***Original Research Article***

**Effects of Polyethylene Terephthalate Microplastics on CA 15-3 Breast Cancer Marker, TNF-α and Breast Tissues of Chronically Exposed Female Albino Rats**

.

ABSTRACT

|  |
| --- |
| Breast cancer remains the leading cause of cancer-related deaths among women, with approximately 670,000 deaths globally. Emerging studies have linked exposure to various substances, including microplastics, to organ damage and cancer development. Polyethylene terephthalate (PET) is a common plastic polymer in Nigeria and a popular choice for beverage bottles, food packaging, and synthetic fibers. Over time, PET products can degrade into microplastics, contributing significantly to pollution and human toxicity.  **Aim:** This study evaluated the effects of chronic exposure to PET-derived microplastics on CA 15-3 breast cancer marker, Tumor Necrosis Factor-alpha (TNF-α), and breast tissues in female albino rats.  **Methodology:** Thirty-five female albino rats weighing 150–170g were used for the study. The rats were acclimatized for 14 days and randomly divided into five groups with 7 rats in each group. PET pellets were crushed to microplastics, dissolved in water and filtered before use. Group 2, 3, and 4 rats received 40mg/kg, 80mg/kg, and 120mg/kg PET-microplastics respectively, administered orally using gavage tubes for 90 days. Group 5 rats were given water exposed to sunlight between 10 am to 4pm daily in PET1 containers for 30 days, while Group 1 served as the negative control with standard food and water. After treatment, blood samples were collected to assess CA 15-3 and TNF-α using ELISA method, and breast tissues were also collected for histological examination. Statistical analysis was performed using GraphPad Prism (Version 9.0.0), with significance set at *P*≤0.05.  **Results:** Results obtained showed that the mean levels of TNF-α in the rats exposed to PET were significantly higher than the levels in the control group at *P*<0.001. However, CA 15-3 levels showed no significant difference between PET-exposed rats and the control rats at *P*=0.077, indicating that CA 15-3 was not notably affected by PET exposure. Histological analysis revealed fibrocystic changes in the breast tissues of PET-exposed rats.  **Conclusion:** These findings suggest that chronic PET microplastics exposure induced inflammatory responses and histological breast tissue alterations such as fibrocystic changes, which are significant indicators of tissue remodeling and potential disease risk. This highlights the potential health risks associated with prolonged PET exposure. |

*Keywords: Breast cancer, Inflammation, Polyethylene Terephthalate (PET), Microplastics*

1. INTRODUCTION

Breast cancer, which is an abnormal, uncontrolled growth of cells in the breast tissue, is currently the 5th cause of cancer-related deaths, with an estimated 2.3 million new cases worldwide (Sung *et al.,* 2021; Kumar *et al.,* 2022). It is one of the most frequently occurring cancers among women, remaining a major public health concern due to its high occurrence and increasing trend (Devi *et al.,* 2021). In 2022, there were 2.3 million women diagnosed with breast cancer and 670 000 deaths globally (WHO, 2024). It is most common in developed countries but rapidly rises in developing countries (Sun *et al.,* 2022). Breast cancer rates vary due to genetic manipulations, lifestyle factors, early detection and treatment access, making understanding risk factors and etiology crucial for prevention, early detection, and treatment. Recently, different natural and chemical substances, including microplastics, have been implicated in causing damage to organs of the body, including inflammatory response and cancer (Yee *et al.,* 2021; Park *et al.,* 2023; Li *et al.,* 2024).

Microplastics are plastic particles with a diameter less than 5 mm, and recently, there has been a global recognition of the potential damage and threat they pose to the environment and human health (Priya *et al.,* 2022; Ziani *et al.,* 2023). With a tremendous increase in the worldwide use of plastics (Li *et al.,* 2023), recently, microplastic particles have been classified as a new environmental pollutant due to their increased toxicity as a result of high production and extremely low natural biodegradation in the ecosystem (Prata, 2018; Li *et al.,* 2023). There are also increasing reports of microplastics in drinking water and food products (Schymanski *et al.,* 2018), which can enter the human body from the environment or through food (Deng *et al.,* 2017; Schwabl *et al.,* 2019).

Major commercial plastic polymers in the market include polypropylene (PP), polyethylene (PE), polyethylene terephthalate (PET), polystyrene (PS), and polyvinyl chloride (PVC) (Sharma *et al.,* 2023). Polyethylene terephthalate (PET) is a widely used plastic polymer, especially in Nigeria, particularly favored for its robustness, clarity, and food safety, making it a popular choice for beverage bottles, food packaging, and synthetic fibers (Dhaka *et al.,* 2022). Over time and through physical wear, exposure to UV light, and other environmental stressors, PET products can degrade and fragment into smaller pieces (microplastics), which can persist in the environment, contributing significantly to pollution and human toxicity (De Vos *et al.,* 2021). Also, it has been reported that when these plastics are used in food packaging and bottling of water, microplastics have the tendency of leaching into foods (serving as a potential source of toxicity affecting human health), and contributing to various responses and diseases in the body, which may include inflammatory response and cancer (Campanale *et al.,* 2020). Due to the adverse effects of microplastics, various international organizations have banned their use, especially phthalates, in various household products such as toys and childcare articles in concentrations above 0.1% (The Danish EPA, 2013; The REACH Regulation, 2019).

