of SOD, CAT, MDA, 8-OHdG expression in hippocampal neurons and blood serum Aβ42 levels. *Neuropsychiatric Disease and Treatment*. 19, 73-83. https://doi.org/10.2147/NDT.S396556

Song, X., Du, L., Sima, L., Zou, D., & Qiu, X., 2023. Effects of micro (nano) plastics on the reproductive system: a review. *Chemosphere*. 336, 139138. https://doi.org/10.1016/j.chemosphere.2023.139138

Speisky, H., Shahidi, F., de Camargo, A.C., Fuentes, J., 2022. Revisiting the oxidation of flavonoids: loss, conservation or enhancement of their antioxidant properties. *Antioxidants*. 11, 1-28. https://doi.org/10.3390/antiox11010133

Susilowati, R., Setiawan, A.M., Zahroh, A.F., Ashari, Z.N., Iffiyana, A., Hertanto, R., Basyarudin, M., Hartiningsih, I., Ismail, M., 2022. Hepatoprotection of *Cinnamomum burmannii* ethanolic extract against high-fat and cholesterol diet in sprague-dawley rats (*Rattus norvegicus*). *Veterinary World*. 15, 930-936. https://doi.org/10.14202/vetworld.2022.930-936

Triwahyudi, H., Soehargo, L., Muniroh, L., Qolbi, R.N., Aini, T.Q., Kurnia, R.F.Z., Putra, P.A.D., Pramudya, M., Muchtaromah, B., Hayati, A., 2023. Potential of red seaweed (*Dichotomania obtusata*) on immune response and histopathology of rat testis exposed to nanoplastics. *Tropical Journal of Natural Product Research*. 7, 2969-2973. https://doi.org/10.26538/tjnpr/v7i5.20

Umamaheswari, S., Priyadarshinee, S., Kadirvelu, K., Ramesh, M., 2021. Polystyrene microplastics induce apoptosis via ROS-mediated p53 signaling pathway in zebrafish. *Chemico-Biological Interactions*. 345, 109550. https://doi.org/10.1016/j.cbi.2021.109550

Xu, W., Yuan, Y., Tian, Y., Cheng, C., Chen, Y., Zeng, L., Yuan, Y., Li, D., Zheng, L., Luo, T., 2023. Oral exposure to polystyrene nanoplastics reduced male fertility and even caused male infertility by inducing testicular and sperm toxicities in mice. *Journal of Hazardous Materials*. 454, 131470. https://doi.org/10.1016/j.jhazmat.2023.131470

Yee, M.S.L., Hii, L.W., Looi, C.K., Lim, W.M., Wong, S.F., Kok, Y.Y., Tan, B.K., Wong, C.Y., Leong, C.O., 2021. Impact of microplastics and nanoplastics on human health. *Nanomaterials*. 11, 1-23. https://doi.org/10.3390/nano11020496

Zhou, L., Yu, Z., Xia, Y., Cheng, S., Gao, J., Sun, W., Jiang, X., Zhang, J., Mao, L., Qin, X., Zou, Z., Qiu, J., Chen, C. 2022. Repression of autophagy leads to acrosome biogenesis disruption caused by a sub-chronic oral administration of polystyrene nanoparticles. *Environment International*. 163, 107220. https://doi.org/10.1016/j.envint.2022.107220

Zhou, Z., Zhou, B., Chen, H., Tang, X., Wang, Y., 2019. Reactive oxygen species (ROS) and the calcium-(Ca^{2+}) mediated extrinsic and intrinsic pathways underlying BDE-47-induced apoptosis in rainbow trout (*Oncorhynchus mykiss*) gonadal cells. *Science of the Total Environment*. 656, 778-788. https://doi.org/10.1016/j.scitotenv.2018.11.306