As these microplastics (MPs) pose significant environmental and health concerns, the long-term effects of MPs on human health and chronic diseases, including cancer, are currently unknown or not extensively studied, especially in this part of the world (Rahman *et al.,* 2021). Few studies have shown that the ingestion of MPs can cause an inflammatory response and can damage the gut, cause organ damage, and affect reproduction and metabolism (Li *et al.,* 2024). Also, Campanale *et al.* (2020) reported that MPs introduced through skin contact can cause skin damage due to local inflammation and cellular toxicity. Further, most plastic products have been known to release estrogenic chemicals or endocrine-disrupting chemicals (EDCs), and exposure to EDCs and estrogenic chemicals released by most plastics may cause hormonal imbalances as well as increasing the risk of cancer, including breast cancer (Rodgers *et al.,* 2018; Kannan and Vimalkumar, 2021). Park *et al.* (2023), in their study on polypropylene microplastics, found that moderate amounts of polypropylene microplastics (PPMPs) significantly accelerated the cell cycle of cancer cells and enhanced the secretion of interleukin 6 (IL-6) in the human breast cancer cell lines MDA-MB-231 and MCF-7, stating that chronic exposure to PPMPs may increase the risk of cancer progression and metastasis.

Currently, there is a dearth of literature on the risks of PET MPs relating to inflammatory response and cancer development or metabolic diseases, especially in Nigeria, where breast cancer remains one of the most common cancers, affecting millions of people with rising incidence rates. This highlights the urgent need for research into all potential risk factors, including environmental pollutants like microplastics; hence the need for this study. This study was therefore conducted to evaluate the effects of chronic exposure to polyethylene terephthalate (PET) microplastics on CA 15-3 breast cancer marker, tumor necrosis factor-α (TNF-α), and breast tissues of female albino rats. Understanding the effects of these particles on human health, particularly in relation to inflammatory response and breast cancer, is crucial for assessing potential risks, effective diagnosis, prevention, and implementing appropriate regulatory measures to improve health and outcomes.

2. material and methods

**2.1 Materials**

Female albino rats used in this study were obtained from the University of Port Harcourt Teaching Hospital and transported in a well-ventilated wired cage to the Animal House at the Department of Animal and Environmental Biology, Rivers State University, Port Harcourt, where the study was carried out. Polyethylene terephthalate (PET, PETE), (C10H8O4)n, (CAS Number 25038-59-9), pellets used in this study were obtained from a petrochemical industry in Port Harcourt, Rivers State. Rat-specific cancer marker CA 15-3 ELISA kit (Cat. No. E1139Ra) was obtained from Bioassay Technology Laboratory, Jiaxing, Zhejiang Province, China. Rat-specific TNF-α (Tumour Necrosis Factor Alpha) ELISA Kit (Catalog No: E-EL-R2856) was obtained from Elabscience Bioinovation Inc. Other equipment used included the MindRay MR-96A Microplate Reader, NewLife Bucket Centrifuge (Model: 800D), digital weighing balance, tissue embedder (LEICA EG 1160), and rotatory microtome (LEICA RM 2125 RTS). All chemicals, stains, and reagents used for all analyses were of good quality and analytical grade.

**2.2 Experimental Animals**

Thirty-five (35) female albino rats weighing approximately 150 g–170 g were used for the study. The rats were acclimatized for 14 days prior to the initiation of the research and were permitted access to standard laboratory feed and uncontaminated drinking water *ad libitum.* The rats were placed in a well-ventilated cage in a temperature-maintained (28 ± 2 °C) and humidity-regulated (47 ± 2%) location, with a typical 12:12 light-dark photocycle. The animal experiments and handling were in consonance with the National Research Council's Guide for the Care and Use of Laboratory Animals Health (National Research Council, 2011) and the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines (Percie du Sert, 2020).

**2.3 Preparation of Polyethylene terephthalate (PET)**

Polyethylene terephthalate pellets were crushed thoroughly using a mechanical grinder to obtain tiny particles (microplastics). Then, the particles from the crushed PET were weighed using a digital weighing balance (Model: TS500), mixed with water, and allowed to stay for 24 hours, after which they were filtered using a micropore sieve before use.

**2.4 Dose Determination and Pilot Study**

A pilot study was carried out to determine the LD50 of polyethylene terephthalate (PET) microplastic administered orally after allowing 14 days of acclimatization using the Lorke’s method of pilot toxicity testing as described by Chinedu *et al.* (2013). A total of 12 rats weighing approximately 150 g–170 g were used for the pilot study. The rats were classified into 6 groups labelled 1, 2, 3, 4, 5, and 6, with groups 1–3 consisting of 3 rats each and groups 4–6 having 1 rat each, and were treated with 20 mg/kg, 50 mg/kg, 80 mg/kg, 100 mg/kg, 115 mg/kg, and 130 mg/kg PET microplastic, respectively. After 24 hours, the rats were observed for signs of PET toxicity, such as changes in feeding behaviour, micturition, restlessness, pupil constriction, and convulsion. The LD50 of the PET administered orally was obtained using Lorke’s formula:

LD50 = √ (D0 × D100)

Where:

D0 = Highest dose that gave no mortality

D100 = Lowest dose that produced mortality

**2.5 Experimental Design**

After allowing fourteen (14) days for adaptation to the new environment (acclimatization), the rats for the study were randomly assigned into five (5) groups labelled group 1 to 5 with seven (7) rats in each group, as shown in table 1.

**Table 1: Experimental Design**

|  |  |
| --- | --- |
| **Groups** | **Treatment** |
| 1 (Negative Control) | Food and water only |
| 2 (Low Dose Group) | 40mg/kg PET microplastics + Food and Water |
| 3 (Medium Dose Group) | 80mg/kg PET microplastics + Food and Water |
| 4 (High Dose Group) | 120mg/kg PET microplastics + Food and Water |
| 5 (Food and water exposed to sunlight in PET containers) | Food + water exposed to sunlight between 10am to 4pm daily for 90 days in PET 1 containers |

The PET microplastic treatment was done daily, administered orally using gavage tube for 90 days.

**2.6 Blood and Tissue Sample Collection and Preparation**

At the end of the 90-day treatment for the respective groups, the animals in all groups were anaesthetized using chloroform, after which a cardiac puncture was performed. Then, 5 ml of blood samples were collected aseptically into plain sample bottles and allowed to clot. The clotted samples were spun using a centrifuge (NewLife bucket centrifuge, Model: 800D) at 4000 rpm for 5 minutes. The serum was collected into another plain bottle and properly labeled for analysis of breast cancer marker CA 15-3 and TNF α. Breast tissues were also collected from rats in all groups to determine the histological changes in the breast tissue after exposure to PET microplastics.

**2.7 Estimation of CA 15-3 Tumour Marker**

Rat-specific cancer marker CA 15-3 ELISA kit (Cat. No. E1139Ra) from Bioassay Technology Laboratory, Jiaxing, Zhejiang Province, China, was used to measure the concentration of rat carbohydrate antigen CA 15-3 present in the samples using ELISA method according to the manufacturer’s instruction/procedure as described by Bioassay Technology Laboratory, (2024). Analysis was performed using a MindRay MR-96A Microplate Reader.

**2.8 Estimation of Rat Tumour Necrosis Factor Alpha**

Rat-specific TNF-α (Tumour Necrosis Factor Alpha) ELISA Kit (Catalog No: E-EL-R2856) from Elabscience Bioinovation Inc. was used to measure the concentration of Tumour Necrosis Factor Alpha in the samples according to the manufacturer’s instruction/procedure as described by Elabscience Bioinovation Inc., (2024). Analysis was performed using a MindRay MR-96A Microplate Reader.

**2.9 Histological Analysis**

Breast tissue was prepared for histological examination using the procedure as described by Kiernan (2001), which involves fixing the breast tissue in 10% neutral buffered formalin, dehydrating in increasing grades of alcohol (70%, 85%, 90%, 100%, and 100%) for two (2) hours each, and clearing in two changes of xylene for 30 minutes each. The tissue was embedded in paraffin wax in a tissue embedder (LEICA EG 1160) and trimmed using a rotatory microtome (LEICA RM 2125 RTS) and sectioned at 3 µm. The sectioned tissues were attached to slides and subsequently dewaxed in xylene and stained in Haematoxylin and Eosin (H&E) using the method as described by Avwioro, (2014) for general tissue architecture. The stained slides were examined under the light microscope at ×100 magnification, and photomicrographs were taken.

**2.10 Statistical Analysis**

Data obtained from evaluation of parameters was presented as mean ± SD. Analysis was computed using GraphPad Prism Software Version 9.0.0 (121), San Diego, CA. Statistical comparison between groups was done using one-way ANOVA, while Tukey’s multiple comparison (post hoc tests) was used to obtain specific significant differences among the various groups. Differences were considered significant at P≤0.05.

3. results and discussion

**3.1 Results**

**3.1.1 Pilot Toxicity Study for Determination of D0, D100 and LD50 of Rats Treated Orally with PET Microplastics**

Results obtained from the pilot toxicity study as presented in table 2 revealed that the highest dose that caused no death (D0) of PET microplastics administered orally was 115 mg/kg; the minimum dose that caused death (D100) was 130 mg/kg, while the lethal dose 50 (LD50) was 122.27 mg/kg.

**Table 2: Pilot Toxicity Study for Determination of D0, D100 and LD50 of Rats Treated Orally with PET Microplastics**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Groups** | **Dose**  **(mg/kg)** | **Number of rats** | **Number of Death** | **Number Alive** |
| 1 | 20 | 3 | 0 | 3 |
| 2 | 50 | 3 | 0 | 3 |
| 3 | 80 | 3 | 0 | 3 |
| 4 | 100 | 1 | 0 | 1 |
| \*5 | 115 | 1 | 0 | 1 |
| \*\*6 | 130 | 1 | 1 | 0 |

**KEY**

\*Highest Dose that caused no death = 115mg/kg (D0)

\*\*Minimum Dose that caused death = 130mg/kg (D100)

* = √( D0 × D100) (Lorke’s Formula).

Therefore:  = =

= 122.27mg/kg

Thus, the mean lethal dose (LD50) = 122.27mg/kg

**3.1.2 Mean ± SD of Levels of Ca 15-3 and TNF α in the Rats According to Groups**

The results of the mean values of Ca 15-3 and TNF α, as shown in table 3, revealed no significant difference in the mean levels of breast cancer marker Ca 15-3 in rats exposed to polyethylene terephthalate (PET) microplastics and the control rats at *P*=0.077. Also, the levels of CA 15-3 in the rats fed with water in PET bottles exposed to sunlight do not differ significantly from the control rats at *P*=0.077. The mean levels of TNF-α in the rats exposed to PET were significantly higher than the levels in the control group at *P*<0.001.

**Table 3. Mean ± SD of Levels of Ca 15-3, IL 1β and TNF α in the Rats According to Groups**

|  |  |  |
| --- | --- | --- |
| Groups | Ca 15-3 (uIU/ml) | TNF α (pg/ml) |
| 1 | 16.83 ± 2.84 | 26.60 ± 2.84a |
| 2 | 18.03 ± 1.54 | 61.52 ± 4.69b |
| 3 | 18.17 ± 2.42 | 80.36 ± 6.19c |
| 4 | 19.07 ± 0.59 | 84.93 ± 3.75c |
| 5 | 15.90 ± 2.18 | 51.88 ± 2.84d |
| p- value | 0.077 | <0.001 |
| F -value | 2.354 | 145.0 |
| Inference | NS | S |

**NB**. **Post Hoc Analysis (Tukey’s Test):** Values with different superscripts within a column indicate significant differences between groups when compared. Values with the same superscript on each column do not differ significantly from each other.

**Key:** NS- Not Significant; S – Significant; Ca 15-3 – Cancer Antigen 15-3; TNF α – Tumour Necrotic Factor α

**3.1.2 Histological Analysis**

|  |  |
| --- | --- |
| C:\Users\Chimzurum\Pictures\CONTROL FEBRUARY.jpg | C:\Users\Chimzurum\Pictures\B2G2 FEBRUARY.jpg |

**A B**

**Plate 1:** **Histo-architecture of the Breast Tissues of Control (A) and Group 2 (B)** **Rats.**

**(A)** Control group showing normal tissue architecture with unremarkable breast ducts (blue) and lobules (red). **(B)** Group 2, showing unremarkable breast ducts (blue) and lobules (red) also implying a normal tissue architecture. H & E ×100.

|  |  |
| --- | --- |
| C:\Users\Chimzurum\Pictures\CONTROL FEBRUARY.jpg | C:\Users\Chimzurum\Pictures\D3 G4 NICHOLAS.jpg |

**A C**

**Plate 2:** **Histo-architecture of the Breast Tissues of Control (A) and Group 3 (C)** **Rats.**

**(A)** Control group showing normal tissue architecture with unremarkable breast ducts (blue) and lobules (red). **(C)** Group 3, showing preserved breast lobules (blue) with ducts containing pinkish luminal secretion. The stroma shows increased fibrosis (black). This is suggestive of fibrocystic changes. H & E ×100.

|  |  |
| --- | --- |
| C:\Users\Chimzurum\Pictures\CONTROL FEBRUARY.jpg | C:\Users\Chimzurum\Pictures\E3 G5 NICHOLAS.jpg |

**A D**

**Plate 3:** **Histo-architecture of the Breast Tissues of Control (A) and Group 4 (D)** **Rats.**

**(A)** Control group showing normal tissue architecture with unremarkable breast ducts (blue) and lobules (red). **(D)** Group 4, showing breast ducts (blue) with pinkish luminal secretion suggestive of fibrocystic changes. H & E ×100.

|  |  |
| --- | --- |
| C:\Users\Chimzurum\Pictures\CONTROL FEBRUARY.jpg | C:\Users\Chimzurum\Pictures\C1 G1 NICHOLAS.jpg |

**A E**

**Plate 4:** **Histo-architecture of the Breast Tissues of Control (A) and Group 5 (E)** **Rats.**

**(A)** Control group showing normal tissue architecture with unremarkable breast ducts (blue) and lobules (red). **(E)** Group 5, showing unremarkable skeletal muscle (red) and fibrous tissue (blue)

**3.2 Discussion**

This study investigated the effects of chronic exposure to polyethylene terephthalate (PET) microplastics on CA 15-3 breast cancer marker, Tumour necrosis factor-α (TNF-α), and breast tissues of female albino rats.

From this study, as presented in Table 3, there was no significant difference in the mean levels of breast cancer marker CA 15-3 in rats exposed to polyethylene terephthalate (PET) microplastics and the control rats (*P*=0.077). The absence of a statistically significant difference in CA 15-3 levels in this study suggests that PET microplastic exposure has no effect on this marker. CA 15-3, a soluble fragment of Mucin 1 (MUC1), is a well-established biomarker for breast cancer and is commonly used to monitor disease progression and treatment response (Duffy *et al.,* 2010). Alternatively, the lack of a significant difference may indicate that any potential carcinogenic effects of PET microplastics are still in the early stages, influencing underlying pathways that have not yet resulted in elevated CA 15-3, which is primarily associated with later disease progression.

While no study has directly assessed CA 15-3 in relation to PET microplastic exposure, previous research has demonstrated that exposure to different types of microplastics has a significant alteration/influence on breast cancer cell lines (Park *et al.,* 2023; Schnee *et al.,* 2024). Park *et al.* (2023), in their study, found that polypropylene microplastics (PPMPs) significantly accelerated the cell cycle in breast cancer cell lines, which does not directly agree with the breast cancer marker result in this study. The reason for this discrepancy may be that while we examined the effects of PET microplastics on serum marker (CA 15-3) in this study, Park *et al.* (2023) examined the effects of polypropylene microplastics on breast cancer cells, which suggests that different types of microplastics may have varying biological effects depending on their composition, exposure route, and target system. Similarly, Schnee *et al.* (2024) observed absorption of microplastic particles in breast cancer cells exposed to polystyrene (PS) particles, suggesting that different plastics can differentially affect cancer cell behavior. Their study focused on cellular responses, including cancer cell absorption and accumulation of microplastics, while our study evaluated systemic biochemical changes in vivo, which may also account for the difference in the results between the studies.

This study also found a significant increase in the levels of tumour necrosis factor alpha (TNF-α) (*P*<0.001) following PET microplastic exposure (table 3). Since the inflammatory cytokine TNF-α is a key mediator of chronic inflammation and tumorigenesis (Hanahan & Weinberg, 2011), its significant elevation suggests that PET microplastics induce a persistent inflammatory response. Chronic inflammation is a well-established driver of various disease conditions, including cancer. The activation of inflammatory pathways in response to PET microplastics may result from the stimulation of macrophages and the recruitment of immune cells (Hu & Palić, 2020). PET microplastics are recognized as foreign particles by macrophages, which respond by releasing inflammatory cytokines such as TNF-α, interleukin-6 (IL-6), and interleukin-1β (IL-1β). The persistent elevation of TNF-α following PET microplastic exposure indicates a long-term inflammatory state that may accelerate disease progression (Hu & Palić, 2020; Deng *et al.,* 2017). This chronic inflammatory environment can also contribute to immune evasion, whereby malignant cells exploit dysregulated immune signaling to escape immune surveillance. Pro-tumorigenic signaling pathways—particularly those involving nuclear factor kappa B (NF-κB) and TNF-α—are activated under such conditions. These pathways promote the transcription of genes responsible for cell survival, proliferation, and angiogenesis, all of which are essential for tumor development and progression (Zhao *et al.,* 2021). Furthermore, sustained inflammation triggered by PET microplastics may lead to excessive generation of reactive oxygen species (ROS), resulting in oxidative stress. ROS-induced damage to cellular components—such as DNA, lipids, and proteins—can further increase cellular dysfunction. Notably, oxidative damage may activate NF-κB, a central transcription factor regulating immune and inflammatory responses. Elevated ROS levels can cause both single- and double-strand DNA breaks, thereby increasing the risk of mutations and genomic instability, particularly in settings where DNA repair mechanisms are impaired. This genomic instability is a hallmark of carcinogenesis, driving uncontrolled cell proliferation (Prüst *et al.,* 2020; Kumar *et al.,* 2024).

This study’s findings are consistent with that of Campanale *et al.* (2020), who reported that inhaled microplastics trigger inflammation and increase cytokine levels. Similarly, Deng *et al.* (2021) found that microplastic leachates upregulate pro-inflammatory pathways, leading to chronic inflammation and increased cancer risk. Moreover, Park *et al.* (2023) observed enhanced IL-6 secretion in breast cancer cells exposed to polypropylene microplastics, reinforcing the link between plastic exposure and inflammatory cytokine production. The increased levels of TNF-α in this study suggest that while PET microplastics may not directly elevate CA 15-3 levels, they may promote an inflammatory microenvironment conducive to disease development, including breast tissue alterations and cancer progression.

Histological results of breast tissues also support these biochemical findings. Sections of breast tissue from control rats (Plate 1A) and Group 2 rats (Plate 1B) that received a very low dose of PET microplastics showed unremarkable breast ducts and lobules, indicating normal tissue architecture with no significant pathological changes. However, Group 3 (Plate 2C) and Group 4 rats (Plate 3D), which were exposed to higher PET doses, exhibited notable histopathological alterations, including ducts containing pinkish luminal secretion and increased stromal fibrosis, suggestive of fibrocystic changes.

Fibrocystic changes, though benign, reflect chronic tissue stress and may be indicative of underlying endocrine disruption and inflammation (Malherbe *et al.,* 2023). Hence, in this study, the observed fibrocystic alterations may result from inflammatory responses triggered by exposure to PET microplastics. This inflammation may serve as a potential mechanism through which PET contributes to breast tissue remodeling and possibly elevates the risk of disease development. Fibrosis, a key feature of tissue remodeling, arises from the excessive accumulation of extracellular matrix (ECM) components—primarily collagen and fibronectin—typically in response to persistent injury or chronic inflammation (Zhao *et al.,* 2022). Chronic inflammation activates a cascade of signaling events that enhance ECM deposition, increase tissue stiffness, and alter the mechanical properties of tissues. Immune cells such as macrophages, neutrophils, and lymphocytes remain persistently activated, with M2 macrophages secreting profibrotic cytokines like transforming growth factor-beta (TGF-β) and platelet-derived growth factor (PDGF), which in turn stimulate fibroblast activation and differentiation into myofibroblasts. These myofibroblasts possess contractile properties and secrete high levels of ECM, leading to fibrotic scarring due to disrupted tissue remodeling which can in turn lead to pathological conditions (Li *et al.,* 2021; Eom *et al.,* 2025).

Furthermore, chronic inflammation disturbs the balance between ECM synthesis and degradation. Dysregulation of matrix metalloproteinases (MMPs), enzymes crucial for ECM turnover, contributes to ECM accumulation and tissue stiffening. This altered mechanical environment can interfere with normal cellular mechanotransduction signals, potentially activating tumorigenic pathways. As fibrosis progresses, the expanding fibrotic matrix limits oxygen diffusion, creating a hypoxic microenvironment that induces hypoxia-inducible factors (HIF-1α and HIF-2α). These factors not only accelerate fibrosis but also stimulate angiogenesis—a process often exploited by tumors for growth and metastasis (Marozzi et al., 2021). The fibrotic microenvironment is inherently pro-tumorigenic. Increased tissue stiffness promotes epithelial-to-mesenchymal transition (EMT), enabling epithelial cells to acquire migratory and invasive characteristics essential for tumor progression (Marozzi *et al.,* 2021). Additionally, the dense ECM can form physical barriers that hinder immune cell infiltration, impairing effective immune surveillance and allowing malignant cells to evade destruction. Fibrosis-induced hypoxia further enhances tumorigenesis by upregulating vascular endothelial growth factor (VEGF), which drives the formation of abnormal and dysfunctional blood vessels. These neovessels may not only supply nutrients to tumor cells but also facilitate their dissemination and metastatic spread (Marozzi *et al.,* 2021; Lee *et al.,* 2024).

The significant increase in TNF-α in this study aligns with the histological findings, suggesting that PET microplastics contribute to a pro-inflammatory microenvironment that may predispose breast tissue to alterations, including tumour development. Group 5 (Plate 4E), which received water stored in PET bottles exposed to sunlight, showed no significant histopathological abnormalities, with unremarkable skeletal muscle and fibrous tissue. The absence of fibrocystic changes in Group 5 indicates that PET microplastics may not have leached in this case to cause a detectable change in the parameters studied as well as breast tissue histological characterization.

1. **CONCLUSION**

The findings from this study revealed that chronic exposure to polyethylene terephthalate (PET) microplastics significantly increases the levels of TNF-α. Histological findings revealed fibrocystic breast changes in PET-exposed groups, suggesting potential long-term risks associated with PET microplastic exposure. The increased inflammatory cytokines observed in this study highlight PET microplastics as contributors to a pro-inflammatory microenvironment, which may predispose breast tissue to pathological alterations and hormone-related disorders, including breast cancer risks. These findings highlight the potential health risks associated with prolonged PET microplastic exposure.

Consent

It is not applicable.

Ethical approval (where ever applicable)

The National Research Council's Guide for the Care and Use of Laboratory Animals Health (National Research Council, 2011) and the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines (Percie du Sert, 2020) were followed in this research.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The author(s) hereby declares that this manuscript is solely the result of the author’s original research. No generative AI technologies such as large language models (ChatGPT, Copilot, etc.) and text-to-image generators have been used during writing of this manuscript.

References

Avwioro, O.G. (2014). Histochemistry& tissue pathology: principles & technique (3rd ed.). Claverianum Press Ltd, Nigeria.

Bioassay Technology Laboratory (2024). Rat Carbohydrate Antigen CA 15-3 ELISA kits (Cat. No E1139Ra) Retrieved from <https://www.bt-laboratory.com/>

Campanale, C., Massarelli, C., Savino, I., Locaputo, V. & Uricchio, V. F. (2020). A Detailed Review Study on Potential Effects of Microplastics and Additives of Concern on Human Health. *International Journal of Environmental Research and Public Health*, *17*(4), 1212. <https://doi.org/10.3390/ijerph17041212>

De Vos, L., Van de Voorde, B., Van Daele, L., Dubruel, P., & Van Vlierberghe, S. (2021). Poly (alkylene terephthalate) s: From current developments in synthetic strategies towards applications. *European Polymer Journal*, *161*, 110840. <https://doi.org/10.1016/j.eurpolymj.2021.110840>

Devi, H. J., Karkuzhali, P., Baskaran, P. K., & Lilly, S. M. (2021). The Detailed Study of CD10: A Stromal Marker in Breast Carcinoma. *Journal of Pharmaceutical Research International,* 33(22B), 62–70. <https://doi.org/10.9734/jpri/2021/v33i22B31399>

Deng, P., Tan, M., Zhou, W., Chen, C., Xi, Y., Gao, P., Ma, Q., Liang, Y., Chen, M., Tian, L., Xie, J., Liu, M., Luo, Y., Li, Y., Zhang, L., Wang, L., Zeng, Y., Pi, H., Yu, Z., & Zhou, Z. (2021). Bisphenol A promotes breast cancer cell proliferation by driving miR-381- 3p-PTTG1-dependent cell cycle progression. *Chemosphere*, *268*, 129221. <https://doi.org/10.1016/j.chemosphere.2020.129221>

Deng, Y., Zhang, Y., Lemos, B., & Ren, H. (2017). Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. *Scientific Reports*, *7*, 46687. <https://doi.org/10.1038/srep46687>

Dhaka, V., Singh, S., Anil, A. G., Sunil Kumar Naik, T. S., Garg, S., Samuel, J., Kumar, M., Ramamurthy, P. C., & Singh, J. (2022). Occurrence, toxicity and remediation of polyethylene terephthalate plastics. A review. *Environmental Chemistry Letters*, *20*(3), 1777–1800. <https://doi.org/10.1007/s10311-021-01384-8>

Duffy, M. J., Evoy, D., & McDermott, E. W. (2010). CA 15-3: uses and limitation as a biomarker for breast cancer. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, *411*(23-24), 1869–1874. <https://doi.org/10.1016/j.cca.2010.08.039>

Elabscience Bioinovation Inc. (2024). Rat TNF-α(Tumor Necrosis Factor Alpha) ELISA Kit (E- EL-R2856) Retrieved from [https://www.elabscience.com/p/rat-tnf-tumor-necrosis- factor-alpha-elisa-kit--e-el-r2856](https://www.elabscience.com/p/rat-tnf-tumor-necrosis-%09factor-alpha-elisa-kit--e-el-r2856)

Eom, Y. W., Hong, J. E., Jung, P. Y., Yoon, Y., Yoo, S. H., Hong, J., Rhee, K. J., Regmi, B., Fatima, S., Kim, M. Y., Baik, S. K., Ryu, H., & Kwon, H. Y. (2025). TGF-β expressed by M2 macrophages promotes wound healing by inhibiting TSG-6 expression by mesenchymal stem cells. *PloS One*, *20*(4), e0316692. <https://doi.org/10.1371/journal.pone.0316692>

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell*, *144*(5), 646–674. <https://doi.org/10.1016/j.cell.2011.02.013>

Hu, M., & Palić, D. (2020). Micro- and nano-plastics activation of oxidative and inflammatory adverse outcome pathways. *Redox Biology*, *37*, 101620. <https://doi.org/10.1016/j.redox.2020.101620>

Kannan, K., & Vimalkumar, K. (2021). A Review of Human Exposure to Microplastics and Insights Into Microplastics as Obesogens. *Frontiers in Endocrinology*, *12*, 724989. <https://doi.org/10.3389/fendo.2021.724989>

Kiernan, J. A. (2001). Histological & histological methods: theory & practice. Butter-worth, oxford.

Kumar, N., Lamba, M., Pachar, A. K., Yadav, S., & Acharya, A. (2024). Microplastics - A Growing Concern as Carcinogens in Cancer Etiology: Emphasis on Biochemical and Molecular Mechanisms. *Cell Biochemistry and Biophysics*, *82*(4), 3109–3121. <https://doi.org/10.1007/s12013-024-01436-0>

Kumar, U., Singh, A., Chandra, K., Atreya, K., Singh, R., & Kumar, M. (2022). Molecular Classification of Breast Carcinoma Based on the Prognostic Marker: A Clinico- pathological Correlation. *Journal of Advances in Medicine and Medical Research,* 34(23), 237–247. <https://doi.org/10.9734/jammr/2022/v34i234859>

Lee, S. A., Cho, G. J., Kim, D., & Kim, D. H. (2024). Biophysical interplay between extracellular matrix remodeling and hypoxia signaling in regulating cancer metastasis. *Frontiers in Cell and Developmental Biology*, *12*, 1335636. <https://doi.org/10.3389/fcell.2024.1335636>

Li, M., Hou, Q., Zhong, L., Zhao, Y., & Fu, X. (2021). Macrophage Related Chronic Inflammation in Non-Healing Wounds. *Frontiers in Immunology*, *12*, 681710. <https://doi.org/10.3389/fimmu.2021.681710>

Li, T., Bian, B., Ji, R., Zhu, X., Wo, X., Song, Q., Li, Z., Wang, F., & Jia, Y. (2024). Polyethylene Terephthalate Microplastic Exposure Induced Reproductive Toxicity Through Oxidative Stress and p38 Signaling Pathway Activation in Male Mice. *Toxics*, *12*(11), 779. <https://doi.org/10.3390/toxics12110779>

Li, Y., Chen, L., Zhou, N., Chen, Y., Ling, Z. & Xiang, P. (2024). Microplastics in the human body: A comprehensive review of exposure, distribution, migration mechanisms, and toxicity. *The Science of the Total Environment*, *946*, 174215. <https://doi.org/10.1016/j.scitotenv.2024.174215>

Li, Y., Tao, L., Wang, Q., Wang, F., Li, G., & Song, M. (2023). Potential Health Impact of Microplastics: A Review of Environmental Distribution, Human Exposure, and Toxic Effects. *Environment & Health (Washington, D.C.)*, *1*(4), 249–257. <https://doi.org/10.1021/envhealth.3c00052>

Malherbe, K., Khan, M., & Fatima, S. (2023). Fibrocystic Breast Disease. In *StatPearls*. StatPearls Publishing.

Marozzi, M., Parnigoni, A., Negri, A., Viola, M., Vigetti, D., Passi, A., Karousou, E., & Rizzi, F. (2021). Inflammation, Extracellular Matrix Remodeling, and Proteostasis in Tumor Microenvironment. *International Journal of Molecular Sciences*, *22*(15), 8102. <https://doi.org/10.3390/ijms22158102>

National Research Council. (2011). *Guide for the Care and Use of Laboratory Animals: Eighth Edition*. Washington, DC: The National Academies Press. <https://doi.org/10.17226/12910>.

Park, J. H., Hong, S., Kim, O. H., Kim, C. H., Kim, J., Kim, J. W., Hong, S., & Lee, H. J. (2023). Polypropylene microplastics promote metastatic features in human breast cancer. *Scientific Reports*, *13*(1), 6252. <https://doi.org/10.1038/s41598-023-33393-8>

Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., ... & Würbel, H. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *Journal of Cerebral Blood Flow & Metabolism*, *40*(9), 1769-1777. <https://doi.org/10.1111/bph.15193>

Prata J. C. (2018). Airborne microplastics: Consequences to human health? *Environmental Pollution (Barking, Essex: 1987)*, *234*, 115–126. <https://doi.org/10.1016/j.envpol.2017.11.043>

Priya, A. K., Jalil, A. A., Dutta, K., Rajendran, S., Vasseghian, Y., Qin, J., & Soto-Moscoso, M. (2022). Microplastics in the environment: Recent developments in characteristic, occurrence, identification and ecological risk. *Chemosphere*, *298*, 134161. <https://doi.org/10.1016/j.chemosphere.2022.134161>

Prüst, M., Meijer, J., & Westerink, R. H. S. (2020). The plastic brain: neurotoxicity of micro- and nanoplastics. *Particle and Fibre Toxicology*, *17*(1), 24. <https://doi.org/10.1186/s12989-020-00358-y>

Rahman, A., Sarkar, A., Yadav, O. P., Achari, G., & Slobodnik, J. (2021). Potential human health risks due to environmental exposure to nano- and microplastics and knowledge gaps: A scoping review. *The Science of the Total Environment*, *757*, 143872. <https://doi.org/10.1016/j.scitotenv.2020.143872>

Rodgers, K. M., Udesky, J. O., Rudel, R. A., & Brody, J. G. (2018). Environmental chemicals and breast cancer: An updated review of epidemiological literature informed by biological mechanisms. *Environmental Research*, *160*, 152–182. <https://doi.org/10.1016/j.envres.2017.08.045>

Schnee, M., Sieler, M., Dörnen, J., & Dittmar, T. (2024). Effects of polystyrene nano- and microplastics on human breast epithelial cells and human breast cancer cells. *Heliyon*, *10*(20), e38686. <https://doi.org/10.1016/j.heliyon.2024.e38686>

Schwabl, P., Köppel, S., Königshofer, P., Bucsics, T., Trauner, M., Reiberger, T., & Liebmann, B. (2019). Detection of Various Microplastics in Human Stool: A Prospective Case Series. *Annals of Internal Medicine*, *171*(7), 453–457. [https://doi.org/10.7326/M19- 0618](https://doi.org/10.7326/M19-%090618)

Schymanski, D., Goldbeck, C., Humpf, H. U., & Fürst, P. (2018). Analysis of microplastics in water by micro-Raman spectroscopy: Release of plastic particles from different packaging into mineral water. *Water Research*, *129*, 154–162. <https://doi.org/10.1016/j.watres.2017.11.011>

Sharma, S., Sharma, V., & Chatterjee, S. (2023). Contribution of plastic and microplastic to global climate change and their conjoining impacts on the environment - A review. *The Science of the Total Environment*, *875*, 162627. <https://doi.org/10.1016/j.scitotenv.2023.162627>

Sun, H., Dai, J., Chen, M., Chen, Q., Xie, Q., Zhang, W., Li, G., & Yan, M. (2022). miR-139- 5p Was Identified as Biomarker of Different Molecular Subtypes of Breast Carcinoma. *Frontiers in Oncology*, *12*, 857714. <https://doi.org/10.3389/fonc.2022.857714>

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, *71*(3), 209–249. <https://doi.org/10.3322/caac.21660>

The Danish Environmental Protection Agency (Danish EPA) (2013). Phthalates Strategy. Copenhagen, Denmark: The Danish EPA. Retrieved from <https://www2.mst.dk/Udgiv/publications/2013/06/978-87-93026-22-3.pdf> Retrieved January, 2025.

The REACH Regulation (Regulation (EC) No 1907/2006) on Registration (2006). Evaluation and Authorisation and Restriction of Chemicals. Retrieved from <https://osha.europa.eu/it/legislation/directives/regulation-ec-no-1907-2006-of-the-> european-parliament-and-of-the-council

World Health Organization. (2024). *Breast cancer*. Retrieved from [https://www.who.int/news- room/fact-sheets/detail/breast-cancer](https://www.who.int/news-%09room/fact-sheets/detail/breast-cancer) Retrieved January, 2025.

Yee, M. S., 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 (Basel, Switzerland)*, *11*(2), 496. <https://doi.org/10.3390/nano11020496>

Ziani, K., Ioniță-Mîndrican, C. B., Mititelu, M., Neacșu, S. M., Negrei, C., Moroșan, E., Drăgănescu, D., & Preda, O. T. (2023). Microplastics: A Real Global Threat for Environment and Food Safety: A State of the Art Review. *Nutrients*, *15*(3), 617. <https://doi.org/10.3390/nu15030617>

Zhao, H., Wu, L., Yan, G., Chen, Y., Zhou, M., Wu, Y., & Li, Y. (2021). Inflammation and tumor progression: signaling pathways and targeted intervention. *Signal Transduction and Targeted Therapy*, *6*(1), 263. <https://doi.org/10.1038/s41392-021-00658-5>

Zhao, X., Chen, J., Sun, H., Zhang, Y., & Zou, D. (2022). New insights into fibrosis from the ECM degradation perspective: the macrophage-MMP-ECM interaction. *Cell & Bioscience*, *12*(1), 117. <https://doi.org/10.1186/s13578-022-00856-w>